

Research Article

Analysis of Some Trace Metals in Fish Species after Preconcentration with Congo Red on Amberlite XAD-7 Resin by Flame Atomic Absorption Spectrometry

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A new procedure for separation and preconcentration of trace amounts of Cu(II), Ni(II), Fe(III), Zn(II), Cr(III), Cd(II), and Pb(II) in fish samples was proposed. The procedure is based on the adsorption of these metal ions on the column of Amberlite XAD-7 as congo red complexes prior to their determination by flame atomic absorption spectrometry (FAAS). Several factors that can affect the sorption and elution efficiency of the metal ions were investigated and optimized. The sorption was quantitative in the pH range of 6.0–9.0 for Cu(II) and Ni(II), 5.5–8.0 for Fe(III), 6.0–8.5 for Zn(II) and Cd(II), and 7.0–8.5 for Cr(III) and Pb(II). The optimum pH for simultaneous retention was 7.5. The sorption capacity of the resin was found to be 0.89, 0.72, 0.82, 0.61, 0.53, 0.84, and 0.78 mg/g for Cu(II), Ni(II), Fe(III), Zn(II), Cr(III), Cd(II), and Pb(II), respectively. The precision of the method was evaluated as the relative standard deviation obtained by analyzing a series of six replicates and below 6% for all seven elements. The validation of the method was performed by the analysis of certified reference materials. The proposed method was successfully applied to separation/preconcentration and determination of these metals in fish samples.

1. Introduction

Due to human activities including agriculture, industrial production, mining, and transportation, metal ions have released into surface and ground waters, soil, and atmosphere. In particular, waters and soils contaminated with toxic metals may lead to accumulation of them in foodstuff. Ultimately, the uptake of metal ions associated with the consumption of these foods by human beings can cause a possible risk to public health. Therefore, World Health Organization (WHO) and European Community recommend controlling toxic metal ions in food sources in order to guarantee food safety [1–5]. Since the health and ecological problems in connection with environmental contamination increase, the quantification of heavy metal ions at trace level in various samples has become an important issue in the analytical laboratories [6]. Flame atomic absorption spectrometry (FAAS) is widely used and preferred for determination of toxic elements, but appropriate separation and preconcentration steps before analysis by

FAAS are usually required because of the complex matrixes and the low concentrations of the metal ions in the real samples [7]. Hence, various separation and preconcentration procedures, including solvent extraction, cloud point extraction (CPE), solid phase extraction (SPE), and coprecipitation, have been reported for trace metal ion analysis in different matrixes [8–14]. Among these preconcentration techniques, SPE has been utilized in many studies due to its advantages such as simplicity, rapidity, easy applicability to the preconcentration methods which increase sensitivity, and good separation capacity for target analytes. SPE has a relatively high preconcentration factor and also is able to apply to large volume samples without contamination [15, 16]. Although many SPE studies have been published employing adsorbents including activated carbon, chelating resins, ion exchange resins, and others, some SPE procedures do not have practicability due to long preconcentration time [17, 18]. Therefore, it can be said that a well-chosen adsorbent determines the efficiency of the SPE technique. Amberlite

XAD® resins which are able to adsorb metal complexes have received a great deal of attention because of versatile usage with or without derivatization [19–22]. In particular, Amberlite XAD-7 resin possessing an intermediate polarity and hydrophilic surface as a highly crosslinked macroreticular acrylic resin (polyacrylic acid ester polymer) has been performed in SPE studies [23–25]. Moreover, the surface area and sorption capacity of Amberlite XAD-7 resin which has a surface area of 450 m²/g are higher than Amberlite XAD-2 whose surface area is 330 m²/g [26].

A member of anionic azo dyes, congo red (CR), and (disodium 4-amino-[4-[4-(1-amino-4-sulfonato-naphthalen-2-yl) diazenylphenyl] phenyl] diazenyl-naphthalene-1-sulfonate) has displayed excellent complexing ability for some metal ions and analytical application of this ligand in the potentiometric and electrical conductivity studies was well established [27, 28]. CR derivatized microspheres were used for the removal of heavy metal ions and metal chelate affinity of albumin [29–31]. A SPE procedure for the separation and preconcentration of trace amounts of Fe(III), Mn(II), Ni(II), and Zn(II) as their complexes in water and food samples on a multiwalled carbon nanotube (MWCNT) disk was published in literature [32]. But the combination of Amberlite XAD-7/CR has not yet been used for the separation/preconcentration of any metal ions.

