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Araştırma Makalesi/Research Article

Preconcentrations of Cd(II) And Cu(II) by Solid Phase Extraction on Thermophilic *Bacillus firmus* Loaded Amberlite XAD-4 Prior to Their Determinationsin Food Samples by ICP-OES

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Abstract: It is well known that sensitivity of ICP-OES is not enough when ultra trace levels are concerned. From this perspective a new method was developed for the determinations of Cd(II) and Cu(II). It was based on the use of thermophilic *Bacillus firmus* loaded Amberlite XAD-4 as a biosorbent for solid phase extraction. The optimum pH, flow rate, eluent concentration and volume, amount of biomass and amount of Amberlit XAD-4 were found as 5.5, 3 mL min^{-1}, 5 mL 1 mol L^{-1} of HCl, 250 mg of biomass and 750 mg of Amberlite XAD-4, respectively for recovery of tested metal ions. Preconcentration factor was achieved as 35 for Cd(II) and 50 for Cu(II). Resuability of the column were tested and same column could be used for 35 cycle. LODs were calculated as 0.09 and 0.08 ng mL^{-1}, respectively for Cd(II) and Cu(II). RSDs were found as lower than 5.9%. The developed method was successfully applied to food samples to determine analyte concentrations.

Keywords: Solid phase extraction; biosorption; preconcentration; Cd; Cu

AMBERLİTE XAD-4 ÜZERİNE TUTTURULMUŞ TERMOFİLİK *Bacillus firmus*'DE KATI FAZ EKSTRAKSİYON İLE Cd(II) VE Cu(II)'IN ICP-OES İLE GIDA NUMUNELERİNDE TAYİN EDİLMEDEN ÖNCE ÖNDERİŞTİRİLMESİ

Özet: Eser elemenler söz konusu olduğunda ICP-OES'in hassasiyetinin yeterli olmadığı iyi bilinmektedir. Bu bakımdan Cd(II) ve Cu(II)'nin belirlenmesi için yeni bir metod geliştirilmiştir. Amberlit XAD-4 üzerine tutturulmuş termofilik *Bacillus firmus* temel baz alınarak katı faz ekstraksiyon için biyosorbent olarak kullanılmıştır. Test edilen metal iyonlarının geri kazanımı için optimum pH, akış hızı, eluent konsantrasyonu ve hacmi, biyokütle miktarı ve Amberlit XAD-4 miktarı sırasıyla; 5.5, 3 ml dk^{-1}, 1 mol L^{-1} 5 mL HCl, 250 mg ve 750 mg olarak tespit edildi. Prekonsantrasyon faktörü Cd için 35 ve Cu için 50 olarak elde edildi. Kolonun geri kullanımı test edildi ve aynı kolon 35 defa kullanılabilir. LODs Cd ve Cu için sırasıyla 0.09 and 0.08 ng mL^{-1} olarak hesaplandı. RSDs'nin %5.9'dan daha düşük olarak bulundu. Geliştirlen yöntem gıda örneklerindeki analit konsantrasyonlarının belirlenmesi için başarılı bir şekilde uygulandı.

Anahtar Kelimeler: Katı faz ekstraksiyonu; biosorpsiyon; prekonsantrasyon; Cd; Cu

Introduction

The rise in industrial employments has become environmental contamination troubles and the disruption of various ecosystems with accumulation of toxic metals.The potential hazards of metal ions contamination on the bio-environment have caused intensive researches over last decades.Several metals are necessary for life at too low concentrations (i.e., copper), whereas several metals such as Cd, Pb, Hg, etc are hazard even at low levels and have a direct and reverse effect on different biological processes (Dasbası et al., 2015).Diet andenvironmental, agricultural and industrial activities are the most important sources of heavy metals for human(Marahel et al., 2011).Therefore, the determination of metal ions at trace concentrationsplays a progressivelysignificant role in environment, industry, food, agriculture, etc. However, the direct detection of metal ions in natural samples still face several limitations because of their tracelevel and matrix interferences (Zhang, 2013).

