

Phallus impudicus loaded with γ -Fe₂O₃ as solid phase bioextractor for the preconcentrations of Zn(II) and Cr(III) from water and food samples

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ABSTRACT

We investigated the application of fungus *Phallus impudicus* loaded γ -Fe₂O₃ nanoparticles as a biosorbent for magnetic solid phase extractions of trace levels of Zn(II) and Cr(III) ions from natural samples before their measurements by inductively coupled plasma optical emission spectrometry. The characterization of magnetized *P. impudicus* was performed using the scanning electron microscope, the energy dispersive X-ray and Fourier transform infrared spectroscopy. Important experimental factors were investigated. The experimental results fitted well to the Langmuir adsorption model and pseudo-second order kinetic model. Limit of detections of targeted ions by magnetic solid phase extraction method based on use of *P. impudicus* were found as 10.5 ngL⁻¹ and 12.6 ngL⁻¹ respectively for Cr(III) and Zn(II). The sorption capacities of the biosorbent were 22.8 mgg⁻¹ for Cr(III) and 25.6 mgg⁻¹ for Zn(II). The preconcentration factors were achieved as 100 for both of ions. RSDs for inter- and intraday precisions were found as lower than 2.0% and 2.1% respectively for both of targeted ions. The accuracy of the recommended process was tested by recovery measurements on the certificated reference materials and successfully applied for quantification recoveries of Cr(III) and Zn(II) ions from water and food samples.

1. Introduction

Due to the toxicity of some metal cations on human health, their removing even at trace levels from an aqueous environment is an important issue. Since human economy has been developing very rapidly, uncontrolled usage of chromium (Cr) and its compounds in various fields such as dyeing, leather tanning, metal surface treatment, chrome mining result in water pollution [1]. Cr has two stable oxidation states including III and VI. Although they are commonly found in nature water, toxicity of these two species is not similar. It has been found experimentally that Cr(VI) is much more toxic than Cr(III) [2]. Cr(VI) is known to be one inducer of skin dermatitis. Moreover, it is considered as carcinogenic and mutagenic [3,4].

Zinc (Zn) is a very important essential element for all living organisms, as it is involved in crucial biochemical events, and its chemicals show great disinfectant efficiency [5,6]. However, when overexposed, Zn can result in major harms in the human body such as distortions in energy metabolism or increased oxidative stress [7].

Several important techniques such as ion-exchange, extraction (cloud point, solid phase, liquid-liquid), adsorptive stripping voltametry, coprecipitation [8–13] have been employed to removing of Cr and Zn ions from an aqueous medium. Among them, solid phase extraction (SPE) is the most preferential method that provides high efficiency, low operational cost, applicability to organic and inorganic pollutants [14,15]. Several agents such as activated carbon [16,17], silica gel [13], biosorbents, activated alumina [18], carbon nanotubes and nanoparticles (NPs) [19] have been used for preconcentrations of toxic metals. Magnetic nano-materials, mainly iron oxides have prominent properties, such as easy preparation, low toxicity, low cost and separability by an external magnetic field which lead to their use in the SPE methods in recent years [20–23]. However, raw magnetic nanoparticles (MNPs) have several drawbacks such as easy oxidization by the surrounding environment and the ability to leach into a low pH medium. MNPs may aggregate in solutions because their surface energy and specific surface area are higher, which obviously decrease their sorption capacity and limit their range of applications [24]. Loading by sorbent from the biological origin is an

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alternative way to solve the problem.

Recently, one more noteworthy future of $\gamma\text{-Fe}_2\text{O}_3$ and Fe_3O_4 , magnetism, was actualized and then, used for environmental remediation. At laboratory scales for removal of toxic metals from contaminated water, magnetic separation was combined with adsorption by taking advantage of the magnetic properties of these adsorbents. Since it overcomes a variety of problems related to centrifugation, filtration, gravitational separation, and reduces energy to carry out separation, magnetic separation is especially desirable in industry.

Biomass such as algae, fungus, bacteria have been used as biosorbent for SPE technique to removal of the toxic metals at trace levels. Silica, nanoparticles, resins etc. have been broadly used as support materials for biosorbents. In a recent article, benefits of fungus-based SPE technique for the preconcentrations of metal ions were examined in a detailed manner [25]. Major advantages of the fungus-based SPE methods include reducing the expending of the number of chemicals and analysis time, low limit of detections (LOD), requesting to use of reusable sorbents, sensitivity, and selectivity.

