Solid Phase Extraction Based on the Use of *Agaricus arvensis* as a Fungal Biomass for the Preconcentrations of Pb and Al Prior to Their Determination in Vegetables by ICP-OES

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ABSTRACT

A solid phase extraction (SPE) method based on the use of a fungal biomass as sorbent was developed for the preconcentrations of Pb and Al. *Agaricus arvensis* was immobilized on Amberlite XAD-4 and loaded onto a column. Important parameters such as pH and flow rate of the solution, amount of biosorbent and resin, volume of sample solution, which affect the efficiency of the preconcentration procedure for Pb and Al, were optimized. The effect of possible interfering ions, i.e., Cd²⁺, Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, and Fe²⁺, was investigated. Experiments on the repeatability of the solid phase column showed that the same column could be used at least 30 cycles without loss of the biosorption efficiency for the recovery of Pb and Al.

The limit of detection (LOD) of Pb and Al was found to be 0.10 ng mL⁻¹ and 0.03 ng mL⁻¹,

respectively. The sensitivity of ICP-OES improved 39.8-fold for Pb and 39.5-fold for Al. Linearity was obtained in the concentration range of 1.25–50.0 ng mL⁻¹ for Pb and 0.5–50.0 ng mL⁻¹ for Al. The relative standard deviation (RSD) of the method under optimum conditions was lower than 8.4% (n=5) for Pb and Al, which was validated through the analysis of certified reference tea and poplar leaves samples. The biosorption capacity of immobilized *Agaricus arvensis* for Pb and Al was found to be 31.2 mg g⁻¹ and 45.7 mg g⁻¹, respectively.

The developed method was applied to determine the concentrations of Pb and Al in various edible vegetable samples (cucumber, okra, tomato, beans, aubergine, watermelon, zucchini, pepper, melon, lettuce, roka, purslane, scallion, cress, parsley, cabbage, sugar cane, and basil) grown along the cultivated banks of the Tigris River in Diyarbakir, Turkey.

in food samples. Various instrumental techniques are applied for the determination of these elements including flame atomic absorption spectrometry (FAAS) (8,9), inductively coupled plasma optical emission spectrometry (ICP-OES) (10-12), and inductively coupled plasma mass spectrometry (ICP-MS) (13,14). The sensitivity of FAAS and ICP-OES is usually insufficient for the direct determination of these elements found at very low concentration levels in complex environmental matrices. If the concentration of the element of interest is lower than the quantification limit, a preconcentration step is needed. ICP-MS instruments often cannot be found in laboratories due to their high instrumental cost in comparison to FAAS and ICP-OES instrumentation. Various preconcentration methods such as cloud point extraction (CPE) (5-17), solid phase extraction (SPE) (18-22), and coprecipitation (23-26) have been developed for use prior to instrumental analysis. Recently, SPE (based on the use of biomass as a sorbent) has attained special interest due to its high enrichment factor, high recovery, simple preparation of solid phase, low cost and low consumption of organic solvents, simplicity and environmentally friendly approaches, etc. (12, 27). Many adsorbents have been used in the SPE procedure such as chelating resins (28), bentonite (29), and activated carbon (30). The advantages and disadvantages of the SPE method are provided in a recent paper (31). The use of biomass for

INTRODUCTION

Heavy metal ions are increasingly being released into the environment and cause serious pollution, particularly as a result of industrialization. Lead (Pb) and aluminum (Al) are well recognized as highly toxic and hazardous heavy metals to human

health (1,2). The toxicity of Al in plants depends on its species (3), while both of these toxic metals are also frequently found in drinking waters and foods (4,5). Therefore, it is important to develop simple, rapid, and efficient methods for identifying and monitoring these toxic metals in the environment (6,7).

The concentration ranges of Pb and Al are generally found to be at levels lower than mg L⁻¹ or mg kg⁻¹

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the preconcentration and separation of traces of heavy metals and some organic materials at trace levels are popular due to the good adsorption properties such as high surface area and high adsorption capacity of the biological substances (32).

Our research group has reported the use of a variety of biomasses as a solid phase sorbent for the preconcentration of metals prior to their determination by ultraviolet visible spectrophotometry (UV-VIS), FAAS, and ICP-OES. The method has been applied to the preconcentration of Cd and Ni (11), Th (32), Cd and Co (33), and U (34).