This limitation problem can be solved by utilize of a separation-enrichment procedures before instrumental analysis, if the sensitivity of the instrument is not sufficient for the metal ions (Ozdemir et al. 2012). Various methods such as are used liquid–liquid extraction, precipitation, membrane filtration, coprecipitation, cation-exchange resins, cloud point extraction, and solid phase extraction, for the separation and preconcentration of metals (Ozdemir et al. 2012, Tuzen et al. 2008).Within the different preconcentration processes, SPE is one of the most influential technics because it ensures more flexible study conditions together with well selectivity, stability, affinity, high concentrating capability, and capacity for metal ions (Ozdemir et al. 2010). There are various kinds of immobilization matrices utilized for all cells examined today. The significant immobilization matrices utilized in biomass immobilization contain alginate, Sepabeads SP 70, sodium alginate, Chromosorb 101, Sepiolit, Diaion SP-850, Silica gel, pumice stone, multiwalled carbon nanotubes, polysulfone, TiO_2 nanoparticles, polyurethane, polyacrylamide and Amberlite XAD resins, (Ozdemir et al. 2013a). Among the Amberlite resins, the Amberlite XAD-4 resin has excellent physical features such as durability of acids, bases, and oxidizing and reducing agents, large surface area, (>750 m^2 g^{-1}), well-characterized medium size (0.49–0.69 mm) and porosity (Hoque et al. 2015).

The main objective of present study was to develop a solid phase sorbent based on the use of bacterial sources. From this perspective thermophilic *Bacillus firmus* loaded onto Amberlite XAD-4 was employed for the preconcentrations of metal ions. It was observed that prepared biomass loaded sorbent had high affinity to Cd(II) and Cu(II) ions. Thus, important experimental parameters were investigated in details prior to their determinations in real samples by ICP-OES.

Materials and Method

Instrumentation and chemicals

Cd(II) and Cu(II) concentrations were determined by PerkinElmer Optima™ 2100 DV (PerkinElmer, Inc., Shelton, CT, USA) (ICP-OES) at 228.804 nm and 327.393 nm respectively. The operating conditions of the ICP-OES were reported in our previous paper (Özdemir et al., 2012).Mettler Toledo MPC 227 (Polaris Parkway, Columbus, OH, USA) digital pH meter was employed to measure the pHs of the solutions. SPE experiments were performed by using filtration column (1.0 cm × 10.0 cm), equipped with polypropylene frites. Watson-Marlow 323 peristaltic pump (Cornwall, England) was used to obtain controlled flow rate for solutions.The spectra were recorded on a Perkin-Elmerinfrared spectrometer as KBr pellets with a resolution of4 cm^{-1} in the range of 400–4000 cm^{-1}.

Certified solutions of Cd(II) and Cu(II) at 1000 µg mL^{-1}were used as stock (High Purity Standards, Charleston, SC, USA). The standard at lower concentrations were prepared by diluting the stock solutions. High purity reagents were used through the experiments. Doubly distilled water was used for all experimental studies. All glass materials were kept permanently full of 1.0 mol L^{-1} of nitric acid when not in use. Concentrated HNO_3 (65%), H_2O_2 (35%), HCl (36.5-38.0%), NH_4OH, $FeCl_36H_2O$ and $FeCl_2$ were supplied from (Sigma Aldrich, Germany). Certified and standard reference samples of fortified water (NWTM-15, High Purity Standard), dogfish muscle (DORM2 National Research Council of Canada), tea leaves (NCSDC 73014China National Analysis Center for Iron and Steel) and simulated fresh water NIST 1643e(NIST) obtained from a local distributor were used to validate the method.

Growth of Thermophilic *Bacillus firmus*

In this research study, *Bacillus firmus* was isolated from mud in spring of Omer, Afyonkarahisar,Turkey. The bacteria was identified by biochemical andmorphological tests and 16S rRNA analyses in OINTEK, ITU, Istanbul, Turkey. Thermophilic *Bacillus firmus*was cultured in autoclaved 1000 mL glass flaskscontaining 250 mL Nutrient Broth (NB.Allglass flasks were inoculated with one-night culture of 6.5 mL cell suspension and were then incubated on shaker at 60 °C and 120 rpm for 24 hours.

Preparation of the Powdered Dried Dead *Bacillus firmus* and loadedBiomass

The culture media were centrifuged at 7000 rpm for 10 min after 24 h incubation. Upper solution were removed and the pellets were then cleaned two times with 0.9 % NaCl. The cleaned pellets dried in an oven 80 °C for 24 h. To get a fine powder, dried cells were ground in a porcelain mortar and then were autoclaved at 121°C for 15 min to assess complete death of the dried cells. The autoclaved cells were cultured into NB medium and the absence of any growth showed positive results (whole death of the cell). 200 mg dried powder B. *firmus* was stirred with 750 mg of Amberlite XAD-4 and 5 mL of distilled water and then, thoroughly mixed. Solid phase extraction column study was experimented from our previous study (Ozdemir et al. 2015).