In the presented study, a novel analytical method including the combination of SPE and FAAS for the preconcentration and separation of Cu(II), Ni(II), Fe(III), Zn(II), Cr(III), Cd(II), and Pb(II) ions on Amberlite XAD-7 as their congo red complexes was developed. After determining optimal experimental conditions, the proposed method was validated by analyzing the certified reference material. The metal ion contents of the fish species were eventually determined by using FAAS and the level of toxicity in fish samples was evaluated.

2. Experimental

2.1. Apparatus. The concentrations of metal ions were determined by FAAS technique according to the standard guideline of the manufacturer (Perkin-Elmer AAnalyst 700, Norwalk, USA). A Sartorius PP-15 model pH meter, with a glass-electrode employed was used for measuring pH values in the aqueous phase. For the sample digestion, Milestone Ethos D microwave system (Sorisole-Bg, Italy) was also used in the experiments.

2.2. Chemicals and Standard Solutions. Analytical reagent-grade chemicals were employed for the preparation of all solutions. Milli-Q water was used to prepare aqueous solutions (Millipore, Milford, MA, USA). Stock metal solutions, 1000 mg/L (Sigma Chemical Co., St. Louis, MO), were used for the preparation of standard and working solutions. Congo red (CR) solutions (1.0 mmol/L) were daily prepared by dissolving the required amounts of CR (Merck, Germany) in water. The pH of the model solution was adjusted to pH 2–3 with hydrochloric acid-potassium chloride, pH 4–6 with sodium acetate-acetic acid, and pH 7–10 with ammonia-ammonium chloride buffer solutions.

Amberlite XAD-7 with surface area and bead size are 450 m²/g and 20–40 mesh was purchased from Aldrich (Milwaukee, USA) and firstly allowed to stand in 1.0 mol/L sodium hydroxide for 1 h and 4.0 mol/L hydrochloric acid for 1 h before use. After separation by filtration, it was rinsed by distilled water and methanol. Finally, the dried XAD-7 resin was ground by means of agate mortar and kept in a desiccator.

2.3. Experimental Procedure. The proposed method was tested with model solutions containing metal ions (30 µg of Pb(II), 30 µg of Cr(III), 20 µg of Fe(III), 15 µg of Cu(II), 15 µg of Ni(II), 10 µg of Cd(II), and 10 µg of Zn(II)) prior to its applications. 10 mL of appropriate buffer solution (in order to adjust the pH between 2 and 10, as given above) and 4.0 mL 1.0 mmol/L CR solution were added to 50–60 mL of model solution. 0.5 g of XAD-7 filled column was pretreated with the relevant buffer solution. Metal-CR complexes were passed through the XAD-7 column at a flow rate of 5–10 mL/min. The adsorbed metal ions on the resin were then eluted with 5 mL of 3.0 mol/L HNO₃ in acetone. The solution was evaporated almost to dryness and diluted to 5 mL with 0.1 mol/L HNO₃. The eluent was analyzed by FAAS for the determination of metal concentrations.

2.4. Sample Collection and Preparation. Fish samples of *Dicentrarchus labrax*, *Sparus aurata*, *Mullus barbatus*, and *Merlangius merlangus euxinus* species were purchased from the fish market in Istanbul, Turkey. Fishes were dissected to separate organs (scale, skin, muscle, gill, and liver) according to FAO methods [33]. The collected samples were packed on ice for transport to the laboratory and stored at –4°C until analyzed.