This paper describes the analytical potential of *Agaricus arvensis* (horse mushroom) as a sorbent for solid phase extraction and preconcentration of Pb and Al. *Agaricus arvensis* is a good, edible, and white rot fungi, which can be found in grassy places in the garden. It is fairly common in parts of Europe, the U.S., and China, and sought after in many other areas (35). The experimental parameters were optimized throughout this study, while the applicability of the method was tested against certified reference samples and edible vegetables. The results of this study show that the sensitivity of ICP-OES was improved for the determination of both Pb and Al.

EXPERIMENTAL

Instrumentation

The concentrations of Pb and Al were determined using a PerkinElmer Optima™ 2100 DV ICP-OES (PerkinElmer, Inc., Shelton, CT, USA) at 220.353 nm for Pb²⁺ and 396.153 nm for Al³⁺. The operating conditions of the ICP-OES are listed in Table I (and in references 33,36,37). The linear calibration equations used were $y=4680.3x-36.2$ in the range of

0.05–2.0 $\mu\text{g mL}^{-1}$ for Pb and $y=447519x-2769.0$ in the range of 0.02–2.0 $\mu\text{g mL}^{-1}$ for Al. The pHs of the solutions were adjusted using a Mettler Toledo MPC 227 digital pH meter (Polaris Parkway, Columbus, OH, USA). Solid phase extraction experiments were performed in filtration columns (1.0 cm $\times$ 10.0 cm) equipped with polypropylene frits. The flow rates of the samples and standard solutions were adjusted to the desired values by using a Waters Marlow 323 peristaltic pump (Waters Corporation, Milford, MA, USA).

Reagents and Solutions

Stock solutions of Pb and Al were prepared from their standard solutions at 1000 $\mu\text{g mL}^{-1}$ (High Purity Standards, Charleston, SC, USA). Working standards were prepared by diluting the stock solutions. All chemicals used were of high purity reagent grade unless stated otherwise. Doubly distilled water was used for all experimental studies. All glassware was kept per-

manently filled with 1.0 mol L⁻¹ of nitric acid when not in use. Concentrated nitric acid (65%), hydrogen peroxide (35%), and hydrochloric acid (36.5–38.0%) were obtained from Sigma Aldrich, Germany. Certified samples of fortified water (NWTM-15, High Purity Standard), wastewater (EU-L-2 SCP Science EnviroMAT), powdered tea (NCSZC 73014 China National Analysis Center for Iron and Steel), poplar leaves (NCSDC 73350 China National Analysis Center for Iron and Steel), obtained from a local distributor, were used for validation of the method. Adsorber resin (polystyrene divinyl benzene) Amberlite XAD-4 was supplied from Sigma Aldrich Co., USA. Contaminants were removed from the XAD-4 resin using the procedure described in our previous studies (11,32,34).

Preparation of Fungal Biomass

Agaricus arvensis, collected from Siirt, Turkey, was used as the biosorbent for this study. It was cleaned twice with distilled water

TABLE I
ICP-OES Operating Conditions

Parameters	
RF power	1450 W
Plasma gas flow rate	15 L/min
Auxiliary gas flow rate	0.2 L/min
Nebulizer gas flow rate	0.8 L/min
Sample flow rate	1.5 L/min
View mode	Axial-Radial
Read	Peak area
Source equilibration time	15 sec
Read delay	60 sec
Replicates	3
Background correction	2-point (manual point correction)
Spray chamber	Scott-type spray chamber (HF resistant)
Detector	CCD
Purge gas	Nitrogen
Shear gas	Air
Gas	Argon
Analytical wavelengths	Pb 220.353 nm, Al 396.153 nm

to remove contaminants and finally dried at room temperature. The dried fungal biosorbent was ground in a porcelain mortar to obtain a fine powder. It was then oven-dried at 80 °C for 24 hours to assess complete death of the dried cells. Finally, the cells were inoculated into a malt agar at 25 °C for 24 hours. The absence of mycelia of *Agaricus arvensis* showed positive results, indicating the complete death of the fungus.

Preparation of the Column

200 mg of dry fungal biomass powder was mixed with 1000 mg Amberlite XAD-4 for 24 hours. The amount of biomass taken up by the resin was determined by measuring the increase in weight of the resin after mixing the paste which was heated in an oven at about 105 °C for 1.0 hour to dry the mixture. The wetting and drying steps were repeated to maximize the contact between biomass and resin, thereby improving the immobilization efficiency. Finally, the product obtained was ground to achieve an original size of 20–60 mesh and then packed into a solid phase column (1.0 cm x 10.0 cm).