General Sorption Studies

Initial solid phase extractions were applied to different cations; Cd(II), Co(III), Cu(II), Pb(II), Ni(II), Fe(II), Zn(II) and Mn(III). By considering the results, further studies focused on Cd(II) and Cu(II) that recovery values of other cations were clearly lower than Cd(II) and Cu(II). Model solutions consisting of a 50.0-mL mixture of Cd(II) and Cu(II) at the concentration of 10.0 ng mL^{-1} were prepared and the pH of the solutions were adjusted to desired value by adding the required amounts of HCl and NH_3. The model solution was passed through the prepared SPE column at an adjusted flow rate with a peristaltic pump. Then, 10.0mL distilled water was passed through the column. The retained metal ions on the column were then eluted with 5.0 mL of 1.0 mol L^{-1}HCl. ICP-OES was used to determine their concentrations in eluate.

Loading Capacity

Batch equilibrium method was applied to 50.0 mL of 100.0 mg L^{-1} of Cd(II) and Cu(II) solution at pH 5.5. 100.0 mg *Bacillus firmus* loaded Amberlite XAD-4 was added to this solutions for 120 minutes at 25^{o}C shaker at 120 rpm. The dried powdered dead cells were then separated by centrifugation at 10000 rpm for 10 minutes. The concentrations of the Cd(II) and Cu(II) in supernatant were directly measured by ICP-OES. The remaining pellet was digested in concentrated HNO_3 acids before measurement. The amount of biosorbed Cd(II) and Cu(II) was calculated according to literature(Ozdemir and Kilinc 2012):

Sample Preparation

pH of the 100 mL of tap water (it was sampled to 1.0 L glass bottle after flushing 1.0 min) was adjusted to 5.5 and developed method was directly applied. Mineral water sample was bought from local market and 5 of its were mixed to obtain homogenous sample. 100 mL portion of mineral water was subjected to method. Black and green teas, potato, tomato cabbage, carrot, walnut, olive, pepper, spinach, rice, cherry and apple juices, biscuit and honey samples were bought from local market and digested by microwave oven (Berghof MWS3 - Berghof, Tubingen, Germany). 1.0 g portions of samples (5.0 g for honey samples) were weighed. 5.0 mL of HNO_3:HCl (1:1, v/v) was added and the mixture heated on a hot plate. It was evaporated until dryness and 6.0 mL of HNO_3:HCl:H_2O_2 (1:1:0.2, v/v/v) was added before transferring to a microwave vessel. They were heated to 170 °C by microwave irradiation and waited for 5.0 min. Then temperature was reached to 200 °C in 15 min and waited for 1.0 min. It was decreased to 100°C and waited 20 min. After digestion the final volume was made up to 50.0 mL volume and the pH adjusted to the desired value before the SPE procedure. The SPE procedure was directly applied to a 50.0-mL portions of fortified NWTM-15 and NIST1643e water sample certified water samples (LGC, Middlesex, UK) after pH adjustment.A 0.5 g portion of certified reference NCSZC 73014 tea leaves and dogfish muscle (DORM2) samples were digested using the same method described for the above mentioned samples.

Results and Discussion

Effect of pH

The acidity of solution has two impacts on biosorption of metal ions: at lower pH, proton may be protonated the binding group of chelating molecules and at higher pH, hydroxide can be binded and precipitated the heavy metals (Tu et al. 2009). For these reasons, pH ranges of sample solution is primary factor to be optimized. In order to test the influence of various solution pH's, were experimented from 2.0 to 8.0 for recoveries of Cd(II), Co(II), Cu(II), Pb(II), Ni(II), Fe(II), Zn(II) and Mn(II) (Fig. 1.). Results for Pb(II), Ni(II), Fe(II) and Zn(II)ions were not presented in Fig. 1 that recovery values were lower than 10%. When the pH degree decreased from 4.0 to 2.0, the recovery percentages of Cd(II), Co(II), Cu(II) and Mn(II) were reduced from 87.9% to 51.3%, 24.8 to 7.4%, 93.6% to 54.9% and 38.7 to 12.6, respectively. Besides, when the pH value rised from 6.0 to 8.0, the recovery percentages of all tested metal were also decreased. The optimum pH was determined as 6.0, 6.0, 5.0 and 5.0 for Cd(II), Co(III), Cu(II) and Mn(II), respectively.

