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Preconcentration with *Bacillus subtilis*–Immobilized Amberlite XAD-16: Determination of Cu^{2+} and Ni^{2+} in River, Soil, and Vegetable Samples

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ABSTRACT Solid-phase extraction (SPE) method was developed for the preconcentration of Cu^{2+} and Ni^{2+} before their determination by inductively coupled plasma optical emission spectrometry (ICP-OES). *Bacillus subtilis*–immobilized Amberlite XAD-16 was used as biosorbent. Effects of critical parameters such as pH, flow rate of samples, amount of Amberlite XAD-16 and biosorbent, sample volume, eluent type, and volume and concentration of eluent on column preconcentration of Cu^{2+} and Ni^{2+} were optimized. Applicability of the method was validated through the analysis of the certified reference tea sample (NCS ZC73014). Sensitivity of ICP-OES was improved by 36.4-fold for Cu^{2+} and 38.0-fold for Ni^{2+} by SPE-ICP-OES method. Limit of quantitation (LOQ) was found to be 0.7 and 1.1 ng/ml for Cu^{2+} and Ni^{2+}, respectively. Concentrations of Cu^{2+} and Ni^{2+} were determined by ICP-OES after application of developed method. Relative standard deviations (*RSD*s) were lower than 4.9% for Cu^{2+} and 7.9% for Ni^{2+}. The Tigris River that irrigates a large agricultural part of Southeast Turkey is polluted by domestic and industrial wastes. Concentrations of Cu^{2+} and Ni^{2+} were determined in water, soil, and some edible vegetables as a biomonitor for heavy metal pollution.

KEYWORDS biosorbent, Cu^{2+}, Ni^{2+}, preconcentration, sensitivity improvement, solid-phase extraction

INTRODUCTION

Vegetables constitute essential components of the human diet by providing protein, vitamins, iron, calcium, and other nutrients that are usually in short supply (Thompson and Kelly 1990). They also act as buffering agents for acid substances released during the digestion process (Bahemuka and Mubofu 1999). However, vegetables contain both essential and toxic elements over a wide range of concentrations. Heavy metals are among the major contaminants of food supply and may even be considered the most important problem,

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especially in developing countries. Heavy metals, in general, are not biodegradable and have long biological half-lives and potential for accumulation in the different body organs, leading to unwanted side effects (Sathawara, Parikh, and Agarwal 2004). The excessive content of heavy metals in food is associated with the etiology of a number of diseases, especially with cardiovascular, kidney, nervous, and other diseases (Jarup 2003).

It has been reported that concentration levels of heavy metals in environmental samples such as vegetable, water, or soil must be lower than the allowable limits (Cayir and Coskun 2010). Determination of heavy metals in natural samples such as water, soil, and food continues to be a challenge for analytical chemists around the world. From that point of view, there is a great need to develop an easy, susceptible, selective, and cheaper method for the determination of heavy metal levels in food and natural water and soil samples (Uluozlu et al. 2010).

Chemical precipitation, oxidation or reduction, filtration, ion exchange, electrochemical treatment, reverse osmosis, membrane technology, and cloud point extraction are common physicochemical methods used to remove heavy metals. Most of these are ineffective or excessively expensive when metal concentrations are less than 100 mg/L (Ahluwalia and Goyal 2007; Ozdemir et al. 2009; Okumus, Basaran, and Onay 2010).

Solid-phase extraction (SPE) method was used for the removal and enrichment of metal ions (Ghaedi et al. 2010). It was highlighted that biosorbent should have high surface area and high adsorption capacities (Ozdemir et al. 2012). In addition to these, they have advantages over synthetic ligands. When biosorbent was immobilized to within a suitable support material such as resin, it presented a naturally functional surface, porous characteristics, reusable matrix, simple operating procedure, and less waste. By considering the advantages of biomass as a biosorbent, they could also be evaluated as environmentally friendly substances (Iqbal and Edyvean 2004). Recently, solid materials such as Amberlite XAD resins have received an increased attention as basic matrices for designing new modified resins by chelating agent (Ince, Kaya, and Yaman 2010; Kilinc et al. 2013). Amberlite XAD series are widely used as support material for preconcentration procedures because of their good physical and chemical properties such as porosity, high surface area, durability, and purity (Ozdemir, Kilinc, and Erdogan 2010; Ozdemir et al. 2013).