General Sorption Studies

Model solutions, consisting of a 50.0-mL mixture of Pb²⁺ and Al³⁺ at the concentration of 10.0 ng mL⁻¹, were prepared and the pH of the solutions was adjusted by adding the required amounts of HCl and NH₃. The model solution was passed through the prepared SPE column at an adjusted flow rate with a peristaltic pump. Then, 10.0 mL distilled water was passed through the column. The retained Pb²⁺ and Al³⁺ on the fungal biomass was then eluted with 5.0 mL of 1.0 mol L⁻¹ HCl. The concentration of Pb²⁺ and Al³⁺ in this solution was determined by ICP-OES.

Loading Capacity

A batch equilibrium method was used to determine the biosorption capacities of Pb²⁺ and Al³⁺ by *Agaricus arvensis*. An amount of 50.0 mL of the tested metal solutions containing 50.0 mg L⁻¹ of Pb²⁺ and Al³⁺ were placed into 250.0-mL Erlenmeyer flasks and used at optimum pH values for Pb²⁺ and Al³⁺ ions. The 100.0 mg dried powdered dead cells were mixed with the metal solutions for 120 minutes at 25 °C in a Julaba SW72 shaker (Seelbach, Germany) at 120 rpm. The dried powdered dead cells were then separated by centrifugation at 10,000 rpm for 10 minutes. The supernatant and pellet (after acid digestion with concentrated HNO₃ acids) were used to estimate the residual metal concentration by ICP-OES. The amount of biosorbed Pb²⁺ and Al³⁺ ions was calculated using the following equation (37):

$$Q = (c_0 - c_e) \times V/w$$

where c_0 is the initial concentration of the ion (mg L⁻¹), c_e is the equilibrium concentration of the ion (mg L⁻¹), V is the volume of the testing solution (L), and w is the amount of sorbent (g). All experiments were carried out in duplicate.

Sample Preparation

Edible vegetable samples were randomly collected from along the cultivated banks of the Tigris River and stored in separate polyethylene bags according to their type and brought to the laboratory for preparation and treatment. They were first washed under fresh running water to eliminate any foreign substances, then washed again with deionized water. The samples were kept in an oven at 80 °C for 24 hours. Then, 1.0 g portions of the powdered samples were weighed, placed into Teflon® vessels, and 5.0 mL of HNO₃:HCl (1:1, v/v) was added, and the mixture heated on a hot plate. This mixture was

evaporated to dryness and 6.0 mL of HNO₃:HCl:H₂O₂ (1:1:0.2, v/v/v) was added before transferring it to a microwave vessel. This solution was digested in a Berghof MWS3 microwave oven (Berghof, Tübingen, Germany) by a procedure described in the literature (33). Briefly, the vessels were heated to 170 °C by microwave irradiation and left standing for 5.0 minutes. Then the temperature was increased to 200 °C during 15 minutes and the mixture left standing for 1.0 minute. The temperature was then decreased to 100 °C and the mixture left standing for 20 minutes. After digestion, the final volume was made up to 50.0 mL and the pH adjusted to the desired value before the SPE procedure. The concentrations of the analytes in the samples were determined by ICP-OES.

The SPE procedure was directly applied to a 200.0-mL portion of fortified NWTM-15 certified water samples (LGC, Middlesex, UK) after pH adjustment.

A certified EU-L-2 wastewater sample of 2.0 mL (SCP Science, Courtaboeuf, France) was diluted to 200.0 mL. After pH adjustment, the SPE procedure was applied for the preconcentration of Pb and Al.

1.0-g portions of certified reference materials NCS ZC 73014 Tea and NCS DC 73350 Poplar Leaves (China National Analysis Center for Iron and Steel, P.R. China.) were digested using the same method described for the edible vegetable samples.

RESULTS AND DISCUSSION

Effect of pH and Flow Rate of the Solution on the Preconcentration of Pb²⁺ and Al³⁺

It is well known that the pH value of the aqueous solution is the most important variable governing the adsorption capacity of the adsorbent for heavy metal ions

(37). In the biosorption phenomenon, the pH value affects not only the metal ion solubility but also the biosorbent total charge on the sorption sites of the biomass type (38).

The effect of pH on biosorption of 10.0 ng mL^{-1} of Pb^{2+} and Al^{3+} on immobilized *Agaricus arvensis* was studied by varying the pH of the metal solution from 2.0 to 10.0. The results in Figure 1 show that the quantitative retention values were found to be >95% for Pb^{2+} and Al^{3+} in the pH range of 5.0–7.0. So, the pH values selected and used for all further studies were 5.0 and 6.0 for Pb^{2+} and Al^{3+} , respectively.

