Biotechnological Journal of
Environmental Microorganisms
(BJEM)

Biotechnological Journal of Environmental Microorganisms(BJEM) 1(2) 2022 68-77

Preparation of Magnetite Nanocomposites based on *Azolla Filiculoides* fern with Environmental Applications for Organic Pollutants

Reyhaneh Kouchakinejad [1], Shahab Shariati [2*], Jafar Abolhasani [3], Ebrahim Ghorbani Kalhor[4], Mohammad Taghi Vardini [5]

[1]Department of Chemistry, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
[2]Department of Chemistry, Rasht Branch, Islamic Azad University, Rasht, Iran
[3]Department of Chemistry, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
[4]Department of Chemistry, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
[5]Department of Chemistry, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

Received:10 May 2022 / Revised: 27 June 2022 /Accepted: 29 June 2022

Abstract

Azolla Filiculoides fern was utilized as a natural adsorbent to develop a magnetic solid phase extraction method for the preconcentration and determination of trace amounts of chlorpyrifos pesticide, which is a primary environmental concern. In this study, magnetic solid phase extraction (MSPE) method using magnetite modified azolla nanocomposites (azolla@Fe_3O_4 NCs) was developed to preconcentrate trace amounts of chlorpyrifos pesticide prior to UV-Vis spectroscopy. The structural characteristics of the synthesized nanocomposites (NCs) were investigated by X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FT-IR) and vibrating sample magnetometry (VSM) methods. In the proposed MSPE method, after optimizing the adsorption / desorption variables in MSPE and obtaining the optimum level of each variable (adsorption step:V_{sample} = 50 mL, contact time = 15 min, solution pH = 3, adsorbent mass = 0.05 g, ionic strength = 0.01 mol L^{-1}; desorption step: eluent type and volume = ethanol (2 mL) for 10 min), the desorbed ethanolic solution was spectrophotometrically investigated in the UV-Vis region. The results confirmed the developed MSPE method can be used for the preconcentration and determination of trace amounts of chlorpyrifos in the aqueous samples.

Key words: *Azolla Filiculoides*, Magnetic solid phase extraction, Nanocomposite, Pesticide, Clorpyrifos

---

*E-mail address: shariaty@iaurasht.ac.ir (Shahab Shariati)

Introduction

The increase in population, urbanization, and subsequent industrialization of human societies have led to the emergence of external pressures on limited natural resources and the occurrence of various types of chemical pollution, including heavy metals, radioactive, organic (dye, drugs, solvents, etc.) and inorganic compounds in them (Mitra and Brukh 2003). Chemicals play a significant role in polluting resources, as they are used in various industries or agriculture, leading to the entrance of these compounds into surface and ground waters, soil, and air (Lin, et al. 2000). Pesticides, which modern agriculture significantly relies on to increase and maintain the production, are a class of important chemical pollutants that affect the aquatic ecosystem. Pesticides are a wide range of chemical compounds used to eliminate living organisms that threaten human health or the resources they use. They are classified into organochlorines, organophosphates, carbamates, pyrethroids, and mineral pesticides based on their chemical composition (Alloway and Ayres 1997). Without using pesticides, one-third of the world's agricultural products will be lost, and widespread famine will occur. Therefore, the use of these compounds in developing countries, despite awareness of their persistence, toxicity, and irreparable effects on humans and various ecosystems, has grown exponentially, leading to their sustainability and timely non-destruction in soil, and ultimately pollution of water resources, soil quality degradation, and biodiversity loss (Matsumura 1985). Processes such as evaporation, water washing, surface runoff, absorption by plants, or penetration into depths have led to their transfer into the environments. Therefore, the need for developing reliable and cost-effective methods with less production of secondary pollutants is always felt (Faraji et al. 2010). Finding solutions to remove or reduce these pollutants from the environment, especially aquatic environments, has attracted the attention of researchers and scientists. In this regard, many studies have been conducted using chemical precipitation, oxidation, reduction, coagulation, ion exchange, reverse osmosis, solvent extraction, flocculation, membrane separation, purification, evaporation, electrolysis, and adsorption to remove and recover the organic pollutants.

