



## Research Paper

# An Environmental-friendly Procedure Based on Deep Eutectic Solvent for Extraction and Determination of Toxic Elements in Fish Species from Different Regions of Iraq



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## ARTICLE INFO

## Keywords:

Deep eutectic solvent

Fish analysis

Liquid-phase microextraction

Toxic elements

Vortex assisted

## ABSTRACT

In this study, an eco-friendly procedure was established by vortex-assisted liquid-phase microextraction based on deep eutectic solvent (VA – LPME – DES) combined with graphite furnace atomic absorption spectroscopy (GFAAS). The performance of this method was demonstrated by the extraction and analysis of lead (Pb), cadmium (Cd), and mercury (Hg) in fish samples. The hydrophobic DES is considered as a green extractant (environmentally friendly and less toxic than common organic solvents) and is a suitable alternative to common toxic organic solvents and is made of *l*-menthol and ethylene glycol (EG) with a molar ratio of 1:1. Under optimized conditions, the method linearity was in the ranges of 0.15–150  $\mu\text{g kg}^{-1}$  with the coefficient of determinations ( $r^2$ ) higher than 0.996. Accordingly, the detection limits for Pb, Cd, and Hg were 0.05, 0.05, and 0.10  $\mu\text{g kg}^{-1}$ , respectively. The analysis of fish samples showed that the concentration of toxic elements in fish caught from the Tigris and Euphrates Rivers is much higher than the concentration of these elements in locally farmed trout fish. Also, the analysis of fish-certified reference materials with presented procedure produced results that were in good agreement with the certified values. The results showed that VA – LPME – DES is a very cheap, fast, and environmental-friendly procedure for the analysis of toxic elements in different types of fish species.

Fish protein has a high biological value and contains a wide range of essential amino acids such as leucine, isoleucine, valine, phenylalanine, threonine, methionine, tryptophan, and lysine, therefore, it has a relatively high digestibility coefficient, which is suitable for all age groups (Safari et al., 2018).

Unfortunately, the rapid growth of the population and the development of residential, commercial, industrial, and agricultural centers has caused the urban, industrial, and agricultural garbage and sewage

to increase year by year and causes pollution of the environment and the water of the seas and oceans (Jovanovic et al., 2017). Among the most important marine pollutants are petroleum hydrocarbons and toxic elements. Heavy metals enter the environment through the process of melting, extraction, combustion of fuel materials, transportation and processing of petroleum materials, discharge of waste materials, and accidental leakage (Pirsaheb & Fattahi, 2015; Hu & Chen, 2016). Following the transfer of these pollutants to aquatic envi-

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<https://doi.org/10.1016/j.jfp.2023.100102>

Received 18 February 2023; Accepted 6 May 2023

Available online 11 May 2023

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ronments, there is a possibility that fish will absorb some toxic elements from the environment through the food chain or through water. Unlike organic compounds, toxic elements are persistent pollutants that do not break down in nature through chemical or biological processes (Perelsonia et al., 2021; Hwang et al., 2019). The stability of toxic elements makes their bioavailability in the food chain high, so that their amount in the food chain can be several times their amount in the environment (Bozorgzadeh et al., 2021). Accordingly, trace extraction and measurement of metal ions in food and aquatic media are highly significant.

A number of analytical instruments have been developed for the analysis of trace amounts of toxic elements, including the inductively coupled plasma-mass spectrometry (ICP-MS) (Fan et al., 2020; Qin et al., 2021; King et al., 2010), inductively coupled plasma-optical emission spectrometry (ICP-OES) (Bozorgzadeh et al., 2021; Cai & Wang, 2022; Vazquez et al., 2015), flame atomic absorption spectrometry (FAAS) ((Ghanemi, Nikpour, Omidvar, & Maryamabadi, 2011; Haq et al., 2021, 2022)), and graphite furnace atomic absorption spectrometry (GFAAS) (Safari et al., 2018; Pirsahab & Fattahi, 2015; Behbahani et al., 2022).

In cases where the amount of toxic elements in the sample is high, acid digestion and sample preparation for compliance with the analysis instrument are sufficient. But in some cases, the concentration of elements in the sample may be lower than the detection limit of the analysis instrument. In this case, extraction and preconcentration should be done. So far, different sample preparation methods such as liquid-liquid extraction (LLE) (Oliva et al., 2002), solid phase extraction (SPE) (Shahryari et al., 2022; Sa'adi & Es'haghi, 2019), co-precipitation (Saracoglu et al., 2003), cloud point extraction (CPE) (Khan et al., 2017; Citak & Tuzen, 2010), single-drop microextraction (SDME) (Cheng et al., 2013), hollow fiber liquid-phase microextraction (HF-LPME) (Pimparu et al., 2016), dispersive liquid-liquid microextraction (DLLME) (Akhtar et al., 2016; Jalbani & Soylyak, 2015), and DLLME based on solidification of floating organic drop (DLLME-SFO) (Li et al., 2015) have been developed for the extraction and preconcentration of toxic elements from various matrices. The advantages and disadvantages of these methods are discussed in the literature (Safari et al., 2018).