For the fish analysis, about 1 gram of sample (scale, skin, muscle, gill, or liver) was digested with 6 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide in microwave digestion system and diluted to 10 mL with Milli-Q water. A blank digest was performed in a similar manner (digestion conditions for microwave system; 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 6 min for 400 W, and 10 min for 500 W, vent: 8 min, resp.). The preconcentration procedure given above was also applied to the real samples. The final volume was 5 mL. In order to assess the accuracy of the developed method, the certified reference material NRCC-DORM-2-Dogfish muscle which was obtained from National Research Council, Canada, was used and digested in accordance with the digestion method described above.

3. Results and Discussion

3.1. pH Effect. Target analytes were separated and preconcentrated on Amberlite XAD-7 in the pH range of 2–10, while keeping all other parameters constant for the model solutions. Blank studies containing no analyte were also performed by the same preconcentration procedure mentioned above. The results showed that the sorption was quantitative for Cu(II) and Ni(II) in the pH range of 6.0–9.0; Fe(III), 5.5–8.0; Zn(II) and Cd(II), 6.0–8.5; and Cr(III) and Pb(II), 7.0–8.5. As can be inferred from the results, the quantitative recovery values for

TABLE 1: Effect of type and concentration of eluting agent on the recovery of analytes ($N = 4$).

Eluent conditions ^a	Recovery (%)						
	Cu(II)	Ni(II)	Fe(III)	Zn(II)	Cr(III)	Cd(II)	Pb(II)
HCl (2.0 mol L ⁻¹)	75.4	69.4	88.0	48.6	94.2	35.2	29.5
HClO ₄ (2.0 mol L ⁻¹)	24.6	57.8	61.2	55.0	46.7	33.3	29.5
H ₂ SO ₄ (2.0 mol L ⁻¹)	68.7	99.5	28.5	64.2	37.8	39.8	62.5
HNO ₃ (1.0 mol L ⁻¹)	93.4	90.1	89.6	88.7	86.9	87.5	85.8
HNO ₃ (2.0 mol L ⁻¹)	97.5	94.3	93.2	94.6	91.5	92.8	91.7
HNO ₃ (3.0 mol L ⁻¹)	100.2	100.4	99.5	99.2	95.8	99.6	98.9

^aVolume of eluent was 5 mL; all solutions were in acetone.

all studied metal ions were obtained in the range of pH 7.0–8.0. Therefore, pH 7.5 was chosen as the optimum pH value in further experiments. In order to adjust this pH, ammonia-ammonium chloride buffer solution was used.

3.2. Effect of the Type, Concentration, and Volume of the Eluent. A proper eluent is required for the quantitative recovery of the retained analytes on the adsorbent. Various acids with different concentrations were examined to determine the appropriate eluent for extraction procedure (Table 1). According to Table 1, 3.0 mol L⁻¹ HNO₃ in acetone is the best eluent for the quantitative elution of metal ions studied. In order to achieve high enrichment factors, the volume of eluent was investigated in the range of 2–6 mL of HNO₃ in acetone. When using 5 mL of 3.0 mol/L HNO₃ in acetone the recoveries were quantitative in the proposed method. Hence 5.0 mL was chosen as the eluent volume.

3.3. Effect of Sample Volume. The maximum applicable sample volume must be established in order to analyze real samples. The effect of sample volume on the sorption of metal ions was studied by passing 250–2000 mL volumes through the adsorbent filled column and was shown in Figure 1. According to these results, it was revealed that the adsorption of metal ions with 0.5 g resin was not affected by sample volume even at 1500 mL. So, the preconcentration factor was found to be 300 for the studied metal ions in the event 5 mL of the final solution after the developed SPE procedure.

3.4. Effect of Flow Rate. The flow rates for sample solution and eluent are crucial factors to achieve quantitative retention and elution, respectively. Therefore, the effects of the sample and eluent flow rates on the retentions and recoveries of metal ions were also investigated in the flow rate range of 1–9 mL/min. Figures 2 and 3 depict the results obtained for sample and eluent flow rates, respectively. According to these results, all analytes were quantitatively sorbed and recovered on the Amberlite XAD-7 adsorbent in the sample and eluent flow range of 1–7 mL/min. So, 5 mL/min was chosen as sample and eluent flow rate for further experiments.