One of the most famous and widely used phosphorus pesticides in the world is chlorpyrifos(O,O-diethyl-O-3,5,6-trichloropyridin2-phosphorothioate), which is considered as one of the most harmful substances to humans due to its dangerous consequences, such as carcinogenicity and inhibition of acetylcholinesterase enzyme (a key enzyme for nerve function) (Hamadeen et al. 2021).

Azolla Filiculoides (Fig. 1), a floating and small fern with global scope, was first identified by Jean-Baptiste Lamarck in 1783 and since then, six different species of this fern have been reported. This plant is native to warm and tropical regions of Africa, Asia, and America (Lumpkin and Plucknett 1980). The uncontrolled growth of *Azolla Filiculoides* has turned it into a weed in freshwater and wastewater due to its 2 to 4-day growth rate and unique ability to tolerate a wide range of environmental conditions (Vafaei et al. 2012). The abundant presence of this fern on the water surface hinders the penetration of light into the water and reduces gas exchange between the surface and subsurface layers, resulting in a severe reduction in dissolved oxygen and ultimately the elimination of some species in the wetland ecosystems. Furthermore, the intrusion of *Azolla Filiculoides* into the rice paddies causes numerous problems in the planting and cultivation phase, such as hindering the growth and emergence of buds, leading to delays in the harvest of the crop.

Surface area and high porosity make nanoparticles and NCs as a desirable option for removal of organic and inorganic contaminants from water resources. Although conventional methods of water treatment remove a large portion of contaminants, the old methods are not enough to protect the industry and agriculture from irreparable damage. Therefore, there is always a tendency to develop highly efficient targeted methods to precisely determine or eliminate toxic compounds (Bockstaller 2004).

Separation technology using magnetic adsorbents is an alternative method for conventional methods of water and wastewater treatment, which has received a lot of attention in the recent years. Magnetism is a unique property that

Fig. 1. Azolla fern images.