In this study, an SPE procedure was developed for determination of trace levels of Cu^{2+} and Ni^{2+}. *Bacillus subtilis* was used as biosorbent and was immobilized on XAD-16. Critical parameters in SPE experiments were optimized. The optimized method was applied to real samples to preconcentrate Cu and Ni before their determination by inductively coupled plasma optical emission spectrometry (ICP-OES). Therefore, this study presents data on the heavy metal levels (Cu^{2+} and Ni^{2+}) in soil, water, and edible parts of selected vegetables grown in the site of the Tigris River.

MATERIALS AND METHODS

Instrumentation

Concentrations of Cu^{2+} and Ni^{2+} were determined by ICP-OES (Perkin-Elmer Optima 2100 DV; Shelton, CT) at wavelengths of 327.393 and 231.604 nm, respectively. Instrumental conditions were as given in the literature (Ozdemir et al. 2009). SPE experiments were performed on polypropylene column (1.0 cm × 10.0 cm). Digital pH meter (Mettler-Toledo, Columbus, OH, USA) was used to measure the pH of the solutions. Flow rates of solutions were adjusted to desired values by peristaltic pump (Waters Marlow, Milford, MA, USA).

Reagents and Solutions

Standard solutions of 1000 mg/L Cu^{2+} and Ni^{2+} were diluted to prepare working standards. All chemicals used were of analytical reagent grade. Doubly distilled water was used in the experiments. Certified reference tea sample was obtained from China National Analysis Center for Iron and Steel (Tuebingen, Germany). All glass materials were kept permanently full with 1.0 mol/L nitric acid when not in use. Concentrated nitric acid (65%; Merck, Darmstadt, Germany) and hydrogen peroxide (35%; Merck) were used to digest the samples. Amberlite XAD-16 (polystyrene divinyl benzene) was purchased from Sigma (St. Louis, MO). Impurities were removed by using the procedure given in literature (Ozdemir, Kilinc, and Erdogan 2010).

SPE Procedure

B. subtilis, which was isolated from campus area of Dicle University, Diyarbakir, Turkey, was used as biosorbent in this study. It was cultivated in 250-ml Erlenmeyer flasks containing 50 ml Nutrient Broth (NB) medium by shaking at 37°C for 24 h (120 rpm). The cultures were centrifuged at 10,000 rpm for 10 min, and then the pellets were washed twice with 0.9% NaCl and dried in an oven at 80°C for 24 h. To obtain a fine powder of the dried cells, they were ground in a porcelain mortar and then were autoclaved at 121°C for 15 min to assess complete death of the dried cells. Finally, the cells were inoculated to liquid medium, and the absence of any growth indicated positive results (complete death of the bacteria). Dry biomass powder (200 mg) was mixed with 0.75 g of Amberlite XAD-16 and 7.5 ml of doubly distilled water and then thoroughly mixed. Advantages of Amberlite XAD-16 resin in SPE studies were discussed in our recent review (Ozdemir et al. 2013). Column packing procedure from our previous study was applied (Ozdemir, Kilinc, and Erdogan 2010).

The solution (50.0 ml) containing 25.0 μg/L Cu^{2+} and Ni^{2+} was subjected to SPE procedure. pH of the solution was adjusted to desired values by adding HCl and NH_3. It was then passed through the SPE column by using a peristaltic pump. After passing this solution completely, 10.0 ml distilled water was passed through the column. Five milliliters of 1.0 mol/L HCl was used as an eluent. Concentrations of Cu^{2+} and Ni^{2+} in this solution were determined by ICP-OES. According to the experiments, the same column could be used repeatedly (up to 15 times) after washing with 1.0 mol/L HCl solution and distilled water, respectively.

Preparation of Samples for SPE Procedure

Soil samples were collected from three different locations in the Tigris River. These samples were dried at 105°C for 2 h and ground to pass through a 200-mesh (0.075-mm) sieve and homogenized for analysis. One gram of the sieved sample was dissolved in 15 ml aqua regia and left to dry. The residue was treated with 5.0 ml of 1.0 M HNO_3, and the suspension was centrifuged. The clear solution was evaporated to 1.0 ml and then diluted to 15 ml with 1 mol/L HNO_3.