The present study investigates the usability of MNPs that presents over features such as high specific surface area, reproducible nanometer dimensions, easy to separate by a magnet. Also, a fungus as biomass, *P. impudicus* were immobilized onto MNPs and used to investigate the magnetic solid phase extractions (MSPE) of Zn(II) and Cr(III) in water and food samples. In the literature, there are very few articles on fungal biomass and MSPE of heavy metals, especially *P. impudicus*. These properties of *P. impudicus* immobilized MNPs make them highly attractive for the removal and preconcentration of metal cations.

2. Material and methods

2.1. Instrumentation

A Perkin Elmer Optima™ 2100 DV inductively coupled plasma optical emission spectrometry (ICP-OES) (Shelton, CT, USA) was used to measure the concentrations of Zn and Cr at 206.200 nm and 267.716 nm, respectively. pH of the solutions was measured by using digital pH meter (Mettler Toledo MPC 227). For SPE experiments, filtration columns (10 mm × 100 mm) with polypropylene frits were used. A peristaltic pump (Watson Marlow 323, Wilmington, MA, USA) was used to adjust to desired flow rate values of the solutions. Surface structures of the prepared biosorbent were investigated by the Fourier transform infrared (FT-IR) spectroscopy (Perkin-Elmer Spectrum 400, Waltham, MA, USA) and Leo 440 scanning electron microscope- energy dispersive X-ray (SEM-EDX). SEM was operated at 20 kV, 400 pA. The analysis was applied under convenient magnification and increasing voltage.

2.2. Reagents and solutions

1.0 mg mL⁻¹ stock Zn(II) and Cr(III) solutions were used for the preparation of working standards by diluting (High-Purity Standard Inc.). All the chemicals used were high purity analytical grade unless otherwise stated. Double-distilled water was used through the experiments. All glassware was kept permanently full of 1.0 mol L⁻¹ of HNO₃ when not in use. HNO₃ (65%), H₂O₂ (35%), and HF (36.5–38.0%) were supplied from Sigma-Aldrich (Darmstadt, Germany). $\gamma\text{-Fe}_2\text{O}_3$ magnetic nanoparticle was synthesized by a procedure given in literature. EnviroMAT wastewater (EU-L-2), standard reference material for trace elements in water (SRM 1643e), dogfish muscle certified reference material for trace metals (DORM2) were supplied from SCP Science, NIST, National Research Council Canada, respectively. The developed method was validated by applying to them.

2.3. Preparation of *P. impudicus* for MSPE process

P. impudicus collected from Afyonkarahisar, Turkey, was washed 2 times using distilled water to remove impurities. Then, it was dried at 25 °C for a week. The dried *P. impudicus* were ground in a porcelain

mortar to obtain a fine powder. Then fungal biomass was held at 80 °C in an oven for 24 h to kill *P. impudicus*. Finally, it was inoculated to malt agar at 25 °C for 24 h. The absence of growth of *P. impudicus* mycelia in culture media demonstrated positive outcomes, which shows the complete death of *P. impudicus*.

2.4. Solid phase extraction procedure

A 200 mg dried powder *P. impudicus* was mixed with 800 mg of $\gamma\text{-Fe}_2\text{O}_3$ MNPs and 5.0 mL of double-distilled water was added and thoroughly mixed. SPE column packing process given in our previous SPE experiences was adopted to this study [26].

pH of a 50 mL of model solution including Sn(II), Ni(II), Cu(II), Hg(II), Fe(II), Fe(III), As(III), Cr(III), Cd(II), Co(II) and Zn(II) at the concentrations of 10.0 ng mL⁻¹ was adjusted to 2.0–8.0 by adding required volume of HCl and NaOH. This solution was passed through the prepared MSPE column. The adsorbed metal cations were eluted with 5.0 mL of 1.0 mol L⁻¹ HCl. Then, the concentrations of them were measured by using ICP-OES. The results were evaluated and decided to optimize the method to the element(s) that had affinity to fungus loaded resin.