Biosorption generally takes place with low removal efficiency at a low pH value. This occurs due to the high concentration of proton ions in the solution. These proton ions compete with the metal ions in forming a bond with the active sites (the functional groups) on the surface of the biosorbent. These bonded active sites become saturated and cause lower biosorption efficiency (39). At a higher pH value, the existence of counter ions might cause lower efficiency of biosorption. It is possible that some metal precipitation could occur at a higher pH (40).

It is reported that the flow rate of the sample solution is a very important parameter for the retention of the elements (34). To exam-

ine the flow rate of a sample solution onto immobilized *Agaricus arvensis*, solutions were passed through the column at a rate of 1.0 to 6.0 mL min^{-1} . The results presented in Table II show that the recoveries of the analytes were unchanged up to a flow rate of 1.0 mL min^{-1} . When the flow rate of the sample solution was increased by more than 3.0 mL min^{-1} , the retention of the analytes decreased. Thus, a flow rate of 3.0 mL min^{-1} was selected for the following studies.

Effect of the Amount of Biosorbent and Resin

Influence of the amount of *Agaricus arvensis* as a biosorbent was also examined. Thus, different amounts of biosorbent (ranging from 100.0 to 350.0 mg) on the recovery of the analytes were studied under optimum conditions. Figure 2 shows the effect of amount of biosorbent on the recovery of the analytes. The recovery of the analytes increased from 86.7% to 96.6% and 78.3% to 99.1%, respectively, for Pb^{2+} and Al^{3+} when the amount of biosorbent was increased from 100 to 300 mg. The Pb^{2+} and Al^{3+} uptake remained unchanged above the 300-mg amount of biosorbent. Therefore, 300 mg of biosorbent was used for all further studies.

The effect of the amount of XAD-4 on the recovery of Pb^{2+} and

Al^{3+} was also investigated under the optimum condition by changing the resin amounts from 250 to 2000 mg. As can be seen from Figure 3, the recovery values increased up to 1000 mg. There was no change in the recovery value above this amount. Thus, 1000 mg of XAD-4 was used for further studies.

Effect of Sample Volume

It is known that sample volume is a major requirement for obtaining high preconcentration factors (19). To determine the optimum sample volume, 25.0 to 250.0 mL of sample solutions containing $0.5 \text{ } \mu\text{g}$ of Pb^{2+} and Al^{3+} were passed through the solid phase column at the optimum conditions. The results in Table III indicate that the retention of Pb^{2+} and Al^{3+} did not change with a sample volume up to

TABLE II
Effect of Flow Rate of Sample Solution on Solid Phase Extraction of 10.0 ng mL^{-1} of Pb^{2+} and Al^{3+}

Flow rate (mL min^{-1})	Recovery (%)	
	Pb^{2+}	Al^{3+}
1.0	97.1 ± 2.4	99.3 ± 0.9
2.0	99.3 ± 1.5	100 ± 1.5
3.0	100 ± 1.8	100 ± 1.9
4.0	92.5 ± 1.9	97.2 ± 2.5
5.0	85.7 ± 2.4	87.9 ± 0.9
6.0	82.4 ± 2.1	85.1 ± 1.1

Fig. 1. Effect of pH on the recovery of 10.0 ng mL^{-1} of Pb^{2+} and Al^{3+} .

Fig. 2. Effect of biosorbent amount on solid phase preconcentration of 5.0 ng mL^{-1} of Pb^{2+} and Al^{3+} .

Fig. 3. Effect of amount of Amberlite XAD-16 on solid phase preconcentration of 10.0 ng mL^{-1} of Pb^{2+} and Al^{3+} .

TABLE III
Effect of Initial Solution Volume on the Recovery of Pb^{2+} and Al^{3+} (Final volume 5.0 mL , $\text{pH } 5.0$ for Pb^{2+} and $\text{pH } 6.0$ for Al^{3+} , Column Loaded with 300 mg of *Agaricus arvensis* on 1000 mg of Amberlite XAD-4)

Initial Volume (mL)	Conc. (ng mL^{-1})	Preconc. Factor	Recovery (%)	
			Pb^{2+}	Al^{3+}
25.0	20.0	5	101.2 ± 1.4	99.8 ± 0.9
50.0	10.0	10	99.8 ± 0.6	99.2 ± 0.4
100.0	5.0	20	98.6 ± 0.9	99.1 ± 0.5
200.0	2.5	40	97.9 ± 1.2	98.3 ± 0.8
250.0	2.0	50	94.1 ± 0.8	92.2 ± 0.8

TABLE IV
Effect of Type and Volume of Elution Solutions on the Recovery of 10.0 ng mL^{-1} of Pb^{2+} and Al^{3+}