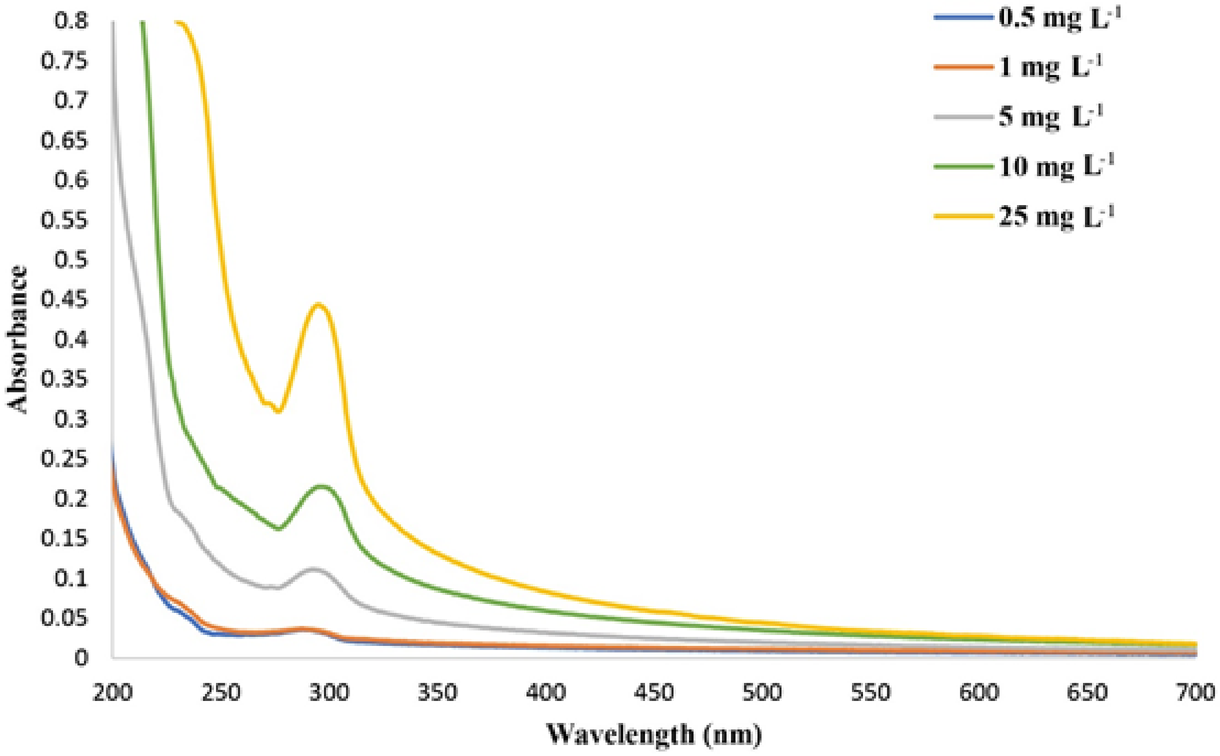

Fig. 2. UV-Vis spectra of chlorpyrifos at concentrations 0.5-25 mg L^{-1} without preconcentration.

can be used in water treatment process by affecting the physical properties of pollutants. Also, combining this property with separation methods facilitates and makes the treatment methods more efficient. Magnetic compounds can adsorb contaminants from effluents and then remove them with applying an external magnetic field without the need for centrifuge or filtration step (Bagheri et al. 2017)(Roberts et al. 2014).

In the recent years, some researchers used modified and unmodified azolla to remove organic pollutants from aqueous samples. In the study of Rakhshaee and co-workers, the adsorption capacity of the adsorbents prepared by attaching zero-valent iron nanoparticles to live and dead *Azolla Filiculoides* for removing methylene blue dye from aqueous solutions was investigated and the results demonstrated the higher adsorption capacity of modified live azolla compared to other forms (adsorption on both iron nanoparticles and biomass) (Rakhshaee 2015).

Alizadeh and coworkers in 2017 studied the adsorption of crystal violet and methylene blue dyes on the azolla and fig leaves modified with magnetite nanoparticles. They prepared magnetite nanoparticles loaded Fig leaves (MNLFL) and magnetite nanoparticles loaded azolla (MNLA) as natural cheap adsorbents via chemical precipitation method. Their results showed the sorption of these dyes on the surface of MNLFL and MNLA adsorbents was best described by a pseudo-second order kinetic model and equilibrium data were fitted to the Langmuir isotherm. Their results showed complete crystal violet removal with sorption capacity equal to 53.47 mg g^{-1} for MNLFL and 30.21 mg g^{-1} for MNLA and complete methylene blue removal with sorption capacity equal to 61.72 mg g^{-1} for MNLFL and 25 mg g^{-1} for MNLA, respectively (Alizadeh et al. 2017).

In 2018, Golzary et al. used *Azolla filiculoides* fern for color, odor, COD, nitrate, and phosphate removal. Their results showed N and P removal at 100 mg L^{-1} of each component in separate medium turned out to be 36% and 44%, respectively, whereas in case of a mixed solution of these two compounds, N and P removal declined to 33% and 40.5%, respectively. Moreover, results have suggested that in the presence of phosphorus, nitrogen absorption decreased. Furthermore, azolla has revealed a high potential of COD removal by 98.8% in 28 days. Finally, they concluded azolla may be one of the most promising agents to remove COD and treat nitrogen-free and phosphorus-rich wastewaters (Golzary et al, 2018).

Using magnetite modified azolla nanocomposites (NCs), Shariati et al. developed a natural adsorbent that was cost-effective and could be used at least 20 times without loss of efficiency in removing the brilliant blue dye from aqueous environments (Shariati et al. 2020)

Besharati et al. employed adsorbents prepared from azolla, fig leaves, eggshell and egg membrane modified with magnetic nanoparticles to remove tetracycline antibiotic from water samples, and the high adsorption capacity for these adsorbents was confirmed due to their high surface area (Besharati et al. 2021).

In another study, Shariati et al. succeeded in removing the reactive yellow dye from aqueous solutions utilizing magnetite (Fe_3O_4) modified azolla powder which demonstrated 95% removal efficiency and 16 times reusability under the optimal conditions (Shariati et al. 2021).