3.5. Effect of Complexing Reagent Concentration. For the purpose of determining the concentration of CR required for quantitative recoveries, the developed preconcentration method was applied. Firstly, the same procedure was studied at pH 7.5 without reagent. It appears that the investigated

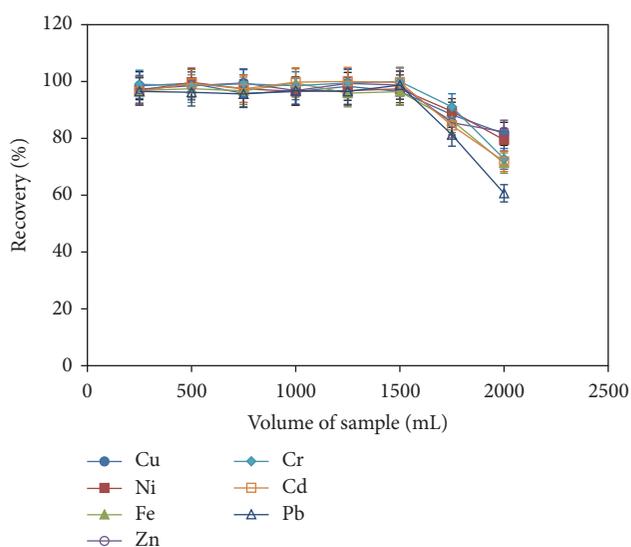


FIGURE 1: Effect of sample volume (pH = 7.5; amount of metal ions: 30 μ g Pb(II) and Cr(III), 20 μ g Fe(III), 15 μ g Cu(II) and Ni(II), and 10 μ g Cd(II) and Zn(II); CR amount: 1.0 mmol/L; amount of resin: 0.5 g; flow rate: 5.0 mL/min; final volume: 5 mL and $N = 3$).

metal ions are not quantitatively recovered without CR. Hence, the experiments were carried out with the various volumes of 1.0 mmol/L CR at pH 7.5. The obtained results showed that there is a positive correlation between recovery of the metal ions and concentration of CR. The recovery values achieved a constant value (95%) when 4.0 mL of 1.0 mmol/L CR was used. Due to low level of the studied metal ion amounts in real samples, further studies were performed at a CR concentration of 4.0 mL of 1.0 mmol/L.

3.6. Total Sorption Capacity. The total sorption capacity of the metal ions was determined by saturating 0.5 g of Amberlite XAD-7 and a suitable aliquot of metal ion complexes. The sorption capacity for each analyte ion on the adsorbent was calculated from the difference between the metal ion concentration before and after desorption. The obtained results are given in Table 2.

3.7. Reusability of the Resin. In order to test the resin reusability, the metal complexes were sorbed and desorbed on 0.5 g of Amberlite XAD-7 several times using a solution (50 mL) with

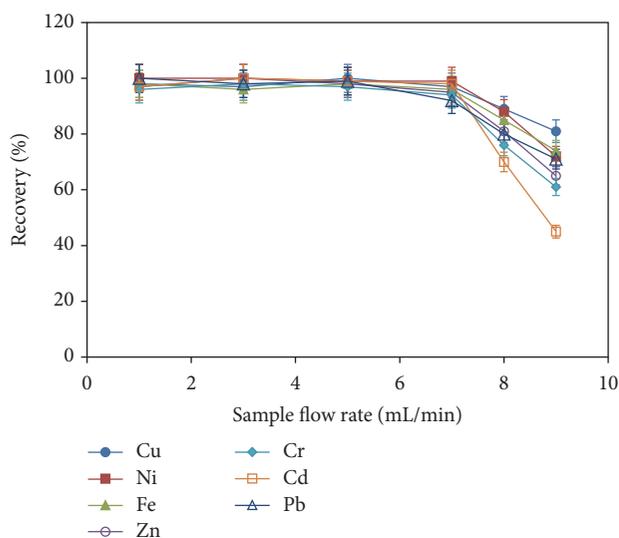


FIGURE 2: Effect of flow rates of sample solution on the retentions of analytes (pH = 7.5; amount of metal ions: 30 μg Pb(II) and Cr(III), 20 μg Fe(III), 15 μg Cu(II) and Ni(II), and 10 μg Cd(II) and Zn(II); CR amount: 1.0 mmol/L; amount of resin: 0.5 g; final volume: 5 mL and $N = 3$).