In recent years, environmentally friendly and inexpensive solvents called deep eutectic solvents (DESs) have attracted the attention of chemists and scientists. DES consists of two components: hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). These two components are connected by hydrogen bond at appropriate temperature and specific molar ratio (Fattahi, Pirsahab, et al., 2022; Yuan et al., 2022; Momotko, et al., 2022). Until now, these solvents have been employed for sample preparation and extraction of different compounds including organic and inorganic (Nemati et al., 2022; Fattahi, Hashemi, et al., 2022; Shishov et al., 2022; Momotko, et al., 2021; Sorouraddin et al., 2020; Makos et al., 2018). Nevertheless, despite the undeniable potential of DESs, there is still controversy about their toxicity. Besides the low number of studies on this topic, there are also some contradicting reports on the biocompatibility of these solvents. Thus, to better apply DESs in green and sustainable chemistry, more studies on their impact on organisms at different trophic levels and the use of proper techniques are required (Marchel et al., 2022).

Recently, Ahmadi Jouybari and co-workers have synthesized a DES using *l*-menthol and ethylene glycol (EG) in a molar ratio of 1:1 and investigated the physical and chemical properties of the synthesized DES and characterized it using Fourier-transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA) (Ahmadi Jouybari et al., 2022). They investigated the application of the synthesized DES in the extraction and preconcentration of triazine herbicides in fruits and vegetables (Ahmadi Jouybari et al., 2022). Considering that the synthesized DES is safe, cheap, environmentally friendly and its synthesis is very simple, we decided to investigate

its application in the extraction of metal ions. The purpose of the current study was to develop a vortex-assisted liquid-phase microextraction (VA-LPME) based on DES followed by GFAAS for the extraction and determination of toxic elements in fish samples. In fact, in this study, our main goal is to prepare a green and cheap DES and use it in the LPME procedure to extraction and enrichment of toxic elements in fish species. It provides a new idea and reference for the extraction, separation, and analysis of toxic elements in fish samples.

## Experimental

### Reagents and standards

Ethylene glycol (EG, analytical grade), *l*-menthol (analytical grade), nitric acid (ultrapur), hydrogen peroxide (suprapur), hydrochloric acid (37%, analytical grade), palladium(II) nitrate, magnesium nitrate, sodium chloride, and diethyldithiophosphoric acid (DDTP) as chelating agent were purchased from Merk Company (Darmstadt). The stock standard solutions of Pb, Cd, and Hg ( $1,000 \text{ mg L}^{-1}$ ) were obtained from Sigma Chem. Co. (St. Louis, USA). Two fish-certified reference materials (CRMs), including DORM-2-dogfish muscle, NRC, Canada and Muscle tissue-NIST SRM 2976, which contained specific amounts of the desired metals, were purchased from National Institute of Standard and Technologies (NIST, Boulder, CO, USA). A mixture of palladium(II) nitrate and magnesium nitrate were used as chemical modifiers with concentrations of  $1,000 \text{ mg L}^{-1}$  and  $300 \text{ mg L}^{-1}$ , respectively.

### Apparatus

An Analytik Jena AG, Jena, Germany model Nov AA 400 AAS with deuterium background correction, equipped with MPE 60 autosampler, were used for the analysis of target metal ions. Pyrolytic graphite-coated graphite tubes (Analytik Jena Part No. 407-A81.026) were used for all measurements. Argon gas with a purity of 99.999% (Air Products, UK) was used at a flow rate of  $500 \text{ mL min}^{-1}$ . Pb was detected at wavelength 283.3 nm with slit width of 0.7 nm and 10 mA lamp current. Cd was detected at wavelength 228.8 nm with slit width of 0.7 nm and 8 mA lamp current. Hg was detected at wavelength 253.7 nm with slit width of 0.2 nm and 5 mA lamp current. Temperature program and other device parameters for AAS are summarized in Table 1. For the digestion of fish samples, a microwave model Multiwave 3000 (Anton Paar, Germany) was employed.

### Sampling and sample preparation

Fish samples were purchased from local fish shops. For this purpose, two samples of fish caught from the Tigris River (snapper and croaker), two samples of fish caught from the Euphrates River (perch and carp), and two samples of farmed trout fish were selected. After transporting the fish samples to the laboratory, they were washed with distilled water and their muscle tissue was separated with a sharp steel knife. To remove the water in the muscle tissue, the samples were air-dried and then oven-dried for 10 h to reach a constant weight. After grinding and sieving, the samples were subjected for further digestion.

For sample digestion, 0.10 g of each fish species was digested in microwave with 2 mL  $\text{HNO}_3$  (65%, ultrapur) and  $\text{H}_2\text{O}_2$  (30%, suprapur) mixture with a molar ratio of 3:1 and a temperature of  $150^\circ\text{C}$  for 20 min. The obtained solution reached a volume of 10 mL with ultra-pure water and filtered with Whatman filter paper ( $0.22 \mu\text{m}$ ). The resulted solution was subjected to VA-DLLME-DES.