Darvishi et al. developed a precise, efficient, and rapid biosensor based on differential pulse voltammetry (DPV) for measuring phthalic acid esters in aqueous solutions by modifying the surface of a copper electrode with a mixed gel made from a combination of electroencephalography gel and prepared azolla powder. The results showed very good detection limits for determination of four phthalic acid esters with a relative recovery of 97.3% to 100% under the optimal conditions (Darvishi et al. 2021).

In another study, Darvishi and coworkers developed two modified impedimetric nanobiosensors using azolla powder and magnetite modified azolla NCs for measuring phthalic acid esters in water samples and compared their responses. The results indicated similar accuracy, speed, reproducibility, and performance for both sensors (Darvishi et al. 2023). In this research in continue with previous studies, we magnetized azolla powder via chemical precipitation to synthesize azolla@Fe_3O_4 NCs. After characterization, the performance of the synthesized magnetite modified azolla NCs in the chlorpyrifos adsorption from aqueous solutions and to extract and preconcentrate it was investigated. One of the main reasons for using *Azolla Filiculoides* in this adsorption system is its abundance, ease of collection from the water surface, economic viability, and ease of transportation due to its low weight after drying.

2.Experimental

2.1. Chemicals and reagents

The chemical reagents including ferric chloride hexahydrate ($FeCl_3.6H_2O$), ferrous chloride tetrahydrate ($FeCl_2.4H_2O$), methanol (MeOH), sodium hydroxide (NaOH), sodium chloride (NaCl) and hydrochloric acid (HCl, 37 wt. %) were obtained with high purity from Fluka (Buchs, Switzerland) and Merck (Darmstadt, Germany). Chlorpyrifos ($C_9H_{11}C_{13}NO_3PS$) with 98% purity was prepared from Sigma-Aldrich (Milwaukee, WI, USA). Absolute ethanol (EtOH) and ammonia (NH_4OH, 25 wt. %) were obtained from Mojallali industrial chemical company (Iran).

The stock standard solution of chlorpyrifos (1000 mg L^{-1}) was prepared in MeOH, stored and refrigerated at 4^{o}C. The solutions of working standards were freshly prepared by diluting the prepared stock solution with double distilled water to the required concentrations.

2.2. Instruments and equipment

A digital WTW pH meter (Inolab7110, Germany) with a combined glass electrode was utilized to adjust the pH of the solutions. The crystallinity of the synthesized materials was determined on a Philips X'Pert PRO (PANalitical, Netherlands) X-ray diffraction (XRD) instrument using a Cu Kα radiation source ($\lambda = 0.154$ nm) in the 2θ range of 10–70°. Absorption measurements were carried out using an Agilent UV–Vis spectrophotometer (Cary 60, USA). The field emission scanning electron microscopy (FESEM) was used for investigating the surface morphology of the azolla@Fe_3O_4 NCs (model TESCAN Mira 3, Cezicha). The Fourier transform-infrared (FT-IR) spectra of the samples were obtained using Thermo Avatar FT-IR spectrophotometer (USA) with KBr pellets in the wavenumber range of 400-4000cm^{-1}. A 1.4 T permanent super magnet (1 × 3 × 5 cm) was applied to separate NCs.

2.3. Preparation of nano-adsorbent

2.3.1.Preparation of azolla powder

The azolla fern (Fig. 1) was obtained from Anzali lagoon (Guilan province, Iran), rinsed several times with water to remove impurities, and immersed in water overnight to wash away the dirt completely. It was then dried in an oven at 70 °C for three days after being rinsed five times with distilled water. The azolla was pounded in a mortar and crushed completely. To remove coarse particles, the powder was sieved using a sieve with a mesh size of 70 (210 µm). To obtain a powder with a particle size of less than 74 µm, the remaining powder was then sieved again through a 200-mesh sieve (Darvishi et al. 2021).