Samples were collected along the cultivated banks of the Tigris River and stored in polyethylene bags according to their type and then brought to the laboratory for preparation and treatment. They were first washed with tap water to remove the pollutions from soil and then rinsed by distilled water. Then, they were dried at 80°C for 24 h in oven. The samples were ashed at 480°C in a furnace for 3.0 h, and the ashed samples were dissolved with a mixture of nitric acid–hydrogen peroxide (2:1, *v/v*). Three milliliters of the acid mixture was then added; the process was repeated two times with occasional shaking on a hot plate. After drying, 4.0 ml of 1.0 mol/L nitric acid was added to dissolve the remaining residue. It was then diluted up to 50.0 ml with distilled water and SPE procedure was applied. The same procedure was also applied to the certified reference tea sample, NCS ZC73014.

RESULTS AND DISCUSSION

Effect of pH

It is well known that initial pH of the solution is one of the most important experimental parameters in SPE procedure that affects the protonation of the functional groups on the biomass as well as the metal chemistry (Ozdemir et al. 2010). At pH values lower than 4.0, the cell wall of the biomass should be positive, which prevents the binding of positively charged metal ions. These results showed that the functional groups such as amino, phosphate, and carboxyl on the surface of the biomass and their ionic states at these pHs determine the extent of biosorption. In addition to this, at lower pH values, the cell wall of biosorbent would be closely associated with the hydronium ions (H_3O^+), which restrict access to ligands by metallic ions as a result of repulsive forces (Nadeem et al. 2008). To investigate the effect of pH, a series of experiments were carried out using 50.0 ml of 25.0 μg/L Cu^{2+} and Ni^{2+}, with varying pH from 2.0 to 8.0. The optimum pH was found to be 7.0 for Cu^{2+} and 5.0 for Ni^{2+} ions (Figure 1). It was also found that at higher pH values, the biosorption yields for Cu^{2+} and Ni^{2+} ions decreased. This might be attributed to the coordination of OH^- with the

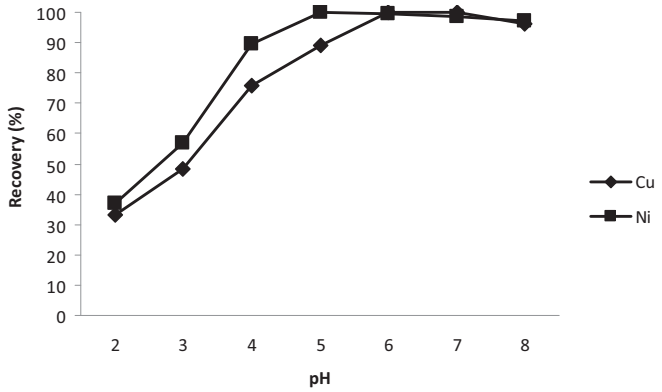

FIGURE 1 Variation of recovery of 25.0 μg/L of Cu^{2+} and Ni^{2+} with pH.

metal ion (Reddy et al. 2010). From that point of view, all the following biosorption studies were carried out at pH 5.0 and 7.0 for Cu^{2+} and Ni^{2+} ions, respectively.

Effect of Sample Flow Rate

It was reported that flow rate of sample solution plays an important role in view of the interaction of metal ions with biosorbent (Duran, Tuzen, and Soylak 2009). It should be optimized in the studies. The effect of sample flow rate was examined over the range of 1.0–8.0 ml/min at pH 7.0 and 5.0 for Cu^{2+} and Ni^{2+}, respectively. The results of this study are given in Figure 2. It was found that 2 ml/min was the optimal flow rate. In further studies, flow rate of 2 ml/min was used. At higher flow rates, recovery values decreased. The decrease in the values was attributed to less interaction of the analyte with sorbent.

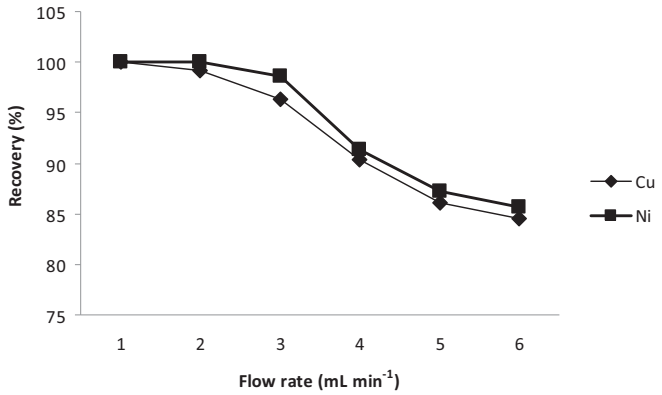

FIGURE 2 Influence of flow rate on recovery of 25.0 μg/L of Cu^{2+} and Ni^{2+}.