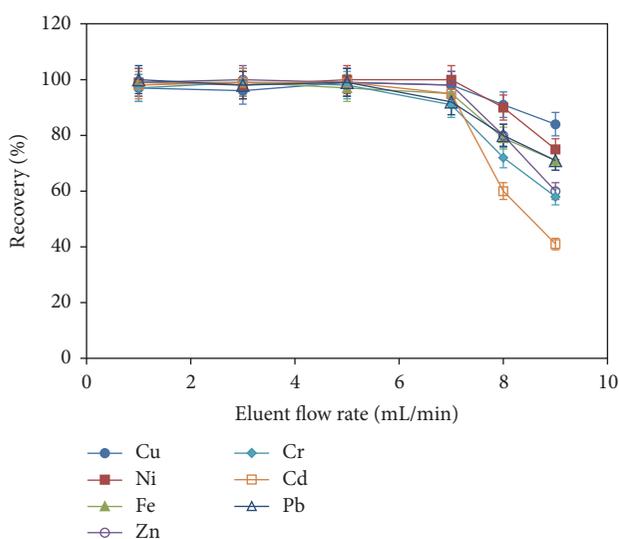


FIGURE 3: The influence of flow rates of eluent solution on the recoveries of the analyte ions with CR on Amberlite XAD-7 (pH = 7.5; amount of metal ions: 30 μg Pb(II) and Cr(III), 20 μg Fe(III), 15 μg Cu(II) and Ni(II), and 10 μg Cd(II) and Zn(II); CR amount: 1.0 mmol/L; amount of resin: 0.5 g; final volume: 5 mL and $N = 3$).

a concentration of 5–50 $\mu\text{g}/\text{mL}$ under the optimised experimental conditions. As can be seen in Figure 4, it appears that the sorption capacity after 60 cycles of sorption and desorption does not vary more than 2.5%. As a consequence, it is possible to reuse the resin without any change in its sorption behavior up to 60 cycles.

3.8. Effect of Foreign Ions on Recovery. In order to evaluate the analytical applicability of the developed method, the effect

TABLE 2: Total sorption capacity of the metal ions on the Amberlite XAD-7 (amount of resin: 0.5 g, metal ions: 50–800 μg , and pH: 7.5).

Metal	Capacity ($\mu\text{g}/\text{g}$ resin)
Cu(II)	890
Ni(II)	720
Fe(III)	820
Zn(II)	610
Cr(III)	530
Cd(II)	840
Pb(II)	780

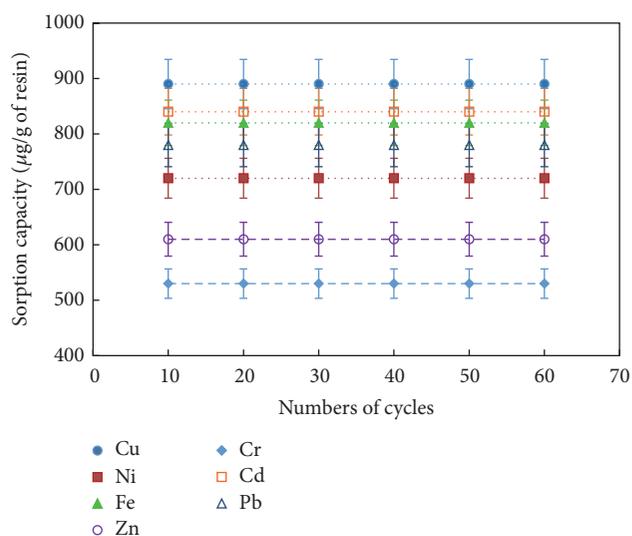


FIGURE 4: The effect of column reuse on recovery of metal ions by Amberlite XAD-7 resin at optimum conditions.

of some foreign ions was investigated. As is also understood from the literature, the effect of humic substances was less studied in the reported procedures [34, 35]. Different anions and cations which are concomitant with the metal ions present at the trace level in several samples and humic substances had no interference in the sorption of these metal ions up to considerable concentrations (Table 3). The tolerance limit is defined as the ion concentration causing a relative error smaller than $\pm 4\%$ related to the preconcentration and determination of the analyte ions.