Elution Solution	Volume (mL)	Concentration (mol L^{-1})	Recovery (%)	
			Pb^{2+}	Al^{3+}
HCl	1.0	0.5	84.3 ± 0.5	87.6 ± 0.4
	3.0	0.5	91.4 ± 0.4	92.1 ± 0.5
	5.0	0.5	95.7 ± 0.2	96.2 ± 0.3
	1.0	1.0	90.8 ± 0.3	91.6 ± 0.6
	3.0	1.0	93.1 ± 0.2	95.2 ± 0.4
	5.0	1.0	98.6 ± 0.4	99.1 ± 0.2
HNO_3	1.0	0.5	82.1 ± 0.1	83.4 ± 0.2
	3.0	0.5	86.5 ± 0.5	88.1 ± 0.3
	5.0	0.5	91.7 ± 0.3	93.9 ± 0.6
	1.0	1.0	87.5 ± 0.1	88.6 ± 0.5
	3.0	1.0	90.2 ± 0.6	92.6 ± 0.3
	5.0	1.0	96.2 ± 0.2	97.6 ± 0.1

200.0 mL . Above 200.0 mL , the recovery decreased for Pb^{2+} and Al^{3+} . This could be due to the low concentration of the metal ions in the solution when the sample volume was increased (19). Therefore, 200.0 mL of the sample solution was selected as maximum sample volume for the preconcentration of the analytes. The preconcentration factor using 5.0 mL of eluent was found to be 40.0 for 200.0 mL of initial solution volume.

Effect of Eluents

In order to determine the most suitable eluent to desorb heavy metals from the biosorbent surface, different concentrations of HCl and HNO_3 were used ranging from 1.0 – 5.0 mL of the eluent. As seen in Table IV, when the volume of HCl and HNO_3 was increased from 1.0 mL to 5.0 mL at a concentration of 0.5 mol L^{-1} , the desorption efficiency for Pb^{2+} and Al^{3+} increased, respectively. It was also observed

that 5.0 mL of 1.0 mol L^{-1} HCl desorbed the Pb^{2+} and Al^{3+} ions from the biosorbent surface by 98.6% and 99.1% , respectively. For further studies, 5.0 mL of 1.0 mol L^{-1} HCl was used as the eluent.

Effect of Foreign Ions

It is known that high concentrations of interfering ions cause matrix effects in the spectroscopic determination of metal ions in environmental and food samples (41). The influence of foreign ions such as Cd^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and Fe^{2+} on the recovery of Pb^{2+} and Al^{3+} was examined under optimum experimental conditions. These elements were selected based on their presence in vegetable samples. The recoveries of Pb^{2+} and Al^{3+} were all higher than 95% , even when the concentration ratio of the interfering elements to the concentration of Pb^{2+} and Al^{3+} was up to 200-fold (Table V). A significant decrease in recovery values was observed when the ratio was higher than 100 for Cu^{2+} , Cd^{2+} , Ni^{2+} , Co^{2+} , Zn^{2+} and at 200 for Na^+ , K^+ , Ca^{2+} , Mg^{2+} and Fe^{2+} . It can, therefore, be concluded that the developed method can be applied to real samples without interference from other ions.

Repeatability of Solid Phase Column

The repeatability of the SPE column loaded with *Agaricus arvensis* was studied by using the same column until the recovery values for the metals was lower than 95%.

The results presented in Figure 4 show that the recovery of Pb^{2+} and Al^{3+} was 98.5% and 97.6%, respectively, after use of the column for 30 times. It was also determined that the retention for repeated recovery on the same solid phase

column did not change by more than 3%. This means that the repeatability factor of the solid phase column was at least for 30 cycles without loss in the biosorption capacity for the recovery of Pb^{2+} and Al^{3+} .

TABLE V
Effects of Major Components
on Recoveries of 10.0 ng mL⁻¹ of Pb^{2+} and Al^{3+}