Influence of Amount of Biosorbent and Resin

The variation of preconcentration of Cu^{2+} and Ni^{2+} depending on the amount of biosorbent was studied in the range of 100.0–350.0 mg *B. subtilis* by passing 50.0 ml of a solution containing 25.0 μg/L Cu^{2+} and Ni^{2+} through the SPE column at optimum conditions. The results are presented in Figure 3. By increasing the amount of biosorbent, uptake of Cu^{2+} and Ni^{2+} ions also increased. Maximum uptake of Cu^{2+} and Ni^{2+} ions was observed when the amount of biosorbent was 300.0 mg. Cu^{2+} and Ni^{2+} uptake had not increased above the 300.0 mg amount of biosorbent. Thus, further studies were performed using 300 mg of biosorbent.

Another optimization parameter was the amount of resin (XAD-16), which was examined in the range of 25–2000 mg (Figure 4). The results showed that recoveries of metal ions increased up to 1000 mg of resin, but further addition did not influence the recovery of metal ions. Hence, 1000 mg of resin was selected as optimum.

Effect of Sample Volume

To obtain a high preconcentration factor, the sample volume should be higher. However, time per analysis will be longer when the sample volume is higher. On the other hand, limitation in sample amount should be considered. From that point of view, the influence of the sample volume on metal biosorption was examined by passing 25.0–300.0 ml of Cu^{2+} and Ni^{2+} at the concentration of 3.12–50.0 μg/L through the SPE column at the optimum pH at a flow rate of

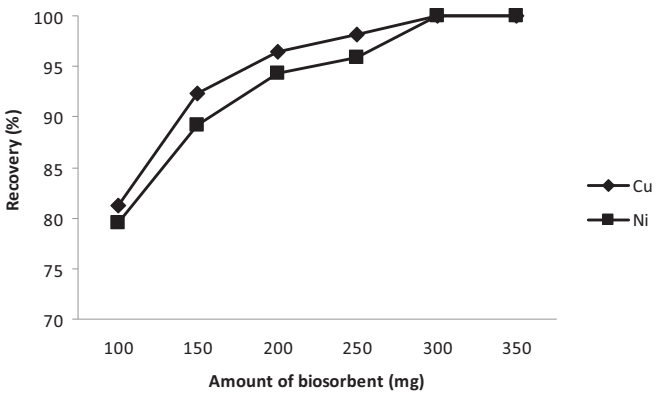

FIGURE 3 Effect of amount of biosorbent on recovery of 25.0 μg/L of Cu^{2+} and Ni^{2+}.

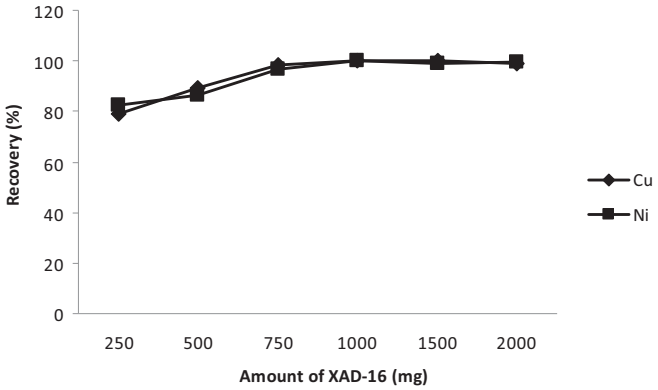

FIGURE 4 Effect of amount of Amberlite XAD-16 on recovery of 25.0 μg/L of Cu^{2+} and Ni^{2+}.