**Table 1**  
Temperature program and optimum operating parameters of investigated toxic elements

| Step            | Temperature (°C) |       |       | Time (s) |      | Gas flow (mL min <sup>-1</sup> ) |
|-----------------|------------------|-------|-------|----------|------|----------------------------------|
|                 | Cd               | Pb    | Hg    | Ramp     | Hold |                                  |
| Prewarming      | 60               | 60    | 55    | 1        | 2    | 500                              |
| Inject modifier | 85               | 85    | 85    | 5        | 15   | 500                              |
| Inject sample   |                  |       |       |          |      |                                  |
| Drying I        | 110              | 120   | 90    | 10       | 15   | 500                              |
| Drying II       | 130              | 240   | 100   | 5        | 20   | 500                              |
| Ashing          | 380              | 650   | 200   | 10       | 10   | 500                              |
| Gas stop step   | 380              | 650   | 200   | 0        | 1    | 0                                |
| Atomization     | 1,800            | 1,900 | 1,000 | 0        | 3    | 0                                |
| Cleaning        | 2,300            | 2,200 | 1,600 | 0        | 2    | 1,100                            |

### Preparation of DES

For the synthesis of DES, *l*-menthol and EG are mixed together with a molar ratio of 1:1, and then, the mixture is kept on a thermostatic magnetic at a temperature of 40°C until it becomes completely clear. After the DES synthesis, which takes about 30 min, the obtained solvent is cooled to room temperature and kept for further use. The characterization of the synthesized DES using NMR, FT-IR, TGA, and DSC has been done before (Ahmadi Jouybari et al., 2022).

### VA-LPME-DES procedure

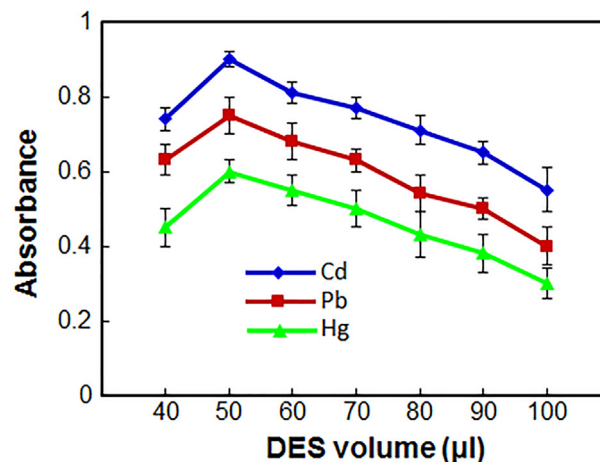
Firstly, the prepared sample solution or 10 mL of distilled water (spiked with 0.5, 0.5, and 1.0 µg L<sup>-1</sup> of Pb, Cd, and Hg, respectively) was put in a glass tube and 100 mg NaCl was added. Then, 50.0 µL of DES containing 15.0 µL DDTP was added into a test tube, maintained at 35°C in a water bath and at the same time, it was vortexed for 10 min. Very fine drops of the DES are spread in the sample solution so that the solution becomes cloudy. Afterward, the target ions were reacted with ligand (DDTP) and transferred into extraction solvent. The obtained solution is centrifuged at a speed of 4,500 rpm for 5 min to collect the extraction solvent containing the analytes on the surface of the solution. The test tube was placed in the freezer for solidification of extraction solvent. The resulting DES was moved into a clean vial and melted at room temperature. Finally, 20.0 µL of the DES using autosampler was injected into the GFAAS.

### Results and discussion

In this research, the VA-LPME-DES was designed and used for the preconcentration of metal ions from fish species. Pb, Cd, and Hg were selected as model analytes to study the possibility of the VA-LPME-DES procedure. To reach a high EF and extraction efficiency (EE) with the employment of VA-LPME-DES, the conditions of VA, LPME, and DES steps should be optimized. Due to the fact that the conditions and parameters of DES were optimized in the previous study (Ahmadi Jouybari et al., 2022), its results were used in the current work. Only important and significant parameters were studied and optimized in VA and LPME stages.

#### Effect of the DES volume

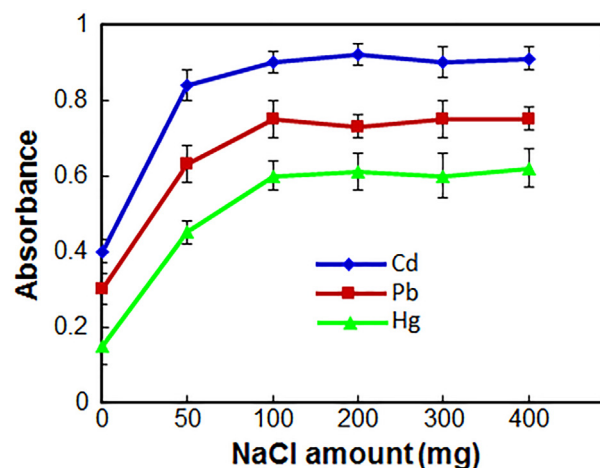
For investigation of the volume of DES, various DES volumes (40–100 µL at 10-µL intervals) were tested. Figure 1 shows the variation of the absorbance of metal ions versus the DES volume. As shown in the figure, analytical signal of target ions increases with increasing the DES volume from 40 to 50 µL and slightly decreases with a further increase of the DES volume. It is clear that the analytes are not well extracted in volumes less than 50 µL, and in volumes greater than 50 µL, the absorbance intensity of analytes decreases due to the dilution effect. Therefore, the best sensitivity was achieved with the employment of 50 µL DES.