Ion	Ratio of interfering Ion Concentration (ng mL ⁻¹)	Recovery (%)	
		Pb^{2+}	Al^{3+}
Na	-	100.1±1.1	100.1±1.3
	20	99.3±0.8	99.6±1.5
	200	97.8±1.5	98.7±0.9
K	-	100.2±0.8	100.2±0.9
	10	99.4±0.9	99.6±1.3
	200	98.7±0.9	99.4±1.1
Ca	-	100.4±1.1	99±1.5
	20	99.5±1.2	99.8±2.3
	200	97.9±1.5	98.2±1.2
Mg	-	99±0.9	100.0±2.1
	20	97.1±0.8	98.1±1.4
	200	96.3±1.2	96.8±2.3
Fe	-	100.1±1.3	100.3±1.1
	20	99.2±1.1	99.4±1.2
	200	95.4±1.5	95.3±1.5
Cd	-	99.6±1.3	94.5±2.6
	10	99.1±1.4	95.6±1.8
	100	94.5±0.9	96.5±2.5
Cu	-	98.1±0.9	98.7±3.4
	10	98.0±1.3	97.9±2.0
	100	96.5±1.2	99.7±2.1
Ni	-	101.3±0.7	96.9±1.4
	10	99.7±0.9	97.6±0.9
	100	93.2±0.7	98.1±1.3
Zn	-	96.7±0.8	99.0±1.1
	10	95.4±0.5	99.8±1.4
	100	94.6±1.6	99.3±1.1
Co	-	96.5±1.3	98.9±1.3
	10	95.6±0.9	99.1±1.1
	100	95.1±1.2	99.2±1.2

Fig. 4. Reusing the column for the solid phase preconcentration of 10.0 ng mL⁻¹ of Pb^{2+} and Al^{3+} .

FT-IR Studies for Surface Functionality of *Agaricus arvensis*

FT-IR was employed to determine the surface functionality of *Agaricus arvensis* and to discuss the interactions with Pb and Al. The overlay spectra are presented in Figure 5. The double peaks at 3100 cm⁻¹ are attributed to the C-H vibrations in the aromatic rings and in Amberlite XAD-4 (Figure 5a). C = vibrations in the aromatic rings gave double signals at approximately 1500–1600 cm⁻¹ (Figure 5a). Figure 5b shows that the broad peak at about 3500 cm⁻¹ could arise from the -OH and/or -NH₂ groups. The peaks at 3100 cm⁻¹ could be due to the presence of the aromatic groups of the C-H vibrations. Strong peaks at about 1600 cm⁻¹ could arise from the carboxyl groups. The peaks at 1470 and 1300 cm⁻¹ were due to the C-H vibrations. In Figure 5c, the strong peaks of about 3050 cm⁻¹, 1600 cm⁻¹, 1200 cm⁻¹ (double peaks), and 750 cm⁻¹ are attributed to the immobilization of *Agaricus arvensis* to Amberlite XAD-4. Figure 5d shows a strong peak at 1470 cm⁻¹ after Pb^{2+} was biosorbed on *Agaricus arvensis* and immobilized to Amberlite XAD-4. Figure 5e shows a decrease in peak intensity at 2900 cm⁻¹ and a strong peak at 1400 cm⁻¹, attributed to the complexation of Al^{3+} with the biosorbent. However, it is difficult to explain the exact mechanism of the biosorption of Pb^{2+} and Al^{3+} only with FT-IR. Further studies are needed in order to determine the nature of the exact interaction mechanisms when *Agaricus arvensis* is used as the biosorbent.

Fig. 5. FT-IR spectra of (a) Ambelite XAD-4, (b) *Agaricus arvensis*, (c) *Agaricus arvensis* immobilized on Ambelite XAD-4, (d) Pb^{2+} sorbed on *Agaricus arvensis* immobilized on Ambelite XAD-4, (e) Al^{3+} sorbed on *Agaricus arvensis* immobilized on Ambelite XAD-4.

Analytical Figures of Method

The biosorption capacity of immobilized *Agaricus arvensis* for Pb^{2+} and Al^{3+} was found to be 31.2 mg g^{-1} and 45.7 mg g^{-1} , respectively. It could be said that the biomass-based SPE column developed in this study showed a higher biosorption capacity than all of the other sorbents listed in Table VI.

The analytical characteristics of the developed SPE method were evaluated by considering the linear range, slope, intercept, and correlation coefficient of the linear plot, limit of detection (LOD), limit of quantification (LOQ), and relative standard deviation (RSD). Three times the standard deviation of the slope ratio was employed to calculate the LOD, while 10 times the ratio was accepted as the LOQ. The %RSD was calculated from the ratio of the standard deviation to the mean signal. For the preconcentration of the Pb^{2+} and Al^{3+} ions, 200.0 mL of the aqueous solution containing 0.2, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, and 20.0 ng mL^{-1} of Al^{3+} and 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, and 20.0 ng mL^{-1} of Pb^{2+} were passed through the solid phase extraction column at the 3.0 mL min^{-1} flow rate of the sample using pH 6.0 and 5.0, respectively. Linear calibration equations by SPE-ICP-OES were found as $y=186445x-78.6$ in the range of $1.25-50.0 \text{ ng mL}^{-1}$ for Pb and $y=17690000x-3813.2$ in the range of $0.5-50.0 \text{ ng mL}^{-1}$ for Al. The correlation coefficients were higher than 0.9995. Table VI lists the analytical features of the developed SPE method in comparison with different SPE methods. The LOD values were calculated as 0.25 ng mL^{-1} for Pb(II) and 0.07 ng mL^{-1} for Al^{3+} . The LOQ values were calculated as 0.84 ng mL^{-1} for Pb^{2+} and 0.24 ng mL^{-1} for Al^{3+} . The RSD values were found to be lower than 8.4% for Pb^{2+} and 6.4% for Al^{3+} (for the lowest concentrations of linear calibration plots). The theoretical