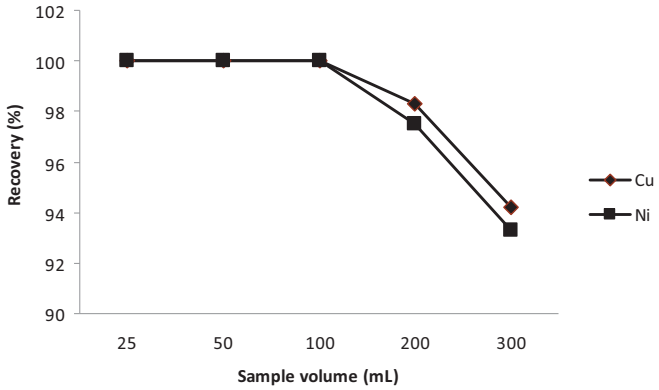

FIGURE 5 Variation of recovery of Cu^{2+} and Ni^{2+} at different volumes.

2.0 ml/min. The effect of sample volumes on the recovery values is presented in Figure 5. It was found that 98.3% and 97.5% recovery values were achieved for Cu^{2+} and Ni^{2+}, respectively, at a sample volume of 200 ml. When the sample volume was increased, the recovery value decreased. Therefore, 200.0 ml of the sample solution was accepted as the furthest sample volume for the preconcentration of Cu^{2+} and Ni^{2+}. As the final solution was 2.0 ml, the preconcentration factors were 100.0 for both Cu^{2+} and Ni^{2+}.

Effect of HCl and HNO_3 Volume/ Concentration for Elution

HCl and HNO_3 were examined as eluent. Variations in recovery values of metal ions with different concentrations and volumes of acids were investigated (Table 1). When concentration of HCl was increased from 0.5 to 1.0 mol/L by a volume of 5.0 ml, recovery values of Cu^{2+} and Ni^{2+} increased from 93.7% to 99.7% and from 93.2% to 99.1%, respectively. From that point of view, 5 ml of 1.0 mol/L HCl was selected as the optimum volume and concentration for elution of Cu^{2+} and Ni^{2+}.

Analytical Features of SPE Method

To examine the biosorption capacity of *B. subtilis* immobilized on Amberlite XAD-16, 0.05 g of immobilized cells was added to 100 ml solution containing 10 mg of metal ion and incubated for 120 min at 37°C on a shaker at 120 rpm at pH 7.0 and 5.0 for Cu^{2+} and Ni^{2+} ions, respectively. Immobilized cells were separated by centrifugation at 10,000 rpm for 10 min. The supernatants were used to estimate residual metal concentration by ICP-OES. Biosorbent capacity is the most important factor, for it limits how much biosorbent is required to quantitatively remove a certain amount of metal ions from aqueous solutions (Wang et al. 2011). Thus, the maximum biosorption capacity of *B. subtilis* immobilized on Amberlite XAD-16 was determined as 12.4 and 10.3 mg/g for Cu^{2+} and Ni^{2+}, respectively.

TABLE 1 Effects of the Type and Volume of Elution Solutions on the Recovery of Cu^{2+} and Ni^{2+}

			Recovery (%)	
Type of elution solution	Volume (ml)	Concentration (mol/L)	Cu^{2+}	Ni^{2+}
HCl	3.0	0.5	91.5 ± 0.4	89.8 ± 0.3
	5.0	1.0	94.3 ± 0.3	93.2 ± 0.5
	3.0	0.5	94.2 ± 0.1	92.7 ± 0.2
	5.0	1.0	99.7 ± 0.2	99.1 ± 0.3
HNO_3	3.0	0.5	85.8 ± 0.3	83.9 ± 0.6
	5.0	1.0	94.6 ± 0.5	92.1 ± 0.2
	3.0	0.5	90.7 ± 0.6	88.6 ± 0.3
	5.0	1.0	96.7 ± 0.4	94.9 ± 0.7

TABLE 2 Analytical Characteristics of Methods

Method	Element	Linear range	Slope	Intercept	R^2	LOD (ng/ml)	LOQ (ng/ml)	E^a
ICP-OES	Cu^{2+}	0.015–1.0 μg/ml	294,240	−1349	0.9989	3.60	12.0	—
	Ni^{2+}	0.020–1.0 μg/ml	60,130	346	0.9996	4.50	15.0	—
SPE-ICP-OES[b]	Cu^{2+}	1.000–50.0 ng/ml	10,710	1198	0.9991	0.21	0.7	36.4
	Ni^{2+}	1.300–26.0 ng/ml	2285	−25	0.9969	0.33	1.1	38.0

Note. LOD = limit of detection; LOQ = limit of quantitation.
[a]Enhancement factor was calculated from slope ratio of SPE-ICP-OES method to ICP-OES method.
[c]Initial volume was 200.0 ml; eluent was 5.0 ml of 1.0 mol/L HCl.