**Figure 1.** Effect of the volume of DES on the absorbance of the metal ions.

#### Effect of the salt addition

When the DES is added into the sample solution, a water-in-row emulsion is formed and the dispersion of the DES in the sample solution is not satisfactory. Also, during phase separation, the DES (organic phase) is not completely separated from the aqueous phase. This problem is solved by increasing the salt and the ionic strength of the environment because salt breaks the emulsion. For this purpose, different experiments were performed by adding different amounts of NaCl in the range of 0–400 mg. As shown in Figure 2, when increasing the NaCl amount up to 100 mg, the absorbance of target ions increased



**Figure 2.** Effect of the salt addition on the absorbance of the metal ions.

and then decreased with further addition of NaCl. Because the emulsion is not broken well in salt amount less than 100 mg and in greater than 100 mg, the distribution coefficient of the analytes in the DES decrease. Thus, 100 mg of NaCl was chosen as the optimum amount of NaCl.

#### Effect of the sample solution pH

Sample solution pH plays an important role in the formation of metal-chelate ligand and following extraction efficiency. In this research, the molecular form of DDTP ligand is completely converted to ammonium salt using ammonia and the effect of pH on complex formation is investigated in the range of 1–7 (Safari et al., 2018). Figure 3 shows the effect of the pH on the absorbance intensity of target ions. As you can see in the figure, the analytical signal of target analytes is almost unchanged and maximum in pH = 1–4 and decreases at higher pH. Considering that the DDTP solution itself is acidic (pH ~ 2.3 in 10.0 mL sample solution), in the present work, no acid or buffer was added to pH adjustment, to prevent possible contamination.

#### Effect of the vortex time

In conventional DLLME, a disperser solvent is used to disperse the extraction solvent in the sample solution. One of the disadvantages of DLLME method is the large consumption of the disperser solvent, which in most cases is one milliliter or more. One of the best alternatives for disperser solvent is the use of a vortex, which can disperse the extraction solvent in the sample solution. The vortex increases the contact surface of the analytes with the DES by breaking the emulsion and improves the extraction efficiency. For this purpose, the vortex time was investigated in the range of 0–30 min. As shown in Figure 4, as the vortex time increases from 0 to 20 min, the analytical signal of metal ions increases and with a further increase in the vortex time, absorbance is constant. So, 20 min was selected as the optimum vortex time.

#### Effect of the DDTP concentration

The effect of DDTP concentration on the complexation and extraction of target ions was evaluated in the range of 0.01–0.30% (v/v). The results showed that with the increase of DDTP concentration from 0.01% to 0.15% (v/v), the absorbance of desired ions increases. When the ligand concentration exceeds 0.15%, the signal of ions remains

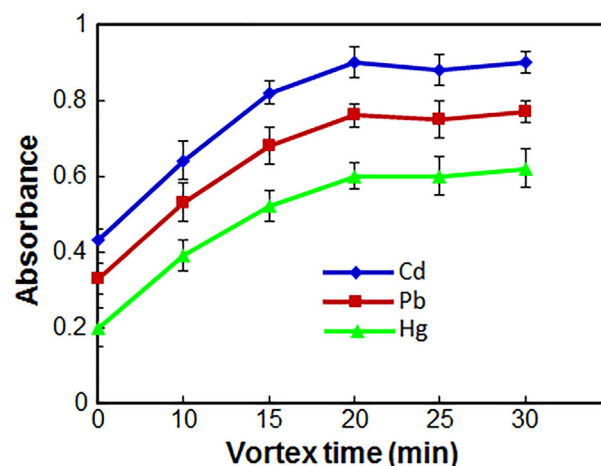


Figure 4. Effect of the vortex time on the absorbance of the metal ions.

almost constant. Thus, the 0.15% (v/v) of DDTP was selected as the best concentration.

#### Interference studies

The VA–LPME–DES–GFAAS procedure was employed for the recovery of target ions in the presence of various amounts of interfering ions in aqueous solution. In these experiments, 10.0 mL of solutions contains  $1.0 \mu\text{g L}^{-1}$  of Hg and  $0.50 \mu\text{g L}^{-1}$  of Pb and Cd, and various amounts of interfering ions were treated according to the recommended procedure. A given ion was considered to interfere if it resulted in a  $\pm 5\%$  variation of the signal of  $1.0 \mu\text{g L}^{-1}$  of Hg and  $0.50 \mu\text{g L}^{-1}$  of Pb and Cd. The results are summarized in Table 2.