3.9. Accuracy of the Method. The accuracy of the proposed method was controlled by the analysis of certified reference material (NRCC-DORM-2-Dogfish muscle). As can be seen in Table 4, the results obtained showed good agreement with the certified value. Also, it can be said that the developed SPE method is free from matrix effects.

The relative standard deviations (RSD, $N = 6$) with regard to the determinations in fish samples were found to be lower than 6%. The detection limits (LOD, $N = 6$) based on the 3Sb criterion for Cu(II), Ni(II), Fe(III), Zn(II), Cr(III), Cd(II),

TABLE 3: Tolerance limit of foreign species (in binary mixtures) on sorption of metal ions (column parameter: 100 mL of solution; 10 $\mu\text{g/L}$ metal ions; 0.5 g resin).

Foreign species	Tolerance ratio [foreign species ($\mu\text{g/L}$)/metal ion ($\mu\text{g/L}$)]						
	Cu(II)	Ni(II)	Fe(III)	Zn(II)	Cr(III)	Cd(II)	Pb(II)
Na ⁺ (NaCl)	2.46×10^6	3.51×10^6	1.84×10^6	1.99×10^6	1.67×10^6	1.77×10^6	1.68×10^6
K ⁺ (KCl)	1.98×10^6	3.12×10^6	4.02×10^6	1.55×10^6	1.98×10^6	1.82×10^6	0.97×10^6
NH ₄ ⁺ (NH ₄ Cl)	1.09×10^6	2.31×10^6	3.68×10^6	1.38×10^6	1.66×10^6	1.65×10^6	1.02×10^6
Cl ⁻ (NaCl)	2.98×10^6	3.98×10^6	4.77×10^6	1.79×10^6	1.51×10^6	7.19×10^6	1.75×10^6
SO ₄ ²⁻ (Na ₂ SO ₄)	3.78×10^6	3.94×10^6	4.02×10^6	4.12×10^6	7.89×10^6	8.14×10^6	3.75×10^6
NO ₃ ⁻ (NaNO ₃)	3.9×10^6	3.47×10^6	3.88×10^6	4.17×10^6	8.13×10^6	1.5×10^6	4.12×10^6
PO ₄ ³⁻ (Na ₃ PO ₄)	6.8×10^5	6.72×10^5	7.75×10^5	4.43×10^5	6.76×10^5	2.62×10^5	4.68×10^5
CO ₃ ²⁻ (Na ₂ CO ₃)	2.47×10^6	2.43×10^6	2.83×10^6	1.39×10^6	7.41×10^6	1.35×10^6	2.12×10^6
Citrate (sodium citrate)	1.34×10^4	2.49×10^4	3.53×10^4	2.12×10^4	9.52×10^4	2.17×10^4	2.71×10^4
Tartrate (NaKtartrate)	1.98×10^3	6.99×10^3	8.81×10^3	3.17×10^3	6.41×10^3	3.65×10^3	6.23×10^3
Oxalate (Na ₂ C ₂ O ₄)	1.51×10^3	7.14×10^3	8.62×10^3	1.85×10^3	4.12×10^3	6.17×10^3	1.98×10^3
Fulvic acid	18	77	35	31	52	29	30
Humic acid	33	134	74	87	83	45	68
CH ₃ COO ⁻ (CH ₃ COONa)	2.17×10^5	7.23×10^5	8.12×10^5	3.43×10^5	2.78×10^5	1.47×10^5	3.12×10^5
Ca ²⁺ (CaCl ₂)	4.53×10^5	6.98×10^5	4.22×10^5	4.52×10^5	6.23×10^5	2.13×10^5	3.98×10^5
Mg ²⁺ (MgCl ₂)	4.02×10^5	7.75×10^5	6.23×10^5	4.98×10^5	5.14×10^5	1.35×10^5	5.17×10^5

TABLE 4: Analysis of certified reference material (NRCC-DORM-2-Dogfish muscle) ($N = 5$).