2.5. Sample preparation

All of the samples were bought from local market in Diyarbakır, Turkey. 1 g portions of canned tuna fish, canned anchovy fish, black tea, green tea, cow milk, honey, bread, biscuit, flour, rice and dogfish muscle as certified reference materials were added in 5.0 mL of HCl:HNO₃ (1:1, v/v), and the mixture was heated. Before transferring to a microwave vessel, it was evaporated until dryness and it was added to 6.0 mL of HCl:HNO₃:H₂O₂ (1:1:0.2, v/v/v). This solution was digested in the Berghof MWS3 microwave oven (Berghof, Tübingen, Germany). Then, vessels were heated to 170 °C by microwave irradiation and waited for 5.0 min. After 15 min the temperature reached up to 200 °C and they were waited for 1.0 min. Finally, the temperature was reduced to 100 °C for 20 min. After digestion, the final volume and pH were adjusted to the requested value before the MSPE process. 50 mL of tap water, Tigris River water, wastewater (EU-L-2), standard reference material for trace elements in water (SRM 1643e) were applied the MSPE procedure after pH adjustment.

3. Results and discussion

3.1. Characterization of biosorbent

The overlay FTIR spectra of *P. impudicus*, $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles and *P. impudicus* loaded with magnetized $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles are shown in Fig. 1a, b, and c. The peak at ~2900 cm⁻¹ was attributable to sym./asym. stretch of -CH₃, -CH₂ groups. The peaks at ~2360 and 1080 cm⁻¹ were aromatic nitrile and cyanate stretch, respectively. The peaks observed at ~1630 cm⁻¹ was carboxylate, ~1380 cm⁻¹ was possible sulphur-oxy compounds. Also, ~700 cm⁻¹ belonged to C-S stretch and Fe-O vibrations of magnetic nanoparticles were observed on 697 cm⁻¹. By considering the hard and soft acid and base theory, it could be discussed that *P. impudicus* present hard and borderline basic functionality [27]. As can be seen in Fig. 1d and 1e that, there were no significantly differences on biosorbent surface after interaction with Cr(III) and Zn(II).

Surface macrostructure of *P. impudicus* onto $\gamma\text{-Fe}_2\text{O}_3$ magnetic nanoparticle with and without Zn(II) and Cr(III) were monitored by SEM-EDX. Images were presented in Fig. 2 a,b,c. The porous surface structure allows the higher adsorption capacity. In EDX spectra, specific peaks for Zn(II) and Cr(III) were recorded in addition to impurities. From SEM images it was not possible to clearly discuss the changes on surface macrostructure before and after Zn(II) and Cr(III) biosorption. Because we know the interactions of metal cations with functional groups on biomass are at the molecular level and does not contribute to the differences in macro and/or microstructure. However, SEM is not a way to show the complexation or adsorption of dissolved metal cations.