#### Quantitative analysis

The analytical performance of the method including the precision (repeatability and reproducibility), linearity, coefficients of determination ( $r^2$ ), limits of detection (LODs), and enrichment factor (EF) was measured (Table 3). The repeatability and reproducibility of the VA–LPME–DES combined with GFAAS (based on seven replicate measurements) for  $0.50 \mu\text{g L}^{-1}$  of Hg and  $0.10 \mu\text{g L}^{-1}$  of Pb and Cd were determined to be 1.6–3.5% and 3.1–6.8%, respectively. Linear range was observed in the range of  $0.3–100 \mu\text{g kg}^{-1}$  for Hg and  $0.15–150 \mu\text{g kg}^{-1}$  for Pb and Cd with coefficients of determination higher than 0.996. The limit of detection (calculated as three times the standard deviation of seven blank measurements, divided by the slope of the calibration curve) was obtained in the range of  $0.05–0.10 \mu\text{g kg}^{-1}$ . The enrichment factor and enhancement factor (calculated as the ratio of the slopes of the calibration graphs with and without preconcentration) of metal ions were from 97–148 and 88–110, respectively.

#### Determination of toxic elements in fish samples

The presented method was successfully applied to the measurement of toxic elements in fish samples purchased from local fish shops. For this purpose, two samples of fish caught from the Tigris River, two samples from the Euphrates River and two samples of locally farmed trout fish were prepared and analyzed. The results are collected in Table 4. It should be noted that the Hg measured in this study is total Hg because organic Hg is converted to inorganic Hg during sample preparation. The results showed that the concentrations of Cd, Pb, and Hg in Euphrates River fish were in the range of 31.2–58.3, 183.6–212.5, and 326.1–518.2, respectively. The concentrations of Cd, Pb, and Hg in the Tigris River fish were in the range of 25.3

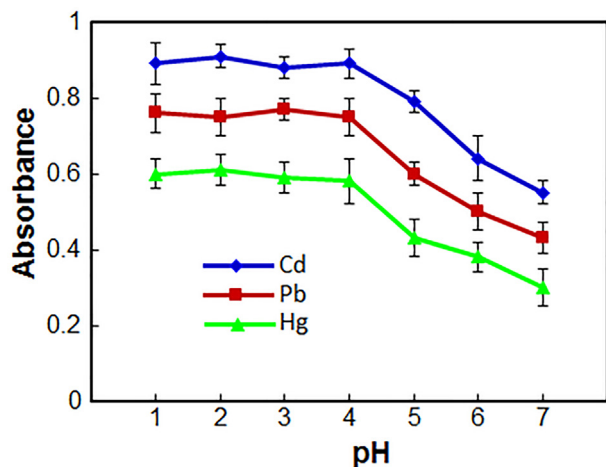


Figure 3. Effect of the sample solution pH on the absorbance of the metal ions.



**Table 2**  
Effect tolerance limits of the coexisting ions

| Interference                  | Interference to metalions ratio <sup>a</sup> | Recovery (%) |     |     |
|-------------------------------|--|--------------|-----|-----|
|                               |  | Cd           | Pb  | Hg  |
| Na <sup>+</sup>               | 10,000                                       | 93           | 93  | 102 |
| K <sup>+</sup>                | 2,000  | 91           | 94  | 96  |
| Ca <sup>2+</sup>              | 1,000  | 104          | 105 | 91  |
| Zn <sup>2+</sup>              | 1,000  | 102          | 101 | 89  |
| Zn <sup>2+</sup>              | 500  | 90           | 95  | 93  |
| Mn <sup>2+</sup>              | 1,000  | 97           | 108 | 90  |
| Mn <sup>2+</sup>              | 100  | 96           | 93  | 94  |
| Cu <sup>2+</sup>              | 1,000  | 92           | 91  | 103 |
| Al <sup>3+</sup>              | 500  | 105          | 107 | 95  |
| Co <sup>2+</sup>              | 500  | 107          | 92  | 107 |
| As <sup>5+</sup>              | 500  | 96           | 107 | 89  |
| As <sup>5+</sup>              | 100  | 106          | 101 | 94  |
| Fe <sup>3+</sup>              | 500  | 102          | 96  | 102 |
| Cr <sup>3+</sup>              | 200  | 93           | 99  | 108 |
| As <sup>3+</sup>              | 200  | 98           | 104 | 91  |
| Sn <sup>2+</sup>              | 100  | 101          | 90  | 106 |
| Ni <sup>2+</sup>              | 100  | 90           | 91  | 86  |
| Ni <sup>2+</sup>              | 50   | 104          | 106 | 103 |
| Cl <sup>-</sup>               | 100,000                                      | 101          | 97  | 97  |
| NO <sub>3</sub> <sup>-</sup>  | 10,000                                       | 95           | 99  | 99  |
| SO <sub>4</sub> <sup>2-</sup> | 10,000                                       | 91           | 103 | 104 |
| SCN <sup>-</sup>              | 10,000                                       | 96           | 96  | 98  |

<sup>a</sup> Concentration of Cd and Pb ions is 0.50 µg L<sup>-1</sup> and Hg ion is 1.0 µg L<sup>-1</sup>.