Element	Certified value ($\mu\text{g/g}$)	Amount found ^a ($\mu\text{g/g}$)	Recovery (%)
Cu(II)	2.34 ± 0.16	2.31 ± 0.13	99
Ni(II)	19.4 ± 3.1	19.10 ± 1.31	98
Fe(III)	142 ± 10	137.50 ± 5.82	97
Zn(II)	25.6 ± 2.3	24.90 ± 1.43	97
Cr(III)	34.7 ± 5.5	34.20 ± 1.61	99
Cd(II)	0.043 ± 0.008	0.042 ± 0.002	98
Pb(II)	0.065 ± 0.007	0.063 ± 0.003	97

^aMean \pm SD (standard deviation).

and Pb(II) were found to be 0.10, 0.30, 2.50, 1.20, 1.03, 0.08, and 0.16 $\mu\text{g/L}$, respectively.

3.10. Application of the Presented Procedure. The developed procedure was applied to the determination of investigated metal ions in the microwave digested fish samples as described above. The results obtained are presented in Table 5. European Community has established the maximum allowable levels in fishes of 0.10 and 0.30 mg/kg of Cd and Pb, respectively [3]. According to Table 5, Cd and Pb levels in the majority of analyzed fish samples were found higher than their maximum levels. It can be inferred that these fishes can cause toxicological effects on human health.

3.11. Comparison with Other Methods. Determination of metal ions in particularly fishes by the SPE procedure and FAAS detection was compared with other methods and the results are shown in Table 6. It can be seen that the detection limits for the studied metal ions are satisfactory to those of

some separation/preconcentration methods for fish analyses. Preconcentration factor of the method also is superior and comparable to those presented by methods showed in the table.

4. Conclusion

The proposed separation/preconcentration method has some advantages such as simplicity and time saving as well as accuracy, sensitivity, and precision. This method is also low-cost because the reusability of Amberlite XAD-7 is as high as 60 cycles without any significant lost of its adsorption properties. The analytical performance of the developed method is comparable with other SPE methods in the literature. The presented method also shows high tolerance to interferences from the matrix ions. The system is also successfully applied to fish samples for the determination of trace amount of Cu(II), Ni(II), Fe(III), Zn(II), Cr(III), Cd(II), and Pb(II) ions.

TABLE 5: Concentration (mg/kg) of metals in different parts of the fish species ($N = 5$).

Fish	Cu(II)	Ni(II)	Fe(III)	Zn(II)	Cr(III)	Cd(II)	Pb(II)
<i>Dicentrarchus labrax</i>							
Scale	5.8 ± 0.22 ^a	4.0 ± 0.04	284.0 ± 6.0	3.1 ± 0.10	15.8 ± 0.05	0.8 ± 0.02	0.8 ± 0.03
Skin	1.3 ± 0.07	0.5 ± 0.01	42.0 ± 1.7	1.6 ± 0.07	3.2 ± 0.08	0.2 ± 0.01	0.1 ± 0.01
Muscle	0.7 ± 0.01	<LOD	6.1 ± 0.42	1.2 ± 0.02	2.5 ± 0.03	8.7 ± 0.14	<LOD
Gill	2.7 ± 0.04	<LOD	33.1 ± 0.51	2.6 ± 0.04	4.8 ± 0.07	1.1 ± 0.08	0.1 ± 0.01
Liver	12.3 ± 0.17	0.2 ± 0.01	47.2 ± 0.42	2.1 ± 0.03	1.4 ± 0.02	12.3 ± 0.07	<LOD
<i>Sparus aurata</i>							
Scale	0.9 ± 0.03	0.7 ± 0.02	21.2 ± 0.60	<LOD	1.6 ± 0.05	12.2 ± 0.31	0.2 ± 0.01
Skin	5.1 ± 0.18	2.4 ± 0.04	378.3 ± 2.00	1.1 ± 0.01	<LOD	3.2 ± 0.04	<LOD
Muscle	2.1 ± 0.08	0.6 ± 0.01	0.7 ± 0.03	<LOD	<LOD	7.2 ± 0.02	0.2 ± 0.03
Gill	1.4 ± 0.04	1.5 ± 0.03	120.1 ± 2.00	<LOD	<LOD	6.1 ± 0.02	<LOD
<i>Mullus barbatus</i>							
Scale	5.2 ± 0.04	2.2 ± 0.05	400.5 ± 3.01	<LOD	7.4 ± 0.07	6.3 ± 0.12	0.6 ± 0.04
Skin	3.8 ± 0.07	<LOD	52.5 ± 0.72	<LOD	4.7 ± 0.04	14.2 ± 0.07	0.2 ± 0.02
Muscle	5.4 ± 0.15	0.4 ± 0.02	12.3 ± 0.08	<LOD	<LOD	13.7 ± 0.04	<LOD
Gill	6.1 ± 0.21	0.7 ± 0.04	308.4 ± 11.0	0.7 ± 0.02	<LOD	10.0 ± 0.06	0.4 ± 0.01
<i>Merlangius merlangus euxinus</i>							
Skin	0.2 ± 0.04	0.2 ± 0.01	18.5 ± 0.60	<LOD	0.2 ± 0.03	6.1 ± 0.13	0.8 ± 0.02
Muscle	0.1 ± 0.01	<LOD	0.2 ± 0.02	<LOD	<LOD	11.6 ± 0.09	0.1 ± 0.01
Gill	<LOD	<LOD	130.2 ± 4.31	<LOD	<LOD	14.2 ± 0.17	0.1 ± 0.01