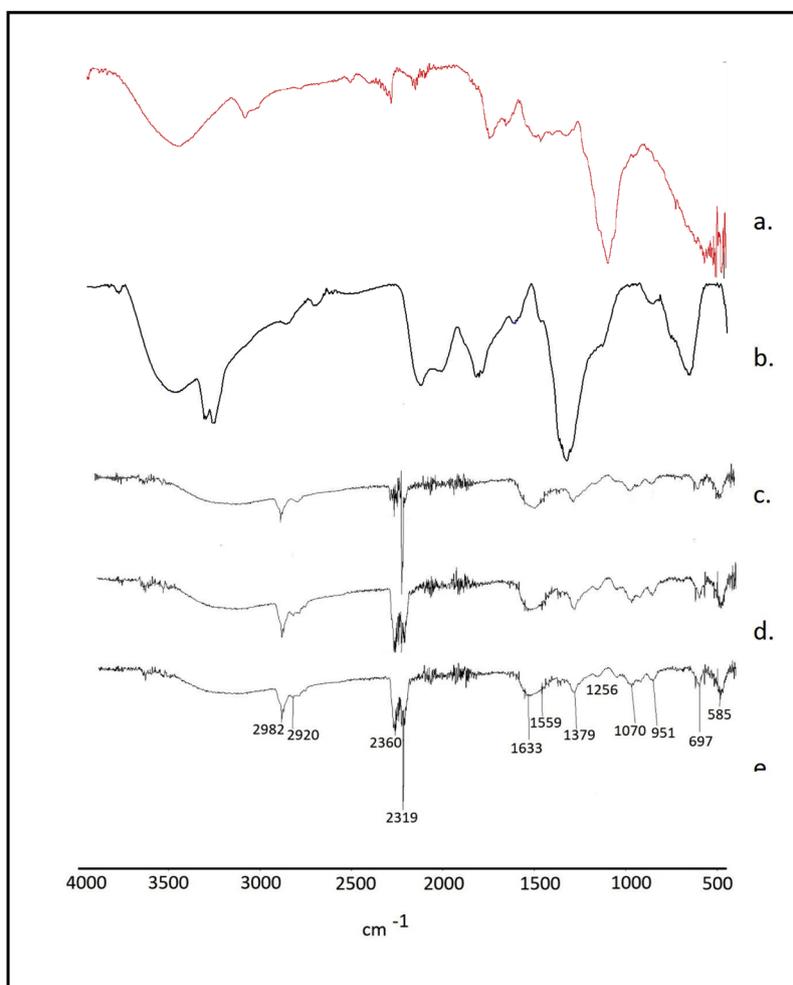


Fig. 1. FT-IR spectral comparison of a. *P. impudicus*, b. MNPs, c. *P. impudicus* immobilized MNPs, d. *P. impudicus* immobilized MNPs loaded with Cr(III), e. *P. impudicus* immobilized MNPs loaded with Zn(II). **Fig. 2.** SEM-EDX investigations of a. *P. impudicus* immobilized MNPs, b. *P. impudicus* immobilized MNPs after MSPE of Cr(III), c. *P. impudicus* immobilized MNPs after MSPE of Zn(II).

3.2. Effects of pH

The impact of pH as a critical parameter on the recoveries of Zn(II) and Cr(III) on *P. impudicus* immobilized MNPs was investigated in the pH range of 2.0–9.0 (Fig. S1). Because, pH of the solution affects the protonation degree of functional groups on the surfaces of magnetized *P. impudicus* and also speciation of metal ions [28]. The quantitative recoveries were obtained for Zn(II) at the pH range of 5.0–7.0 while the recovery of Cr(III) was quantitative at the pH range of 6.0–8.0. According to these results, pH 6.0 was used as the best pH for further works.

3.3. Effects of flow rate

Equilibrium between the metal cation and biosorbent is also depended on flow rate. It affects the binding of toxic metals to the biosorbent surface and the biosorption yield [29,30]. In order not to prolong the MSPE process period, the flow rate also should not be low. The impacts of the flow rate on the recovery of Zn(II) and Cr(III) on *P. impudicus* immobilized MNPs were also examined in the range of 1–6 mL min⁻¹. As seen in Fig. S2, the quantitative recoveries for analytes were obtained at 1–2 mL min⁻¹ flow rates. When the solution flow rate increased from 2 mL min⁻¹ to 3 mL min⁻¹, the recoveries efficiencies of Zn(II) and Cr(III) on *P. impudicus* immobilized MNPs were decreased from 98.1%–92.7% and from 97.3%–90.6%, respectively. Further works were performed at 2 mL min⁻¹ flow rate.

3.4. Influences of amounts of *P. impudicus* and γ -Fe₂O₃ magnetic nanoparticle

The effects of amounts of *P. impudicus* on the recoveries of Zn(II) and Cr(III) were also examined in the range of 50–250 mg of *P. impudicus* (Fig. S3). The recoveries were found as quantitative after 100 mg of *P. impudicus*. The amount of biosorbent less than 100 mg means less binding sites and the recoveries decreases. A larger amount of biosorbent causes an increase in the electrostatic interactions between the cells, causing precipitation and inhibiting binding sites. So, 100 mg of *P. impudicus* was used for the next experiments. Due to the necessity of iron oxide nanoparticles for magnetic separation, the influences of the amount of γ -Fe₂O₃ magnetic nanoparticle on the recoveries of Zn(II) and Cr(III) were tested (Fig. S4). The recovery values for Zn(II) and Cr(III) were not quantitative till 75 mg of iron nanoparticle. After 100 mg of iron nanoparticle, quantitative recoveries for both analytes were achieved. 100 mg of γ -Fe₂O₃ magnetic nanoparticle was selected for further experiments.