2.3.2.Magnetization of azolla powder to prepare azolla@Fe_3O_4 NCs

To prepare the azolla@Fe_3O_4 NCs, 6.3 g $FeCl_3.6H_2O$ and 4.0 g $FeCl_2.4H_2O$ were combined with 3 mL HCl (37%) to give a stock solution of Fe^{3+} and Fe^{2+} ions. This stock solution was then made up to 50 mL with distilled water. To stop the oxidation of iron (II) ions, this solution was deoxygenated in a separatory funnel by blowing N_2 gas (99%) for 10 min. At the other hands, 250 mL of ammonia solution (1.5 M) and 1.5 g of the prepared azolla powder were added to the flask. After purging of N_2 gas, upon heating to 80 °C on a magnetic stirrer, the solution of iron ions was added dropwise over 40 min with vigorous stirring (at 1000 rpm). After precipitation, the black azolla@Fe_3O_4 NCs were magnetically separated using a strong super magnet 1.4 T (1 × 3 × 5 cm), rinsed three times with distilled water, and then stored in 0.1 M NaOH solution for 24 h. After a second wash in distilled water, the azolla@Fe_3O_4 NCs were dried at 60 °C.

2.3.3.Magnetic solid phase extraction process for the extraction of chlorpyrifos

In the current study, azolla@Fe_3O_4 NCs were synthesized and used as adsorbent for the extraction and preconcentration of chlorpyrifos using magnetic solid phase extraction (MSPE). For this purpose, stock solution of chlorpyrifos (1000 mg L^{-1}) was prepared in MeOH, stored and refrigerated at 4^{o}C. Working standard solutions of chlorpyrifos were prepared in doubly distilled water. The

UV-Vis spectra of chlorpyrifos were studied at 1 and 5 mg L^{-1} concentration levels and the maximum absorption wavelength of chlorpyrifos was determined to be 290 nm. For quantitative analysis, the absorbance of chlorpyrifos solutions in the concentration range of 1 to 25 mg L^{-1} was measured (Fig. 2) at a wavelength of 290 nm and a direct calibration curve was plotted. Then, 50 mL solutions with different concentrations of chlorpyrifos were prepared to study its adsorption on the surface of azolla@Fe_3O_4 NCs. The initial optical absorbance of the solutions was measured at 290 nm after adjusting the pH at 3.0 with 0.1 M NaOH or HCl solutions and ionic strength at 0.01 M NaCl. Then, 0.05 g (1.0 g L^{-1}) azolla@Fe_3O_4 NCs as adsorbent was added and contacted with the chlorpyrifos solution for a certain time (5-30 min) to adsorb chlorpyrifos. After the required time, the super magnet was used to separate the adsorbent from the solution, and the final optical absorbance was determined spectrophotometrically (Fig.3). After adsorption step in MSPE, for desorption of chlorpyrifos from the surface of nano-adsorbent, 3 mL EtOH as eluent, was added to the azolla@Fe_3O_4 NCs and the mixture was stirred for 10 min to release adsorbed chlorpyrifos from the surface of each nano-adsorbent. The UV-Vis spectra of the residual aqueos phase and desorbed ethanolic phase were then obtained and the absorbance was measured in the wavelength range of 285-295 nm. The net absorbance of each solution was obtained using the difference of the measured absorbance and blank absorbance.

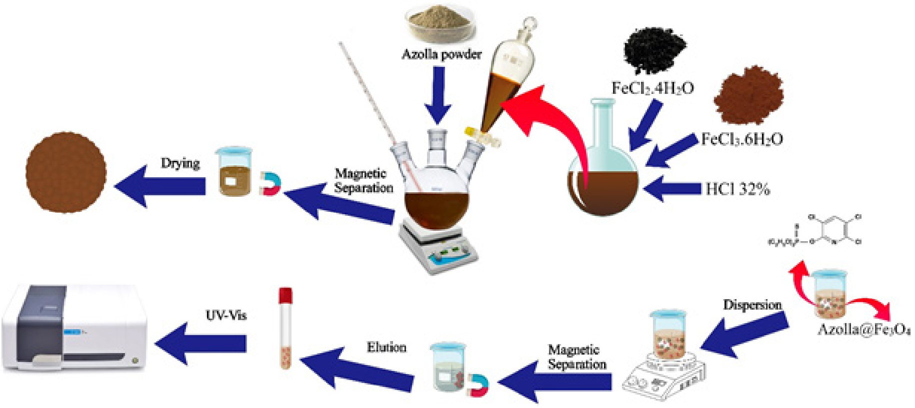

Fig. 3. Schematic for the application of azolla@Fe_3O_4 NCs for the preconcentration of trace amounts of chlorpyrifos using MSPE prior to UV-Vis spectroscopy.