**Table 3**  
Figures of merit of the proposed method

| Analyte | Enrichment factor | Enhancement factor | Detection limit (µg kg <sup>-1</sup> ) <sup>a</sup> | RSD% <sup>b</sup> (intraday, n = 7) | RSD% (interday, n = 7) | Linear range (µg kg <sup>-1</sup> ) | r <sup>2</sup> |
|---------|-------------------|--------------------|---|-------------------------------------|------------------------|-------------------------------------|----------------|
| Cd      | 148               | 110                | 0.05  | 2.8                                 | 4.5                    | 0.15–150                            | 0.997          |
| Pb      | 125               | 100                | 0.05  | 1.6                                 | 3.1                    | 0.15–150                            | 0.998          |
| Hg      | 97                | 88                 | 0.10  | 3.5                                 | 6.8                    | 0.30–100                            | 0.996          |

<sup>a</sup> Detection limits were calculated based on 3S<sub>b</sub>/m.

<sup>b</sup> Percent relative standard deviation for seven replicate measurements of the elements with the concentration of 1.0 µg L<sup>-1</sup> for Hg and 0.50 µg L<sup>-1</sup> for Cd and Pb.

–45.3, 178.2–228.5, and 198.6–449.7, respectively. Finally, the concentrations of Cd, Pb, and Hg in locally farmed trout fish were in the range of 4.2–5.4, 11.5–28.2, and 43.6–55.7, respectively. According to the obtained results, the contamination of Tigris River and Euphrates River fishes with toxic elements is much higher than that of locally farmed trout fish. The reason can be the entry of environmental pollutants from industrial activities into the Rivers.

To assess the matrix effects and the accuracy of the proposed method, all samples were spiked with the target ions' standard solutions at different concentration levels. Also, to check the matrix effect of the samples with each other, the samples were spiked two by two with two identical concentration levels and analyzed with the proposed method. The corresponding relative recoveries are summarized in Table 4. As seen, the relative recoveries for all metal ions in spiked fish samples are in the range of 89–111%. For further validation of the presented method, two fish-certified reference materials (CRMs), including DORM-2-dogfish muscle, NRC, Canada, and Muscle tissue-NIST SRM 2976, which contained specific amounts of the desired metals, were extracted by this method and analyzed with an GFAAS. Based on the results summarized in Table 5, the obtained results were in satisfactory agreement compared to the certified values. These results demonstrate that the fish samples, in our present context, have no significant effect on VA–LPME–DES and GFAAS for analysis of the toxic elements.

#### Comparison with other extraction methods

A comparison between the introduced method and the currently published methods for the analysis of toxic elements in fish species

is presented in Table 6. As seen in this table, the detection limit of the presented method is better than other methods, except for the DLLME–SFO method, which is comparable to our method. Meanwhile, in the DLLME–SFO method, methanol is used as a disperser solvent. The linear range of this method is wider than other methods and the precision is better. One of the most important advantages of this method compared to other methods is the very low consumption of organic solvent. Although the organic solvent used is safe and environmentally friendly. The amount of sample consumed in this method is very small and with only 0.1 g of sample, very good accuracy, precision, and detection limit are obtained.

In conclusion, a hydrophobic DES, consisting of *l*-menthol and EG at a molar ratio of 1:1, was used as extraction solvent in LPME. Vortex was used to spread the extraction solvent in the sample solution, increase the contact surface, and quickly transfer the analytes into the organic phase. The VA–LPME–DES procedure was successfully employed for the analysis of toxic elements in fish species. The method linearity was in the ranges of 0.15–150 µg kg<sup>-1</sup> with coefficient of determinations (r<sup>2</sup>) higher than 0.996. Accordingly, the detection limits for Pb, Cd, and Hg were 0.05, 0.05, and 0.10 µg kg<sup>-1</sup>, respectively. One of the important advantages of this method compared to the conventional DLLME is the elimination of disperser solvent and reducing the consumption of organic solvents. The current procedure was employed for the extraction and determination of metal ions in fish species. The results showed that the concentration of toxic elements in fish caught from the Tigris and Euphrates Rivers is much higher than the concentration of these metals in locally farmed trout fish. The reason for this is industrial activities and the entry of toxic elements into Rivers.