3.5. Elution process

The recovery efficiencies of analytes and the structure of functional groups on the surface of the biosorbent are influenced by the eluent type, concentration, and volume. Therefore, it is important to choose the most suitable eluent. The elution of the adsorbed analytes on the *P. impudicus* immobilized MNPs was examined by using HNO₃ and HCl at different volume and concentrations as eluent shown in Table S1. Zn(II)

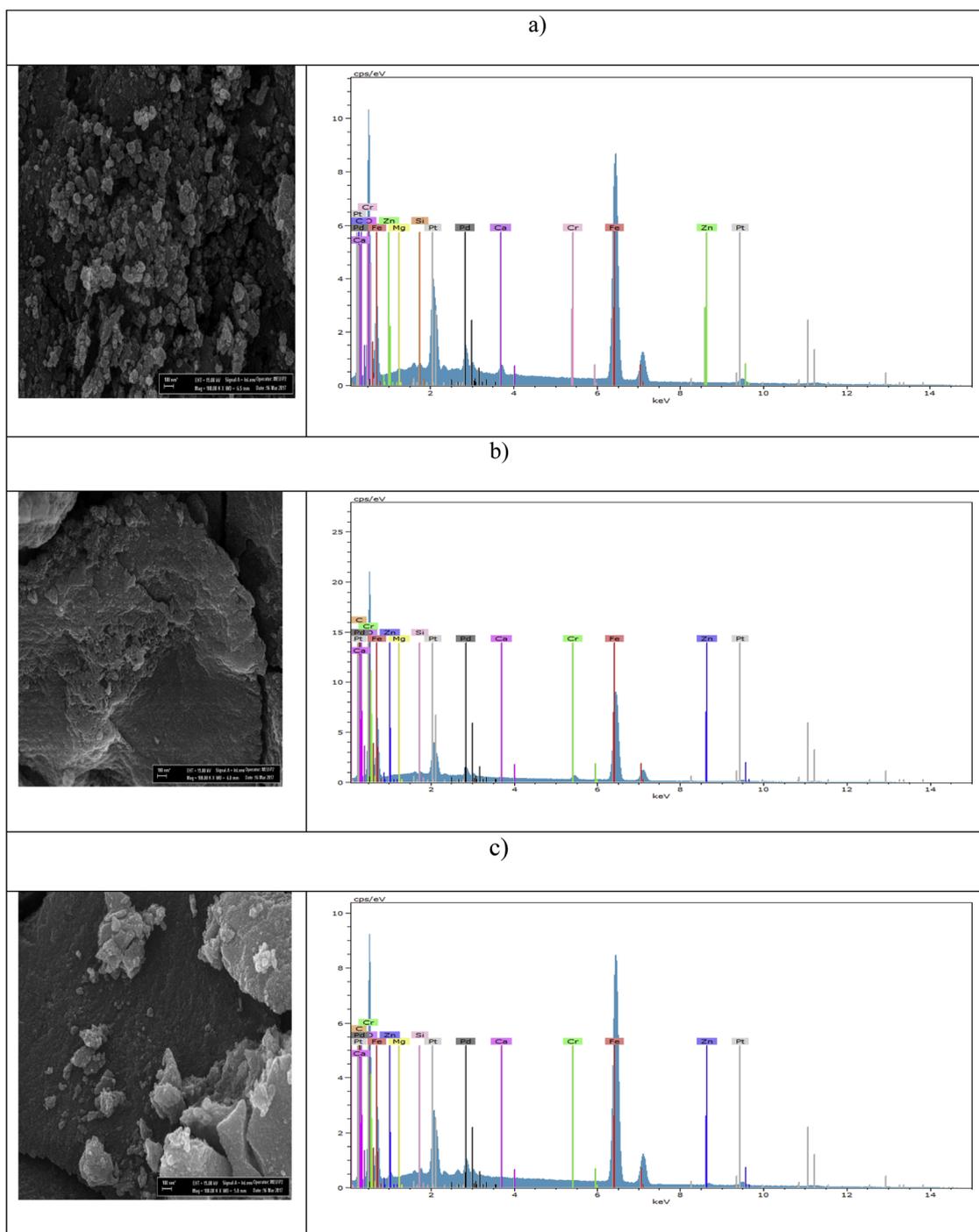


Fig. 2. SEM-EDX investigations of a. *P. impidicus* immobilized MNPs, b. *P. impidicus* immobilized MNPs after MSPE of Cr(III), c. *P. impidicus* immobilized MNPs after MSPE of Zn(II).