preconcentration factor was 40.0 for Pb^{2+} and Al^{3+} for an initial sample volume of 200.0 mL and a final volume of 5.0 mL. The experimental preconcentration factors for Pb^{2+} and Al^{3+} were calculated from the slope ratio of the linear calibration plot of the SPE-ICP-OES method to ICP-OES. The sensitivity of ICP-OES was improved by 95.6 times for Pb, and by 94.9 times for Al. From Table VI, it can be concluded that the developed SPE method had a higher analytical performance with a lower LOD, a higher capacity, and a higher preconcentration factor.

This SPE-ICP-OES method was validated through the analysis of certified reference samples. The results presented in Table VII show that two water samples and two plant samples were subjected to the proposed SPE procedure. A comparison of the certified and determined values shows that the results were satisfactory. The RSD values were found to be lower than 8.5% for Pb^{2+} and 9.8% for Al^{3+} . Each of the certified reference samples was spiked with known amounts of Pb and Al. The recovery values were found higher than 95.1% for Pb^{2+} and 97.3% for Al^{3+} . From these results it can be concluded that the developed SPE method can be applied to real samples without loss of Pb^{2+} and 97.3% for Al^{3+} .

Determination of Pb and Al in Vegetables

The developed method was also applied to the determination of Pb and Al in edible vegetable samples (cucumber, okra, tomato, beans, aubergine, watermelon, zucchini, pepper, melon, lettuce, roka, purslane, scallion, cress, parsley, cabbage, sugar cane, and basil) collected along the cultivated banks of the Tigris River in Diyarbakır. The samples were digested in a microwave oven prior to applica-

tion of the SPE method. A comparison of the results in Table VIII shows that there is a wide concentration range of Pb from <LOQ to 161 ng g^{-1} and Al from <LOQ to 528 ng g^{-1} in the vegetables. The highest amount of Al was determined in watermelon. The Pb and Al concentrations are not reported for parsley, okra, scallion, cress, sugar cane, and basil since their concentrations were lower than the LOQ.

CONCLUSION

In the presented study, an alternative solid phase extraction (SPE) method based on the use of *Agaricus arvensis* immobilized Amberlite XAD-4 as sorbent was developed for the inductively coupled plasma optical emission spectrometry (ICP-OES) preconcentration of Pb and Al. Important parameters of the SPE procedure were optimized and satisfactory results were obtained from validation studies and the analysis of certified reference tea and poplar leaves samples. Spiking experiments showed that both Pb and Al were recovered from the matrix with good recovery values of over 95%. The method was applied to the determination of Pb and Al in different types of edible vegetables collected along the cultivated banks on the Tigris River in Diyarbakır, Turkey. The sensitivity of ICP-OES was improved 95.6 times for Pb and 94.9 times for Al. It was also found that the same SPE column can be used 30 times without loss of efficiency.

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TABLE VI

Comparison of Analytical Figures of Merit for Preconcentration of Pb and Al by Different SPE Methods