As the recovery values of Co(III) and Mn(II) was low, recoveries ofCo(II) and Mn(II)were not experimented.In the following studies, pH 5.0 and 6.0 were selected the most suitable pH values for recovery of Cd(II) and Cu(II), respectively. By performing further experiments at pH 5.5, it was decided to continue future experiments at pH 5.5 that results were quantitative, >95%.

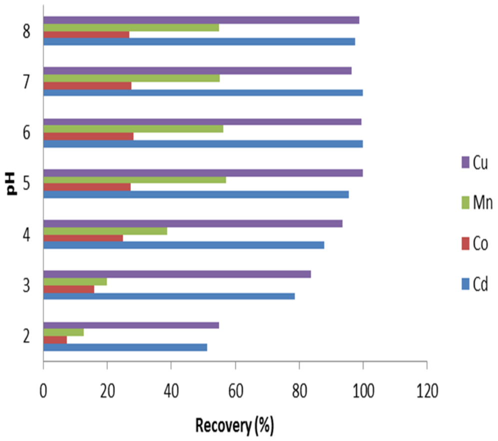


Fig. 1. Effect of pH on the recoveries of Cd(II) and Cu(II)

Effect of flow rate

The sample solution flow rate can also affect the recovery of heavy metal by biosorbent. Mass transfer from the sample solution to the binding groups on the surface wall of biosorbent is influenced by the solution flow rate and must be checked for the yield of biosorption process (Ozdemir and Kilinc, 2012) and biotechnological approach. Therefore, the impact of the solution flow rate on the retention of Cd(II) and Cu(II) was tested at pH 5.0 and 6.0, respectively at different flow rate from 1.0 to 6.0 mL min^{-1}. Results were given in Fig. 2. The retention of Cd(II) and Cu(II) did not affect significantly up to flow rate of 3 mL min^{-1} for both metal ions. When the solution flow rate was increased over 5 mL min^{-1}, the percentage of biosorption yield decreased to a significant extent. Further studies flow rate of 3 mL min^{-1} was applied for both heavy metals.

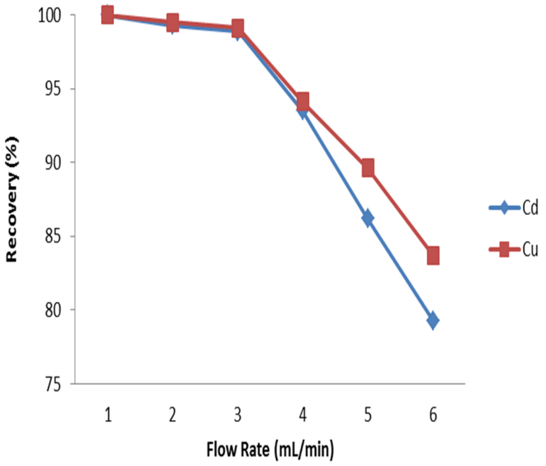
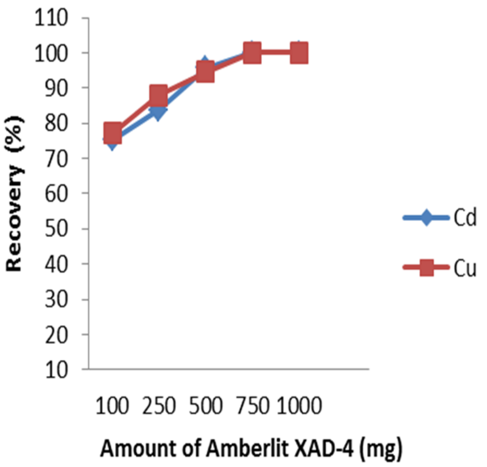


Fig. 2. Effect of flow rate on the recoveries of Cd(II) and Cu(II)

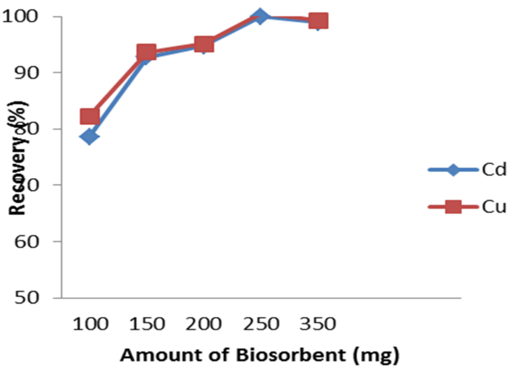
Effect of the biosorbent and resin concentrationsfor recovery of Cd(II) and Cu(II)