and Cr(III) recoveries were found 87.9% and 86.4% with 3 mL 0.5 M HCl. Zn(II) and Cr(III) recoveries were also found 83.7% and 82.8% with 3 mL 0.5 M HNO₃. The quantitative recoveries for both analytes were obtained after both 5 mL of 1 M HCl (100% for Zn(II), 99.6% for Cr(III)) and 5 mL of 1 M HNO₃ (96.3% for Zn(II), 95.1% for Cr(III)). It was decided to select 5 mL 1 M HCl as eluent for next experiments.

3.6. Sample volume

The impacts of the sample volume on Zn(II) and Cr(III) recoveries by using *P. impidicus* immobilized MNPs were carried out in the sample volume range of 25–500 mL at pH 6.0. The results are depicted in Fig.

S5. It was found out that Zn(II) and Cr(III) quantitatively recovered to a high rate up to 500 mL of sample volume. Considering 500 mL as the sample volume and 5.0 mL as the best eluent volume, a preconcentration factor of 100 was reached for Zn(II) and Cr(III).

3.7. Effect of the matrix

This study also investigates the effects of some alkaline earth, alkaline and transition elements on the recoveries of targeted ions on *P. impidicus* immobilized MNPs. The recoveries were found quantitative at the levels given in Table S2 for alkaline, alkaline earth and transition elements. These results showed that the procedure presented are

Table 1Kinetic parameters obtained from pseudo-first-order (q_{e1} : mg g⁻¹, k_1 : min⁻¹) and pseudo-second-order (k_2 : g mg⁻¹.min⁻¹, q_{e2} : mg g⁻¹) for Zn(II) and Cr(III).

Metal	Temp., °C	Pseudo-first-order kinetic			Pseudo-second-order kinetic		
		k_1	q_{e1}	r^2	k_2	q_{e2}	r^2
Cr(III)	25	0.0195	61.9	0.9244	0.00076	89.28	0.9931
	35	0.0174	72.6	0.9848	0.00048	90.91	0.9946
	55	0.0149	65.3	0.9511	0.00060	86.95	0.9952
	60	0.0079	62.1	0.9132	0.00140	67.11	0.9956
Zn(II)	25	0,0254	82.1	0.9514	0.00038	98.04	0.9941
	35	0,0157	72.9	0.9587	0.00046	89.29	0.9913
	55	0,0111	70.3	0.9439	0.00053	80.65	0.9930
	60	0,0074	66.5	0.8979	0.00108	65.36	0.9960

Table 2

Analytical features of the method.

Parameter	Cr(III)	Zn(II)
Linear range, ng mL ⁻¹	0.2-10	0.2-10
r^2	0.9984	0.9994
RSD, %	2.04	2.08
LOD, ng L ⁻¹	10.5	12.6
LOQ, ng L ⁻¹	35.1	42.2
Biosorption capacity	22.8 mg g ⁻¹	25.6 mg g ⁻¹

applicable for the real samples that contain the matrix elements given in Table S2.

3.8. Biosorption characteristics of magnetized *P. impudicus*

The biosorption capacities of magnetized *P. impudicus* with iron oxide nanoparticle were determined by batch method in which 0.05 g magnetized *P. impudicus* were equilibrated in 100 mL of 50 mg L⁻¹ Zn(II) and Cr(III) at pH 6.0, 120 rpm and room temperature for 2 h using a shaker. The magnetized *P. impudicus* with iron particles was then separated from the solution by using a magnet. The supernatant liquid was used to determine the amounts of Zn(II) and Cr(III) for biosorption capacities. Zn (II) and Cr(III) quantities binding on the magnetized *P. impudicus* were also calculated from the following equation:

$$Q = V(C_i - C_f) / 1000m$$

where Q is the specific metal reception (mg of metal/g of biosorbent), V is the volume of metal solution (mL), C_i is the initial metal concentration in solution (mg L⁻¹), C_f is the final metal concentration in solution (mg L⁻¹), and m is the amount of biosorbent (g) [12,31]. The biosorption capacities of magnetized *P. impudicus* with iron nanoparticle were found 25.6 mg g⁻¹ and 22.8 mg g⁻¹ for Zn(II) and Cr(III), respectively.