Element	Sorbent for SPE	Experimental Details	Detection	Co-preconc. metals	LOD (ng mL ⁻¹)	Column reuse	PF ^a	Capacity of Sorbent (mg g ⁻¹)	Ref.
Pb	Chromosorb 108 as bathocuproinedi-sulfonic (BCDS) acid chelates	pH:9.0, eluent: 5.0 mL of 1.0 mol L ⁻¹ HNO ₃ in acetone, eluent flow 5.0 mL min ⁻¹ , 400.0 mL of sample volume	FAAS	Cu, Cd, Zn, Mn, Fe, Cr, Ni, Co	0.60	200	80	6.8	42
Pb	Blackstone (Pierre noire)	pH:7.0, eluent: 0.15 mL of 0.5 mol L ⁻¹ HCl, eluent flow 2.5 mL min ⁻¹ , 50.0 mL of sample volume	ICP-OES	Cd, Co, Ni	1.1	50	20	22.2	43
Pb	Epoxy resin-based monoliths	pH:4.0, eluent: 10.0 mL of 1.0 mol L ⁻¹ HNO ₃ , eluent flow 2.0 mL min ⁻¹ , 500.0 mL of sample volume	FAAS	-	1.2	20	50	106.8	44
Pb	Nanometer-sized γ-alumina coated with sodium dodecyl sulfate-1-(2-pyridylazo)-2-naphthol (SDS-PAN)	pH:7.0-8.0, eluent: 2.0 mL of 2.0 mol L ⁻¹ HNO ₃ , eluent flow 4.0 mL min ⁻¹ , 500.0 mL of sample volume	FAAS	Cd	0.17	-	250	16.4	19
Pb	<i>Agaricus arvensis</i> immobilized on Amberlite XAD-16	pH:5.0, eluent: 5.0 mL of 1.0 mol L ⁻¹ HCl, eluent flow 3.0 mL min ⁻¹ , 200.0 mL of sample volume	ICP-OES	Al	0.1	30	50	31.2	This study
Al	o-aminophenol immobilized Amberlite XAD-1180	pH:6.0, eluent: 20.0 mL of 4.0 mol L ⁻¹ HNO ₃ , eluent flow 2.5 mL min ⁻¹ , 300.0 mL of sample volume	FAAS	Cd, Co, Cu, Ni	4.3	100	50	46.8	45
Al	Eriochrome Cyanine R modified anion-exchange resin	pH:6.0, eluent: HCl and HNO ₃ , 100.0 mL of sample volume	ICP-OES ET-AAS	-	0.11	-	20	4.1	46
Al	nanometer-sized TiO ₂ -silica composite modified with 4-aminophenylarsonic acid	pH:3.0, eluent: 2.0 mL of 0.5 mol L ⁻¹ HCl, eluent flow 1.5 mL min ⁻¹ , 300.0 mL of sample volume	ICP-OES	-	0.06	16	150	56.58	47
Al	<i>Agaricus arvensis</i> immobilized on Amberlite XAD-16	pH:6.0, eluent: 5.0 mL of 1.0 mol L ⁻¹ HCl, eluent flow 3.0 mL min ⁻¹ , 200.0 mL of sample volume	ICP-OES	Pb	0.03	30	50	45.7	This study

^a PF: Preconcentration factor.

TABLE VII
Results for Pb²⁺ and Al³⁺ in Certified Reference Samples (n=3)

Sample	Metal Ion	Added (µg g ⁻¹)	Found (µg g ⁻¹)	Certified (µg g ⁻¹)	Recovery (%)
NWTM-15	Pb	-	11.6±0.9 ¹	11.8 ¹	98.3
		10.0	21.6±1.2 ¹	-	-
	Al	-	21.4±1.4 ¹	21.7 ¹	98.6
		10.0	31.5±3.1 ¹	-	-
EU-L-2	Pb	-	39.9±2.1 ¹	41 ¹	97.3
		10.0	49.4±3.4 ¹	-	-
	Al	-	50.6±4.4 ¹	52 ¹	97.3
		10.0	61.0±4.5 ¹	-	-
NCSZC73014	Pb	-	1460±112	1500±200	97.3
		200.0	1660±234	-	-
	Al	-	1897±152	-	-
		200.0	2100±199	-	-
NCSDC 73350	Pb	-	1427±103	1500±300	95.1
		200.0	1686±143	-	-
	Al	-	1023±48	1040±60	98.3
		200.0	1204±65	-	-

TABLE VIII
Total Pb and Al Levels in Vegetables (n=3)

Vegetable	Present Study Heavy Metals (ng g ⁻¹)		Literature Heavy Metals (ng g ⁻¹)		Ref.
	Pb	Al	Pb	Al	
Cucumber	<LOQ	136±3	-	-	-
Okra	<LOQ ^a	178±22	-	-	-
Tomato	<LOQ	142±6	<LOQ-31.2	-	48
			30.3-67.7	-	49
Beans	<LOQ	192±11	-	-	-
Aubergine	<LOQ	124±7	-	-	-
Watermelon	<LOQ	528±24	-	-	-
Zucchini	161±14	178±6	-	-	-
Pepper	<LOQ	132±7	<LOQ-97.7	-	48
			18.1-70.6	-	49
Melon	<LOQ	230±2	-	-	-
Lettuce	<LOQ	<LOQ	4.1-59.8	-	48
Purslane	142±5	<LOQ	-	-	-
Cabbage	<LOQ	<LOQ	<LOQ	-	50

^a <LOQ: Under the limit of quantification.

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