**Table 4**  
Toxic elements concentration in the fish species

| Fish sample         | Analyte | Added ( $\mu\text{g kg}^{-1}$ ) | Found ( $\mu\text{g kg}^{-1}$ ) $\pm$ SD (n = 3) | Relative recovery (%) |
|---------------------|---------|---------------------------------|--|-----------------------|
| Snapper fish        | Cd      | –                               | 31.2 $\pm$ 1.2                                   | –                     |
|                     |         | 10                              | 40.1 $\pm$ 3.3                                   | 89                    |
|                     |         | 100                             | 133.8 $\pm$ 9.2                                  | 103                   |
|                     | Pb      | –                               | 212.5 $\pm$ 17.8                                 | –                     |
|                     |         | 10                              | 223.2 $\pm$ 19.6                                 | 107                   |
|                     |         | 100                             | 305.4 $\pm$ 17.5                                 | 93                    |
|                     | Hg      | –                               | 518.2 $\pm$ 35.1                                 | –                     |
|                     |         | 10                              | 527.5 $\pm$ 6.6                                  | 93                    |
|                     |         | 100                             | 622.7 $\pm$ 32.1                                 | 105                   |
| Croaker fish        | Cd      | –                               | 58.3 $\pm$ 4.7                                   | –                     |
|                     |         | 10                              | 67.7 $\pm$ 5.3                                   | 94                    |
|                     |         | 100                             | 159.4 $\pm$ 7.5                                  | 101                   |
|                     | Pb      | –                               | 183.6 $\pm$ 14.3                                 | –                     |
|                     |         | 10                              | 194.2 $\pm$ 13.4                                 | 106                   |
|                     |         | 100                             | 280.5 $\pm$ 18.7                                 | 97                    |
|                     | Hg      | –                               | 326.1 $\pm$ 25.8                                 | –                     |
|                     |         | 10                              | 335.7 $\pm$ 28.6                                 | 96                    |
|                     |         | 100                             | 431.4 $\pm$ 31.5                                 | 105                   |
| Perch fish          | Cd      | –                               | 25.3 $\pm$ 1.8                                   | –                     |
|                     |         | 20                              | 44.7 $\pm$ 3.7                                   | 97                    |
|                     |         | 50                              | 74.6 $\pm$ 5.3                                   | 99                    |
|                     | Pb      | –                               | 178.2 $\pm$ 12.5                                 | –                     |
|                     |         | 20                              | 200.1 $\pm$ 16.3                                 | 109                   |
|                     |         | 50                              | 225.9 $\pm$ 20.4                                 | 95                    |
|                     | Hg      | –                               | 449.7 $\pm$ 41.7                                 | –                     |
|                     |         | 20                              | 469.3 $\pm$ 32.2                                 | 98                    |
|                     |         | 50                              | 503.6 $\pm$ 38.1                                 | 108                   |
| Carp fish           | Cd      | –                               | 45.3 $\pm$ 3.2                                   | –                     |
|                     |         | 20                              | 63.8 $\pm$ 4.0                                   | 92                    |
|                     |         | 50                              | 97.4 $\pm$ 7.3                                   | 104                   |
|                     | Pb      | –                               | 228.5 $\pm$ 19.6                                 | –                     |
|                     |         | 20                              | 250.1 $\pm$ 22.3                                 | 108                   |
|                     |         | 50                              | 273.6 $\pm$ 22.4                                 | 90                    |
|                     | Hg      | –                               | 198.6 $\pm$ 13.5                                 | –                     |
|                     |         | 20                              | 216.7 $\pm$ 17.4                                 | 91                    |
|                     |         | 50                              | 254.2 $\pm$ 22.7                                 | 111                   |
| Farmed trout fish 1 | Cd      | –                               | 5.4 $\pm$ 0.5                                    | –                     |
|                     |         | 10                              | 15.1 $\pm$ 1.2                                   | 97                    |
|                     |         | 80                              | 86.3 $\pm$ 6.3                                   | 101                   |
|                     | Pb      | –                               | 11.5 $\pm$ 3.7                                   | –                     |
|                     |         | 10                              | 22.4 $\pm$ 14.1                                  | 109                   |
|                     |         | 80                              | 90.6 $\pm$ 8.2                                   | 99                    |
|                     | Hg      | –                               | 55.7 $\pm$ 4.2                                   | –                     |
|                     |         | 10                              | 65.2 $\pm$ 5.5                                   | 95                    |
|                     |         | 80                              | 136.7 $\pm$ 10.6                                 | 101                   |
| Farmed trout fish 2 | Cd      | –                               | 4.2 $\pm$ 0.3                                    | –                     |
|                     |         | 10                              | 14.0 $\pm$ 1.2                                   | 98                    |
|                     |         | 80                              | 85.2 $\pm$ 6.4                                   | 101                   |
|                     | Pb      | –                               | 28.2 $\pm$ 1.9                                   | –                     |
|                     |         | 10                              | 37.9 $\pm$ 2.5                                   | 97                    |
|                     |         | 80                              | 110.7 $\pm$ 9.4                                  | 103                   |
|                     | Hg      | –                               | 43.6 $\pm$ 3.6                                   | –                     |
|                     |         | 10                              | 54.2 $\pm$ 4.4                                   | 106                   |
|                     |         | 80                              | 121.8 $\pm$ 8.1                                  | 98                    |