To understand the mechanism of adsorption, 0.05 g magnetized *P. impudicus* were equilibrated in 100 mL of targeted ions at the concentrations range of 50–150 mg L⁻¹. The results were applied to well-

Table 3

Comparative data from literature for the preconcentration of Cr(III) and Zn(II).

Method	Ion	Linear range ng mL ⁻¹	LOD ng mL ⁻¹	Instrument	Matrix	Ref.
Graphene oxide covalently modified with 2,2'-iminodiacetic acid	Cr(III)	1-200	0.11	ED-XRF	Water	[34]
<i>Moringa oleifera</i> husks as the solid phase with a flow injection system	Cr(III)	–	1.92	F-AAS	Water	[35]
Coprecipitation of chromium(III) with dysprosium hydroxide	Cr(III)	–	0.65	AAS	Water	[36]
Activated carbon was produced from tea-industry wastes	Cr(III)	–	0.27	F-AAS	Water	[37]
MSPE on <i>Phallus impudicus</i> loaded with γ -Fe ₂ O ₃ nanoparticles	Cr(III)	0.2-10	0.011	ICP-OES	Food and water	This method
Graphene oxide covalently modified with 2,2'-iminodiacetic acid	Zn(II)	1-140	0.07	ED-XRF	Water	[34]
Ion-imprinted polymer nanoparticles	Zn(II)	5-9	1	F-AAS	Food and water	[38]
Micro-electrodeposition in the presence of ionic liquid	Zn(II)	10-250	6	EDXRF	Water	[39]
Minicolumn filled with ethyl vinyl acetate (EVA)	Zn(II)	–	0.08	ICP-OES	Water	[40]
MSPE on <i>Phallus impudicus</i> loaded with γ -Fe ₂ O ₃ nanoparticles	Zn(II)	0.2-20	0.013	ICP-OES	Food and water	This method

known Langmuir and Freundlich models. Coefficient of regression for Langmuir model were 0.9909 and 0.9979 for Zn(II) and Cr(III), respectively while they were 0.9264 and 0.9893 for Zn(II) and Cr(III), respectively. It confirms the monolayer adsorption by Langmuir model that high c_{eq} were found as 285.7 and 344.8 mg g⁻¹ for Zn(II) and Cr(III), respectively and K_b , a constant related to the affinity of the binding sites were found as 0.057 and 0.035 for Zn(II) and Cr(III), respectively.

The r^2 values in Tables 1 indicate that the adsorption mechanisms of Zn(II) and Cr(III) on *P. impudicus* with iron oxide nanoparticle followed the pseudo-second-order kinetic model. The pseudo second-order kinetic constant and the theoretical q_e by a type 1 pseudo second-order expression can be calculated from the plots of t/q_t versus t . Moreover q_{e2} values were calculated as close to experimental ones. In the present case, the electron coordination between the adsorbent and metal ions or adsorption through electron exchange concludes [32].

3.9. Reuse of magnetized *P. impudicus*

In the MSPE processes, the reusability of immobilized biosorbent is also one of the most important factors. Therefore, re-usability should be tested in SPE studies [33]. The reuse of *P. impudicus* immobilized MNPs was also tested. The quantitative recoveries of Zn(II) and Cr(III) were obtained after usage of the adsorbent 30 times. The adsorbent could be used at least 30 times without any lost on its biosorption properties (Fig. S6).

3.10. Analytical features and application

The analytical features were determined under the best experimental conditions shown in Table 2. The LODs, calculated from (3sd/m) (where sd is the standard deviation, m is slope), were achieved as 12.6 and 10.5 ng L⁻¹ for Zn(II) and Cr(III), respectively. On black tea sample, RSD for inter-day precisions ($n = 3$) were determined as lower than 2.0% for both of targeted metal ions, while intraday precisions ($n = 3$) were as lower than 2.1% for both of targeted metal ions. By applying the developed procedure ICP-MS and/or GF-AAS could be eliminated for the measurement at ultra trace level. Some analytical features comparing with literature are summarized in Table 3. It is clear

Table 4
Application of the method to certified reference samples.