The concentration of biosorbent is an important parameter for removal of pollutants in solid phase extraction studies (Ozdemir et al. 2013b). In this research study, by rising concentration of bacterial biomass the recovery percentage rised, because of rising in the surface field and accessible binding groups to the biosorption of heavy metals. However, increase in biosorbent concentration cause interference between active binding groups, so reducing the metals recovery (Yahaya and Don, 2014). As represented in Fig 3a, the recovery of Cd(II) and Cu(II) were increased up to 250 mg. When the biosorbent amount increased from 250 mg to 350 mg, recovery of Cd(II) and Cu(II) were decreased 100% to 98.9% and 100% to %99.3, respectively.

Paralel findings were indicated for Cd(II) and Cu(II) on immobilised *P. sanguineus* by Yahaya et al, (2009) and Mashitah et al. (2008). The concentration of resin (XAD-4) is the another significant factor for heavy metal recovery in SPE experiments. The resin concentration was experimented from 100 to 1000 mg. The recovery percentage of Cd(II) and Cu(II) were increased until 750 mg of XAD-4 (Fig 3b), however further addition did not show any effect on the retention of Cd(II) and Cu(II). In further experiments, 250 mg of dried *B. firmus* and 750 mg of XAD-4 was used as biosorbent and solid matrix, respectively.



a.



b.

Fig. 3. a Effect of amount of biosorbent on the recoveries of Cd(II) and Cu(II) **b.** Effect of amount of Amberlite XAD-4 on the recoveries of Cd(II) and Cu(II)

Elution study

Elution processes are utility to reusage the biosorbent and retention heavy metals, and also contribute to explain the natura of the biosorption study (Ziaei et al, 2014).On the other hand elution process must be experimented to minimize the damaging of immobilized biosorbent. Different volume and concentration of HCl (1 mol L^{-1}) and HNO_3(1 mol L^{-1}) were used to detect of suitable elution volume and concentration. Table 1 demonstrates the recovery percentage of Cd(II) and Cu(II) by using various volume and concentration of HCl and HNO_3. When 1 mol L^{-1} 5 mL HCI was passed through the SPE column at 3 mL min^{-1}, the maximum recovery percentage was obtained for Cd(II) and Cu(II).

Table 1. Optimization of elution conditions on the preconcentrations of Cd(II) and Cu(II).

Eluent Type	Volume (mL)	Concentration (mol L^{-1})	Recovery^{a} (%)	
			Cd(II)	Cu(II)
HCl	3	0.5	91.8±0.2	91.1±0.4
	5	0.5	96.6±0.7	95.8±0.3
	3	1	95.9±0.6	95.1±1.0
	5	1	100.0±1.1	100.1±0.8
HNO_3	3	0.5	87.8±0.4	87.1±0.7
	5	0.5	95.4±1.3	95.1±0.6
	3	1	93.1±0.5	92.4±0.9
	5	1	97.8±0.8	97.3±0.5

Sample volume

Sample volume is a important factor to get reliable and reproducible analytical results and high preconcentration factor. In addition to these, natural samples particularly contain too low amounts of the metal ions, so the highest suitable sample volume must be determined in the solid-phase extraction studies (Li et al. 2009). To detect the highest sample volume, various volume of metal solutions passed through to SPE column under the optimum conditions. The impacts of sample volumes on the retentions of Cd(II) and Cu(II) are seen in Fig 4. The results indicated that the highest sample volume can be up to 175 and 250 mL with the retention >95%, for Cd(II) and Cu(II), respectively. Hence the elution volume was 5 mL, the preconcentration factors was found as 35 and 50 for Cd(II) and Cu(II), respectively.