**Table 5**  
Concentrations of toxic elements found in certified references material

| Certified reference material       | Metal | Certified values $\pm$ SD ( $\mu\text{g kg}^{-1}$ ) | Measured values $\pm$ SD (n = 3, $\mu\text{g kg}^{-1}$ ) | Recovery (%) |
|------------------------------------|-------|---|--|--------------|
| DORM-2-dogfish muscle, NRC, Canada | Cd    | 43.0 $\pm$ 8.0                                      | 41.3 $\pm$ 2.4   | 96           |
|                                    | Pb    | 65.0 $\pm$ 7.0                                      | 68.5 $\pm$ 5.3   | 105          |
|                                    | Hg    | 4,640 $\pm$ 260                                     | 4,710 $\pm$ 408  | 102          |
| Muscle tissue-NIST SRM 2976        | Cd    | 820 $\pm$ 160                                       | 861 $\pm$ 62   | 105          |
|                                    | Pb    | 1,190 $\pm$ 180                                     | 1,094 $\pm$ 101  | 92           |
|                                    | Hg    | 61.0 $\pm$ 3.6                                      | 63.4 $\pm$ 4.7   | 104          |

Table 6

Comparison of VA – LPME – DES with other extraction methods for determination of toxic elements in fish samples

| Methods                         | LOD <sup>a</sup> (µg kg <sup>-1</sup> ) | LR <sup>b</sup> (µg kg <sup>-1</sup> ) | RSD <sup>c</sup> % | Extractant volume (mL) | Sample amount (g) | Metal ions        | Samples  | Reference                     |
|---------------------------------|---|--|--------------------|------------------------|-------------------|-------------------|--|-------------------------------|
| SPE-ICP-MS <sup>d</sup>         | 0.0008–0.0032                           | 0.05–30                                | 1.2–3.5            | 3                      | 0.2               | V, Cr, Cu, Cd, Pb | Laver, fish, chicken                               | Qin et al. (2021)             |
| PSCME-GFAAS <sup>e</sup>        | 0.01–0.05                               | 0.03–200                               | 3.1–4.2            | 40 µL                  | 0.5               | Pb, Cd, Hg        | Farmed trout, salmon, tilapia and south trout fish | Safari et al. (2018)          |
| SPE-ETAAS <sup>f</sup>          | 0.16–0.65                               | –                                      | 2–9                | 2                      | 3                 | Pb, Cd            | Fresh fish   | Barciela-Alonso et al. (2014) |
| MAE-ICP-OES <sup>g</sup>        | 5–10                                    | –                                      | –                  | –                      | 0.5               | Cr, Mn, Zn        | Silverside fish                                    | Vazquez et al. (2015)         |
| SLSDE-ILDLLME-FAAS <sup>h</sup> | 90–400                                  | 1,000–5,000                            | 2.3–2.5            | 400 µL                 | 1                 | Pb, Ni            | Local fish   | Jalbani and Soyлак (2015)     |
| SPE-FAAS                        | 0.21–0.63                               | 0.5–60                                 | 1.5–4.8            | 3                      | 2                 | Pb, Cd, Zn, Cu    | Seawater, fish and oysters                         | Ghanemi et al. (2011)         |
| CPE-FAAS <sup>i</sup>           | 0.67–3.42                               | 250–1,000                              | 1.7–4.8            | 2                      | 1                 | Pb, Cu, Co        | Canned fish  | Citak and Tuzen (2010)        |
| DLLME-SFO-GFAAS <sup>j</sup>    | 0.04–0.10                               | 0.1–100                                | 3.5–6.2            | 40 µL                  | 0.5               | Pb, Cd, Hg        | Trout fish   | Pirsaheb and Fattahi (2015)   |
| VA-LPME-DES-GFAAS               | 0.05–0.10                               | 0.15–150                               | 1.6–3.5            | 50 µL                  | 0.1               | Pb, Cd, Hg        | Snapper, croaker, perch, carp and trout fish       | This work                     |

<sup>a</sup> LOD, limit of detection.<sup>b</sup> LR, linear range.<sup>c</sup> RSD, relative standard deviation.<sup>d</sup> Soli phase extraction and inductively coupled plasma–mass spectroscopy.<sup>e</sup> Persistent sample circulation microextraction and graphite furnace atomic absorption spectrometry.<sup>f</sup> Solid phase extraction and electrothermal atomic absorption spectrometry.<sup>g</sup> Microwave-assisted extraction and inductively coupled plasma-atomic emission spectrometry.<sup>h</sup> Solid–liquid–solid dispersive extraction-ionic liquid-based dispersive liquid–liquid microextraction and flame atomic absorption spectrometry.<sup>i</sup> Cloud point extraction and flame atomic absorption spectrometry.<sup>j</sup> Dispersive liquid–liquid microextraction based on the solidification of floating organic drop and graphite furnace atomic absorption spectrometry.

## Ethics approval and consent to participate

This study was approved by the Deputy of Research and Technology, Salahaddin University (Erbil, Kurdistan Region, Iraq).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors thank the Deputy of Research and Technology, Salahaddin University (Erbil, Kurdistan Region, Iraq) for financial support.

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