Sample	Cr(III), ng mL ⁻¹		Zn(II), ng mL ⁻¹	
	Certified	Determined	Certified	Determined
EU-L-2	60	59 ± 2	23	22.6 ± 1.1
NIST 1643e	20.40 ± 0.24	20.10 ± 0.18	78.5 ± 2.2	76.9 ± 2.0
DORM-2	0.45 ± 0.10 ^a	0.45 ± 0.09 ^a	51 ± 2 ^a	50 ± 1 ^a

^a µg g⁻¹.

Table 5
Application of the method to real samples.

Sample	Cr(III) ngg ⁻¹	Zn(II) µg g ⁻¹
Tap water	< LOD	0.71 ± 0.02 ^a
Tigris River water	< LOD	1.9 ± 0.07 ^a
Mineral water	< LOD	0.96 ± 0.05 ^a
Canned Tuna fish	0.41 ± 0.01	14.6 ± 0.98
Canned anchovy fish	1.12 ± 0.09	29.6 ± 1.0
Black tea	8.0 ± 0.3	14.2 ± 0.9
Black tea ^b	12.9 ± 0.2	19.1 ± 1.2
Green tea	5.3 ± 0.1	20.7 ± 1.2
Cow milk	< LOD	3.9 ± 0.06
Honey	< LOD	4.0 ± 0.1
Bread	< LOD	13.7 ± 2.1
Biscuit	< LOD	13.5 ± 1.8
Flour	4.9 ± 0.2	11.8 ± 0.8
Rice	49.3 ± 5.1	25.9 ± 1.5

^a µg mL⁻¹.

^b Spiked with 5.0 µg mL⁻¹ of Cr(III) and Zn(II).

to conclude that the proposed method has over advantages including low LOQ, simplicity, eco-friendly procedures, wide linear range and reusability application.

EU-L-2, SRM 1643e and DORM2 were utilized to control the accuracy of the MSPE procedure. Results were given in Table 4 that agreed with certified values. Thus, the developed method could be used for the preconcentrations of Zn(II) and Cr(III) in real samples.

Tap, river and mineral water samples, canned tuna fish, canned anchovy fish, black tea, green tea, cow milk, honey, bread, biscuit, flour, rice were applied the developed procedure to show the applicability. Microwave oven was used for digestion of the real samples. Then they were subjected to MSPE procedure before ICP-OES measurements of Zn(II) and Cr(III). As it can be shown in Table 5, the recoveries of analytes were found higher than 95%. In addition, the black tea sample was spiked with a known amount of Zn(II) and Cr(III). The spiked amount was preconcentrated as quantitatively. The results indicate that the proposed method is reliable. In addition, inter and intra day precisions were experimented for a respective liquid (tap water) and solid samples (green tea). RSDs for tap water samples were found as 2.3% and 3.1%, respectively for inter and intra day precisions, while they were found as 2.0% and 2.1%, respectively for inter and intra day precisions for green tea samples.

4. Conclusions

Applicability of the *P. impudicus* loaded with γ-Fe₂O₃ MNPs as a biosorbent for MSPE of Zn(II) and Cr(III) before their determinations by ICP-OES was investigated in details. The best experimental parameters were examined. LODs were achieved as 10.5 and 12.6 ngL⁻¹, respectively for Cr(III) and Zn(II). Thus, sensitivity of ICP-OES was improved for Cr(III) and Zn(II). Adsorption mechanism was also investigated by applying Langmuir, Freundlich adsorption model and pseudo first-second order kinetic models. By applying the developed MSPE, this problem was overcome. Accuracy of the method was checked by applying certified and standard reference materials. Additionally,

applicability of the method to real samples was revealed by applying it to water and food samples.

CRedit authorship contribution statement

M. Serkan Yalçın: Conceptualization, Methodology, Writing - review & editing. **Ersin Kılınç:** Investigation, Validation, Visualization. **Sadin Özdemir:** Investigation, Writing - original draft. **Uyan Yüksel:** Writing - original draft. **Mustafa Soylak:** Supervision.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.procbio.2020.03.012>.

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