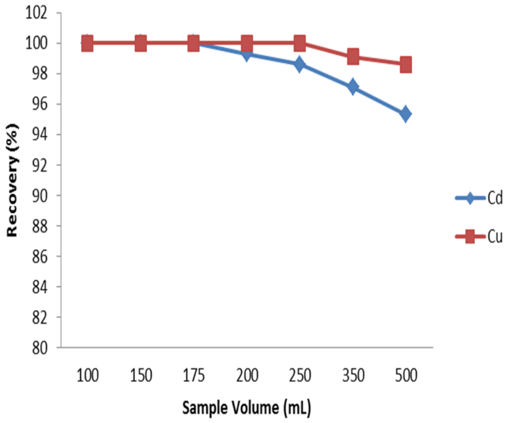


Fig. 4. Effect of sample volume on the recoveries of Cd(II) and Cu(II)

Regeneration times of the immobilized thermophilic *B. firmus*

The biosorption studies presents different superiority, one of which is its capability to reusage and decreasing operation costs in SPE processes. Reusage processes also as indicators on whether a biomass could be safely disposed of into the environment. For this reason, it is significant to regenerate biomass after utilizing the same experiment for metal biosorption (Vijayaraghavan and Yun 2008). Fig. 5 is the dependence of retention of Cd(II) and Cu(II) on the regeneration times of the immobilized thermophilic *B. firmus*. As can be observed, when the 1 mol L^{-1} HCI was used as eluent, the immobilized thermophilic *B. firmus* can be reused for more than 35 times without clear reduce of the retentions of Cd(II) and Cu(II).

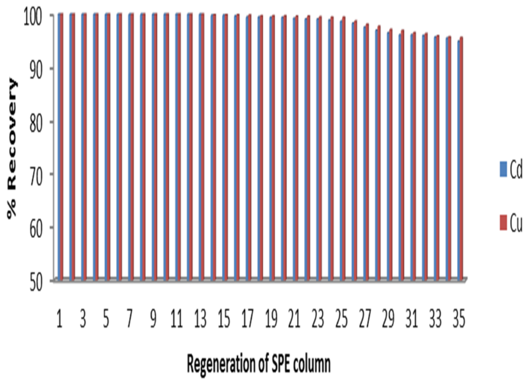


Fig. 5. Reusability of *firmus* loaded Amberlite XAD-4 SPE column on the recoveries of Cd(II) and Cu(II)

Analytical figures of merit

Analytical features of bacteria loaded sorbent is also dependent on its surface structure. Because affinity of metal to surface functionalitiy of the sorbent effects the its. FT-IR was employed to investigate the surface structure. It was comparetively presented in Fig. 6. From Fig 6c, it was concluded that coating of bacteria with resin was achieved by considering the spesifics peaks. After interaction of Cd(II) and Cu(II), no different peaks were observed. Main differences were shifting the signals as approximately 10 cm^{-1} that it could be attributed to complexation of metal cations with surface functional groups.

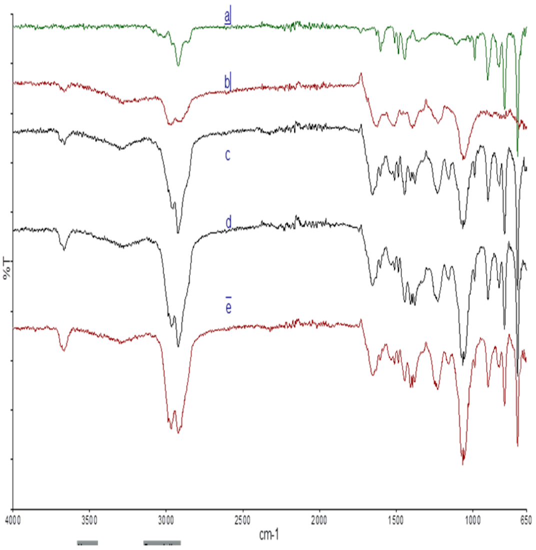


Fig. 6. FT-IR spectral comparison of a. Amberlite XAD-4, b. *firmus,* c. B.s *firmus* on Amberlite XAD-4, d. Cu(II) loaded on B. *firmus*-Amberlite XAD-4, e. Cd(II) loaded on *B. firmus*-Amberlite XAD-4

Analytical figures of merit were summarized in Table 2. Linear calibration curves for Cd(II) and Cu(II) were achieved under the optimized experimental conditions. Linearity was within the range of 0.4–20 ng $mL^{-1}$for Cd(II), 0.6–30ng mL^{-1} for Cu(II) with correlation coefficient as 0.9989 and 0.9992, respectively. LOD and LOQ are defined as LOD=3Sd/m and

LOQ=10SD/m, where sd is the standard deviation (SD) of ten replicate application of the method to the lowest concentrations in the linear range for both of metals. LODs were 0.09 and 0.08 ng mL^{-1} for Cd(II) and Cu(II), respectively.RSDs were calculated as 5.9% and 3.3%, respectively for 1.0 ng mL^{-1} of Cd(II) and Cu(II).