3. Results and discussion

The presence of particles smaller than 40 nm inside the structure of azolla@Fe_3O_4 NCs was detected from the FESEM images (Fig. 4). To detect the presence of magnetite in the nanocomposite structure, an X-ray diffraction (XRD) analysis was performed (Fig. 5). The XRD pattern of azolla@Fe_3O_4 NCs shows that the reflections with Miller indices (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), and (4 4 0) are respectively associated with the peaks at 2θ equals to 30.1°, 35.5°, 43.3°, 53.7°, 57.2°, and 62.8° (Toutounchi, Shariati, and Mahanpoor 2021)(Rahnama, Shariati, and Divsar 2021). This pattern is consistent with the XRD pattern of the Fe_3O_4 standard (JCPDS: 19-0629) and confirms the presence of magnetite nanoparticles with cubic structure in the synthesized azolla@Fe_3O_4 NCs. Fig. 6. displays the FT-IR spectra of the synthesized azolla@Fe_3O_4 NCs. The absorption bands in the wavenumbers of 445 and 630 cm^{-1} are corresponded to the stretching vibrations of Fe-O bond confirming the presence of magnetic nanoparticles in the structure. The bands at 1032, 1404 and 1628 cm^{-1} are related to the C-O bonds, $(CH_2)_n$ bending and C=O stretching vibrations, respectively. To investigate the magnetic properties of the NCs, the VSM residual loop was tested on azolla@Fe_3O_4 NCs at ambient temperature in the range of ±20 kOs. According to the results, the magnetic strength of the prepared nanocomposites was comparable to that of the uncoated Fe_3O_4 NPs. Therefore, the use of magnets to separate the synthesized NCs from the aqueous solutions is straightforward. The results of EDX analysis confirmed the presence of Fe (61.4 %), C (10.6 %), O (26.6 %) and Si (0.1 %) in the azolla@Fe_3O_4 NCs.

To maximize the chlorpyrifos extraction on the the azolla@Fe_3O_4 NCs, four experimental variables affecting the preconcentration of chlorpyrifos during the adsorption step in MSPE including solution pH (3-7), adsorbent mass (0.03-0.10 g), contact time (5-30 min) and ionic strength (0-0.1 M as NaCl concentration) were studied and optimized using Taguchi fractional factorial design. All optimization experiments were done using 50 mL solution containing 1 mg L^{-1} chlorpyrifos and the optimum value of each factor was obtained using UV-Vis absorbance spectra ($\Delta A=A_{peak}-A_{base}$).

The pH of the solution mainly affects the adsorption process. In primary experiments, during the study of the effect of alkaline and acidic pHs, it was found that the adsorption of chlorpyrifos on the surface of azolla@Fe_3O_4 NCs was better at acidic pHs and the highest adsorption was observed at pH=3. At alkaline pH values, chlorpyrifos undergoes hydrolysis and is unstable. Hydrolysis may occur at several reactive centers in its structure, in the presence of OH- or H_2O acting as nucleophilic reagents. The degradation pathway of chlorpyrifos in aquatic environments involves the breakdown of the thiophosphoric esters, forming 3,5,6-trichloropyridinol (TCP) and desethyl chlorpyrifos (DEC) as main metabolites. The stability of chlorpyrifos decreased as the pH increased (Hui, Ariffin, and Tahir 2010). The pH point of zero charge (pH_{pzc}) for the azolla@Fe_3O_4 NCs was obtained as pH = 9, therefore the surface charge of the azolla@Fe_3O_4 NCs is positive at values below the obtained pH_{pzc}, and is negative at pH values above the pH_{pzc}. In acidic solutions, due to the increase of H^+ in the solution, the surface of adsorbent is positively charged that can interact with chlorpyrifos in its anionic form via an electrostatic attraction. The investigating the contact time showed that the adsorption of chlorpyrifos increased up to 15 min. Therefore, this time was selected as the optimum time for adsorption process. The decrease in adsorption at longer times can be attributed to the chlorpyrifos desorption from the surface of adsorbent.