^aMean ± SD (standard deviation) of determination in three separate 1 g tissue type samples.

TABLE 6: Comparison of the proposed method with other SPE methods for determination of metal ions in fish samples by FAAS.

SPE system	Metal ions	PF ^a	LOD ^b ($\mu\text{g/L}$)	Reference
Amberlite XAD-16/ABSA ^c	Cu, Ni, Zn, Co, Cr, Fe, Pb	60–100	0.69–1.91	[19]
Amberlite XAD-4/HBA ^d	Co, Ni, Cu, Zn, Pb	260–460	0.45–1.37	[20]
Activated carbon/BSPD ^e	Cr, Fe, Cu, Ni, Co, Zn	175	0.27–0.33 ng/mL	[36]
Schiff base (L) ^f /silica/magnetite	Pb, Cd, Cu	87.5	0.12–0.19	[37]
Activated carbon/BHAPED ^g	Fe, Cu, Ni, Co, Cr, Zn	219	0.27–0.33 ng/mL	[38]
Polyurethane foam/o-AP ^h	Pb, Cd	250, 319	0.072 and 0.016	[39]
MWCNTs/IDA ⁱ	V, Cr, Pb, Cd, Co, Cu, As	66–101	0.10–3.4 ng/mL	[40]
Dowex Optipore SD-2/MTR ^j	Cu, Ni	50	1.03 and 1.90	[41]
Alumina/Sulfur nanoparticle	Cd, Cu, Zn, and Pb	83.3	0.21–0.63	[42]
Pumice stone/ <i>Penicillium digitatum</i>	Cr, Cd, Mn	50	1.5–2.0 ng/mL	[43]
Activated carbon/HBAHBN ^k	Cu, Zn, Pb	—	0.65–1.90	[44]
Amberlite XAD-7/congo red	Cu, Ni, Fe, Zn, Cr, Cd, Pb	300	0.08–2.50	This work

^aPreconcentration factor.

^bLimit of detection.

^cp-Aminobenzene sulfonic acid.

^dp-Hydroxybenzoic acid.

^eBis salicyl aldehyde, 1,3 propan diamine.

^f3-(4-Methoxybenzylideneamino)-2-thioxothiazolidin-4-one.

^gBis(2-hydroxyacetophenone)ethylenediamine.

^ho-Aminophenol.

ⁱMultiwalled carbon nanotubes, iminodiacetic acid.

^j5-Methyl-4-(2-thiazolylazo)resorcinol.

^k2[2-Hydroxybenzylideneamino] 2-hydroxybenzonal.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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