In SPE processes, reusability of the column is an economically important parameter (Ozdemir, Kilinc, and Erdogan 2010). To examine the reusability of the SPE column, a sample volume of 50 ml, which contained 25.0 μg/L Cu^{2+} and Ni^{2+}, was passed through the column at a flow rate of 2.0 ml/min. It was then found that the resins can be used up to 15 times.

Linear calibration plots for Cu^{2+} and Ni^{2+} were found as $y = 294{,}240x - 1349$, $r^2 = .9989$, and $y = 60{,}130x + 346$, $r^2 = .9996$, by ICP-OES without SPE procedure in the concentration range of 0.015–1.0 μg/ml for Cu^{2+} and 0.020–1.0 μg/ml for Ni^{2+}. Limits of detection (LOD) and limits of quantitation (LOQ) were 3.6 and 12.0 μg/ml for Cu^{2+} and 4.5 and 15.0 μg/ml for Ni^{2+}, which were calculated from the $3s/m$ and $10s/m$, respectively, where s is the standard deviation of the lowest concentration of calibration plot and m is the slope of the calibration plot (Table 2).

From the previous experiments, it had been found that 200.0 ml was the optimum sample volume. To determine the linear working range of the method, concentrations of Cu^{2+} and Ni^{2+} were decreased for a constant volume of 200.0 ml. It was found that linear working ranges of method were 1.0–50.0 and 1.3–26.0 ng/ml for Cu^{2+} and Ni^{2+}. Calibration plots were found as $y = 10{,}710x + 1198$, $r^2 = .9991$, and $y = 2285x - 25$, $r^2 = .9969$, respectively, for Cu^{2+} and Ni^{2+}. LOD and LOQ were calculated as 0.21 and 0.7 ng/ml for Cu^{2+} and 0.33 and 1.1 ng/ml for Ni^{2+} (Table 2).

By considering the analytical characteristics of the method, it could be said that sensitivity of ICP-OES was improved by applying SPE method. Enhancement factors were found to be 36.4 for Cu^{2+} and 38.0 for Ni^{2+}. By considering the 200.0 ml of initial volume and 5.0 ml of final volume, the preconcentration factor was found to be 40.0.

Applicability of the method was validated by applying the method to the certified reference tea sample (Table 3). The obtained values were found to agree with the certified values. Here, 1.0 and 4.0 μg/g of Cu^{2+} and 0.5 and 2.0 μg/g of Ni^{2+} were also added to the sample. The results showed that spiked amounts of Cu^{2+} and Ni^{2+} were quantitatively recovered. Relative standard deviations (*RSD*s) were found to be lower than 4.9% for Cu^{2+} and 7.9% for Ni^{2+}.

Analytical characteristics of recent methods developed for preconcentration of Cu^{2+} and Ni^{2+} are summarized in Table 4. It is clear that our method gave low values of detection limit and wide linear ranges for Cu^{2+} and Ni^{2+}. Therefore, it can be said that the developed method can be used for the trace analysis of Cu^{2+} and Ni^{2+}.

The developed method was applied to real vegetable samples collected along the cultivated banks of the Tigris River to determine the concentrations of Cu^{2+} and Ni^{2+}. The results obtained are given in Table 5. Concentrations of Cu^{2+} were in the range of 2.65–21.64 μg/g. The lowest Cu^{2+} concentration was determined in the tomato, whereas the highest was determined in the red pepper. Ni^{2+} concentrations were found in the range of 0.68–8.41 μg/g. Tomato contained the lowest amount of Ni^{2+}.

Concentrations of Cu^{2+} and Ni^{2+} in white and red cabbage were found as 2.24 and 3.76 μg/g and 2.26

TABLE 3 The Results for Cu^{2+} and Ni^{2+} in the Certified Reference Sample of Tea Leaves ($n = 3$)

Metal	Added (μg/g)	Certified (μg/g)	Found (μg/g)	*RSD* (%)
Cu^{2+}	—	18.6 ± 0.7	18.3 ± 0.9	4.9
	1.0	—	19.5 ± 0.7	3.6
	4.0	—	22.4 ± 0.6	2.7
Ni^{2+}	—	3.4 ± 0.3	3.3 ± 0.2	6.1
	0.5	—	3.8 ± 0.3	7.9
	2.0	—	5.3 ± 0.3	5.7