As a result, the best parameters for the adsorption step were chosen as pH= 3.0, adsorbent mass=0.05 g, ionic strength=0.01 M NaCl, and stirring time=15 min.

After optimizing the adsorption step in MSPE, under the optimum conditions of sorption process, the variables affecting the chlorpyrifos desorption from the surface of NCs including eluent type (ethanol, methanol and distilled water) and volume (1.5, 2 and 3 mL), as well as desorption time (5, 7 and 10 min) were investigated to maximize the desorption efficiency. According to the results, 2 mL EtOH as eluent showed the best desorption of chlorpyrifos from the surface of the azolla@Fe_3O_4 NCs during 10 min contact with the nano-adsorbent.

After optimizing the parameters affecting the chlorpyrifos adsorption, the absorbance of chlorpyrifos solutions before and after MSPE preconcentration steps were compared. According to the results, the initial absorption of 1 and 5 mg L^{-1} chlorpyrifos solutions were obtained as 0.0295 and 0.0718, respectively. After MSPE process using the synthesized azolla@Fe_3O_4 NCs at the optimum adsorption conditions (V_{sample} = 50 mL, pH= 3.0, adsorbent mass = 0.05 g, ionic strength = 0.01 M NaCl and stirring time = 15 min), the absorbance of desorbed solutions was increased to 0.3275 and 0.8375 for 1 and 5 mg L^{-1} chlorpyrifos solutions, respectively. Fig. 7 shows the UV-Vis spectra of the residual solutions after MSPE process for 5 mg L^{-1} chlorpyrifos solutions using azolla@Fe_3O_4 NCs adsorbent during 5-30 min a long with UV-Vis spectra of desorbed solution (2 mL EtOH). As can be seen, the optical absorbance of the desorbed solution (after MSPE step) is more than the absorbance of initial chlorpyrifos solution with an enhancement factor more than 11 in absorbance, confirming the preconcentration of chlorpyrifos occurred during the MSPE process. Chlorpyrifos was preconcentrated in the MSPE step, as evidenced by the size of the absorption band after the process compared to that without it.

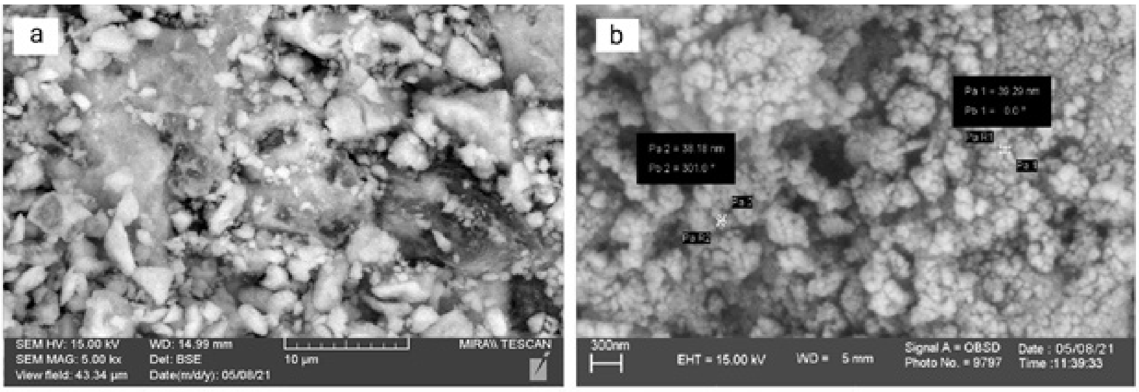

Fig. 4. FESEM image of the synthesized azolla@Fe_3O_4 NCs.