Table 2. Analytical characteristics of the developed method

Parameter	Cd	Cu
LOD, ng mL^{-1}	0.09	0.08
LOQ, ng mL^{-1}	0.29	0.26
Linearrange, ng mL^{-1}	0.4-20	0.6-30
RSD[1]	5.9	3.3
r^2	0.9989	0.9992
PF[2]	35	50

[1] Calculated for1.0 ng mL^{-1} of Cd(II) and Cu(II).
[2] Preconcentraton factor

On the spectroscopic detection of metal ions, very high saline solutions are influenced by the analyte grades, this is described as ‘‘Matrix effect’’ (Soylak and Topalak, 2015).The influences of matrix ions such as Na(I), K(I), Ca(II), Mg(II), Zn(II), Fe(II), Co(II)’ Ni(II) and Al(III) exist in water and food samples on the retention of Cd(II) and Cu(II) were also experimented. The results are represented in Table 3. The matrix ions generally exist in this samples did not exhibit interference effects more than ±5% of the absorbance change. The experimental results display that the suggested separation/preconcentration method can be used to different water and food samples for the determination of Cd(II) and Pb(II).

Comparison of analytical characteristics of the methods developed for the preconcentrations of Cd(II) and Cu(II) were summarized in Table 4 in view of LOD, preconcentration factor and linear range. The developed method offers aneasy procedure with high preconcentratin factor rapid and inexpensive.It was based on the use of bacterial biomass that could be easily grown on high amount.

3.8. Application to real sample

Before application to real sampels, to validate the method, it was applied to certified reference fortified and simulated waters, tea leaves and dogfish muscle samples. As can be seen in Table 5, good

Table 3. Effect of metal ions on recoveries of Cd(II) and Cu(II)

Ion	**Interfericion to metal ion ratio**	**Recovery[a] (%)**	
		Cd (II)	**Cu(II)**
Na(I)	7500	97±0.8	98±1.3
K(I)	7500	98±0.4	99±0.7
Ca(II)	100	99±1.2	100±1.6
Co(II)	10	100±0.9	96±0.9
Fe(II)	150	98±1.4	97±0.8
Mg(II)	250	96±1.0	98±1.0
Ni(II)	10	97±1.3	97±1.2
Zn(II)	10	98±0.6	99±0.7
Al(III)	10	96±0.7	97±1.3

[a] Concentrations of the Cd(II) and Cu(II) ions are 10 µg L^{-1}.

correlation was achieved between the certifid and determined values Therefore, it could be concluded that the recommended bacteria loaded Amberlite XAD-4 sorbent could be used as a reliable solid phase extractant with satisfactory results. Then, concentrations of Cd(II) and Cu(II) in black and green tea, cow and goat milk, tap and mineral water, cherry and apple juice, potato, tomato, cabbage, carrot, walnut, olive, pepper, spinach, rice, biscuit and honey samples were determined after developed method was applied. Results were presented in Table 5. Cd(II) concentrations in green tea, cow and goat milk, tap and mineral water, apple and cherry juice, olive, pepper, biscuit and honey samples were found lower than detection limits. Among them green tea, goat milk, tap water, apple juice, pepper and honey samples were spiked with known amounts of analytes. Spiked amounts were quantitatively determined after developed method was applied.

Table 4. Comparison of analytical characteristics of the preconcentrations methods for Cd(II) and Cu(II)