TABLE 4 Comparison of Analytical Features of Methods for Preconcentration of Cu^{2+} and Ni^{2+}

Method	Procedure	Linear range	Cu^{2+} LOD, ng/ml	Ni^{2+} LOD, ng/ml	Reference
SPE/FAAS	Separation of DDTC complex on Amberlite XAD-4 resin	—	4.00	23.00	Uzun, Soylak, and Elci 2001
SPE/FAAS	Pyrrolidine dithiocarbamate complexes on a short column of Chromosorb-102	—	0.44	3.60	Saracoglu and Elci 2002
Co-precipitation ICP-OES	2,2-Bipyridyl and erythrosine co-precipitation method	200–4000 ng/ml Cu 100–2000 ng/ml Ni	18.0 (ICP-OES)	21.3 (ICP-OES)	Feist et al. 2008
SPE/ICP-OES	Preconcentration by 4-(8-hydroxy-5-quinolylazo) naphthalenesulfonic acid modified silica gel	—	0.85	0.68	Chang et al. 2008
SPE/ICP-OES	Membrane solid-phase microextraction with alumina hollow fiber on line coupled with ICP-OES	—	0.88	0.38	Cui, He, and Hu 2011
SPE/ICP-OES	Activated carbon–loaded mini-column	5.5–11.7	0.10	—	Takara et al. 2005
SPE/ICP-OES	Formation of ternary Cu (II)-CAS-CTAB ion-pair and adsorption of it into a mini-column packed with cotton	0.5–100 ng/ml Cu	0.04	—	Faraji, Yaminia, and Shariati 2009
SPE/ICP-OES	Solid-phase extraction of the metals using a minicolumn of Amberlite XAD-4 modified with DHB	5.0–50.0 ng/ml Cu 5.0–5.0 ng/ml Ni	0.23	0.06	Bezerra et al. 2007
SPE/ICP-OES		1.0–50.0 ng/ml Cu 1.3–26.0 ng/ml Ni	0.21	0.33	This study

Note. LOD = limit of detection; DDTC = diethyldithiocarbamate; CAS-CTAB = chromazurol S (CAS)–cethyltrimethylammonium bromide (CTAB); DHB = dihydroxybenzoic acid.

and 3.40 μg/g, respectively (Feist et al. 2008). In another study, Cu^{2+} amount was found to be 3.66 μg/g, whereas Ni^{2+} amount was found to be lower than 0.2 μg/g (Milacic and Kralj 2003). Recently, heavy metal concentrations in vegetables from urban area of Kayseri, Turkey, were determined. Cu^{2+} concentrations were found as 59.93 μg/g for lettuce, 53.12 μg/g for parsley, 53.83 μg/g for onion, 37.54 μg/g for okra, 37.06 μg/g for green pepper, 32.6 μg/g for tomato, and 73.2 μg/g for bean. Ni^{2+} concentrations were found as 6.3 μg/g for lettuce, 3.47 μg/g for parsley, 4.6 μg/g for onion, 2.7 μg/g for okra, 1.8 μg/g for green pepper, 3.1 μg/g for tomato, and 2.6 μg/g for bean (Demirezen and Aksoy 2006). Cu^{2+} and Ni^{2+} were found in cabbage at concentrations of 2.5 and 1.4 μg/g, respectively (Bezerra et al. 2007). A study of metal contents of plants in vegetable garden sites in Kano Metropolis reported that the Cu^{2+} concentrations were in the range of 3.54–6.74 μg/g for okra, 4.2–7.5 μg/g for onion, 0.63–1.11 μg/g for tomato, 0.69–1.63 μg/g for lettuce, and 0.85–1.33 μg/g for cabbage, and the Ni^{2+} concentrations were in the range of 0.63–1.13 μg/g for okra, 0.41–0.99 μg/g for onion, 0.75–1.2 μg/g for tomato, 1.03–1.52 μg/g for lettuce, and 0.55–0.79 μg/g for cabbage (Audu and Lawal 2006). From the results obtained in our study and the

TABLE 5 Levels of Total Cu^{2+} and Ni^{2+} in Vegetables ($n = 3$)