Method	**Instrument**	**LOD, ng mL^{-1}**		**PF^1**		**Linear range, ng mL^{-1}**		**Ref.**
		Cd(II)	**Cu(II)**	**Cd(II)**	**Cu(II)**	**Cd(II)**	**Cu(II)**	
Batch method on magnetic metal–organic Framework nanocomposite sorbent	FAAS	0.12	-	250	-	0.4-80	-	Hassanpour et al., 2015
Magnetic graphene-dispersive solid-phas eextraction	FAAS	0.16	-		-	0.5-90	-	Ezoddin et al., 2015
SPE on 1-(2-pyridylazo)-2-naphthol-Functionalized organic–inorganic mesoporous material	FAAS	0.04	-	367.5	-	0.1-40	-	Fouladian et al., 2015
SPE on diphenylcarbazone/sodium dodecyl sulfate immobilized on magnetic nanoparticle Fe_3O_4	FAAS	3.71	-	100	-	10-500	-	Mirabi et al., 2015
SPE on *Pleurotus eryngii* loaded Amberlite XAD-16	ICP-OES	0.67	-	50.7	-	1-50	-	Ozdemir et al., 2012
SPE on magnetic Fe_3O_4@C nanoparticles	FAAS	-	1.5	-	50	-	4-400	Samadi and Amjadi 2015
SPE on amino-functionalized Fe_3O_4graphene oxide nanocomposite	FAAS	-	0.9	-	95	-	1-100	Bahar and Karami 2015
SPE on multiwalled carbon nanotubes	FAAS	1.7	2.3	50	50	-	-	Yilmaz and Soylak 2014
SPE on SBA-15 nanoporous silica with dithizone.	FAAS	0.12	0.39	100	100	1-120	1-130	Behbahani et al., 2013
SPE on AEDHB-SG as sorbent	ICP-OES	0.012	0.098	100	100	0.5-4	0.5-4	Durduran et al., 2015
SPE on sorbents equentially modified with polyhexamethylene guanidine and 2-(1,8-dihydroxy-3,6-disulfo-2-naphthylazo)benzene arsonicacid	ICP-OES	0.44	0.31	20	20	-	-	Losev et al., 2015
SPE on multiwalled carbon nanotubes and quinalizarin	FAAS	0.65	0.30	100	100	-	-	Gouda 2014
SPE on *Bacillus firmus* loaded onto Amberlite XAD-4	ICP-OES	0.09	0.08	35	50	0.4-20	0.6-30	Thismethod

1 Preconcentration factor

Table 5. Determinations of Cd(II) and Cu(II) in samples

Samples	**Cd,** mg kg^{-1}	**Cu** mg kg^{-1}
NCS ZC73014, certified	0.062±0.004	18.6±0.7
NCS ZC73014, founded	0.061±0.003	18.6±0.5
NIST 1643e, certified	6.568±0.073[1]	22.76±0.31[1]
NIST 1643e, founded	6.48±0.056[1]	21.99±0.43[1]
DORM2, certified	0.043±0.008	2.34±0.16
DORM2, founded	0.042±0.004	2.30±0.19
NWTM-15, certified	13.2[1]	18.3[1]
NWTM-15, founded	13.1±1.1[1]	18.3±1.6[1]
Black tea	0.20±0.01	9.2±0.8
Greentea	<LOD	6.8±0.4
Green tea[2]	0.098±0.004	6.9±0.4
Cowmilk	<LOD	0.43±0.03
Goatmilk	<LOD	3.9±0.2
Goat milk[2]	0.096±0.006	4.0±0.3
Tap water	<LOD	3.8±0.02[1]
Tap water[2]	0.099±0.9	13.5±0.9
Mineral water	<LOD	12.1±0.9[1]
Cherryjuice	<LOD	126±10[1]
Apple juice	<LOD	88±6.4[1]
Apple juice[2]	0.099±0.6	186±11.1
Potato	0.045±0.002	4.8±0.3
Tomato	0.020±0.001	10.9±0.8
Cabbage	0.090±0.006	1.8±0.1
Carrot	0.043±0.004	4.3±0.2
Walnut	0.023±0.001	12.7±1.1
Olive	<LOD	9.0±
Pepper	<LOD	8.6±0.9
Pepper[2]	0.095±0.007	8.7±0.6
Spinach	0.32±0.02	4.9±0.03
Rice	0.11±0.01	4.3±0.03
Biscuit	<LOD	1.5±0.1
Honey	<LOD	0.64±0.04
Honey[2]	0.098±0.005	0.74±0.05

[1] ng mL^{-1}

[2] Spiked with known amounts of analytes to give final concentrations as 0.1 mg kg^{-1}forCd(II) and 0.1 mg kg^{-1} for Cu(II) (except tap water, it was spiked with 10 ng mL^{-1}of Cu)

4. Conclusion

In this work, an alternative sorbent of composed of *B. firmus* loaded Amberlite XAD-4 was developed. It was used for the simultaneous preconcentrations of Cd(II) and Cu(II). By applying the SPE method, sensitivity of the ICP-OES was improved as 35 and 50 times respectively for Cd(II) and Cu(II). Appropriate figures of merit were obtained with low RSD. The developed method was validated through the analysis of certified reference materials with satisfactory results. Cd(II) and Cu(II) concentrations in real samples were determined after developed method was applied before ICP-OES.

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