Vegetable	Cu^{2+} (μg/g)	Ni^{2+} (μg/g)
Watermelon	5.51 ± 0.160	0.98 ± 0.03
Melon	6.29 ± 0.600	1.14 ± 0.11
Tomato	2.65 ± 0.210	0.68 ± 0.09
Aubergine	3.76 ± 0.040	0.85 ± 0.03
Marrow	9.70 ± 0.120	1.30 ± 0.48
Bean	9.50 ± 0.640	2.87 ± 0.39
Okras	18.74 ± 0.700	1.09 ± 0.09
Red pepper	21.64 ± 0.830	1.96 ± 0.02
Cucumber	16.80 ± 1.070	3.06 ± 0.05
Cabbage	5.55 ± 1.790	1.48 ± 0.14
Lettuce	4.27 ± 0.140	2.22 ± 0.48
Parsley	11.16 ± 0.090	2.19 ± 0.29
Onion	13.79 ± 0.924	8.41 ± 0.76
Sweet basil	15.96 ± 0.950	4.41 ± 0.26
Eruca	7.57 ± 0.920	3.18 ± 0.18
Dill	11.08 ± 0.880	5.08 ± 0.74
Purslane	15.51 ± 0.780	2.88 ± 0.13

literature, it is possible to say that the concentrations of Cu^{2+} and Ni^{2+} in vegetable samples were close to each other.

The range of standard levels of Cu^{2+} in soil and water was 5.0–5.6 mg/kg and 0.3–40 mg/L, respectively. The range of Ni^{2+} values in soil and water was 10–50 mg/kg and 100–6800 mg/L, respectively (Türkdoğan et al. 2002; Maris et al. 2000; Ramos et al. 1999). The mean metal concentrations in the Tigris River are generally lower than the estimated world averages (Table 6) of metal concentrations in other regions of world and even lower than assumed metal background concentrations determined for industrially developed countries (Lithner 1989).

Cu^{2+} and Ni^{2+} concentrations may be explained by natural sources and processes. In water samples (Table 6), mean Cu^{2+} and Ni^{2+} levels were lower than the standard levels. The results in Table 4 indicate a high degree of contamination in soils from the cultivated sites of the Tigres River, when compared with the standard values. The highest concentration of Cu^{2+} was found in the soil sample.

Total contents of heavy metals in soil (Table 4) were higher than those reported in earlier similar studies (Gil, Boluda, and Ramos 2004). In this context, high values of Cu^{2+} and Ni^{2+} obtained in horticultural soils may be due to an excess use of agrichemicals, such as fungicides with Cu^{2+}, often applied by farmers in the agricultural soils of Southeast Turkey, especially by irrigation, which is the main management system applied to horticultural soil. The variation in the metal contents observed in soil samples may also depend on the physical and chemical nature of the soil. The results presented in Table 4 suggest a high bioavailability of heavy metals in the soil of the study areas.

CONCLUSION

SPE method based on the use of *B. subtilis*–immobilized Amberlite XAD-16 as biosorbent was developed for the preconcentration of Cu^{2+} and Ni^{2+}. Metal concentrations were determined by ICP-OES. By applying the developed method to 200.0 ml of initial sample volume, the preconcentration factor was found to be 40.0 in case of final volume of 5.0 ml. Thus, sensitivities of conventional ICP-OES for Cu^{2+} and Ni^{2+} were improved as 36.4 and 38.0 times for Cu^{2+} and Ni^{2+} by SPE-ICP-OES method. The method was validated through the analysis of certified reference tea sample. The result obtained from validation studies agreed with certified values for Cu^{2+} and Ni^{2+}. The concentrations of Cu^{2+} and Ni^{2+} in vegetable samples were determined by ICP-OES after the developed method was applied. It is recommended from our results that further investigations are needed to control the safety of food chain and reduce the exposure to heavy metals.

TABLE 6 Heavy Metals in the Water and Soil Sampled at Sites of the Tigris River

Site	Cu^{2+} (mg/L)	Ni^{2+} (mg/L)	Cu^{2+} (mg/kg)	Ni^{2+} (mg/kg)
Hevsel	0.027	0.012	134.70	84.97
Ongözlü	0.015	0.013	36.00	95.18
Dicle Köprü	0.019	0.013	152.20	96.84
Mean ± *SD*	0.0203 ± 0.0035	0.0127 ± 0.00033	107.63 ± 36.17	92.33 ± 3.71

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