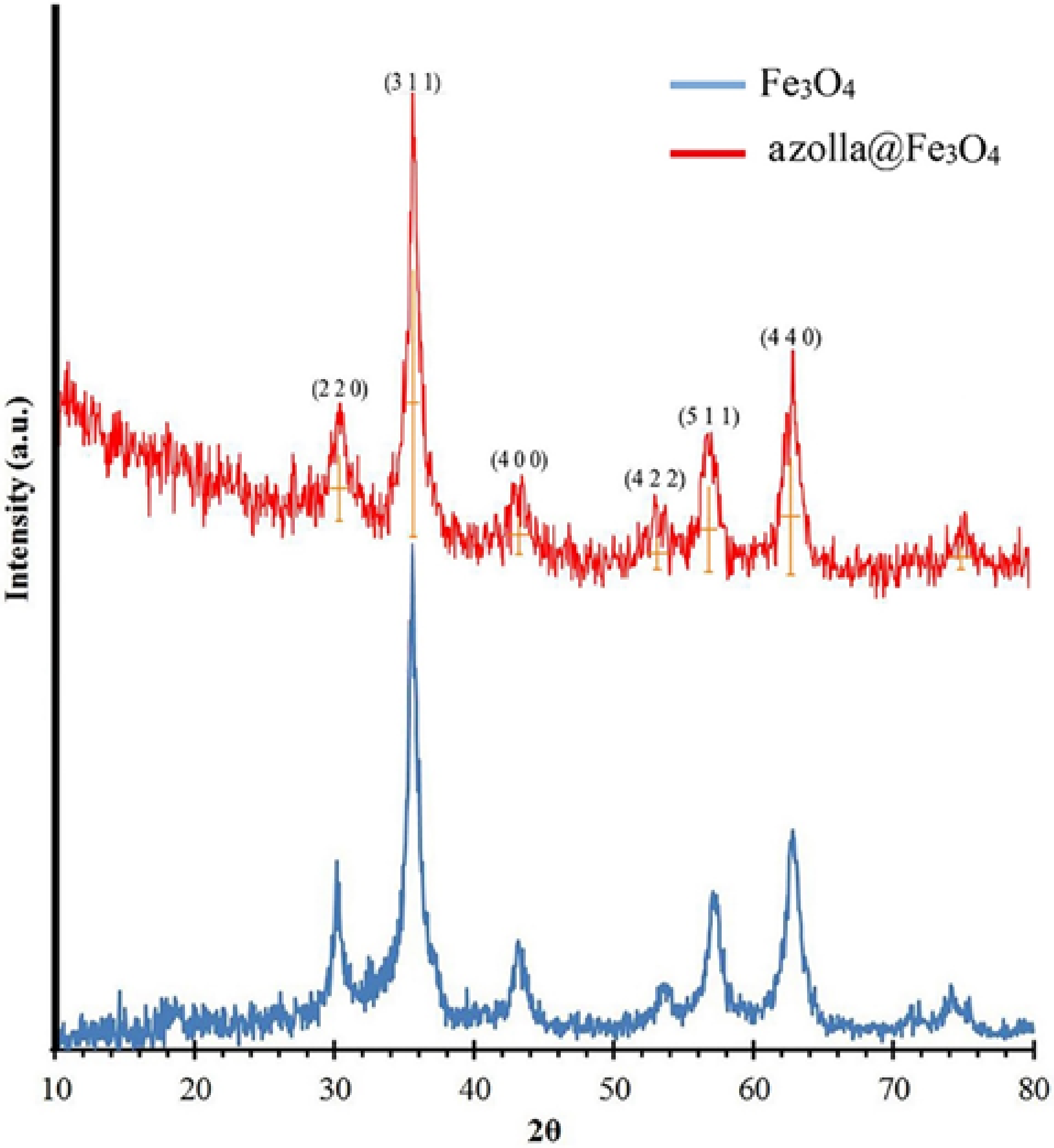

Fig. 5. XRD pattern for azolla@Fe_3O_4 NCs.

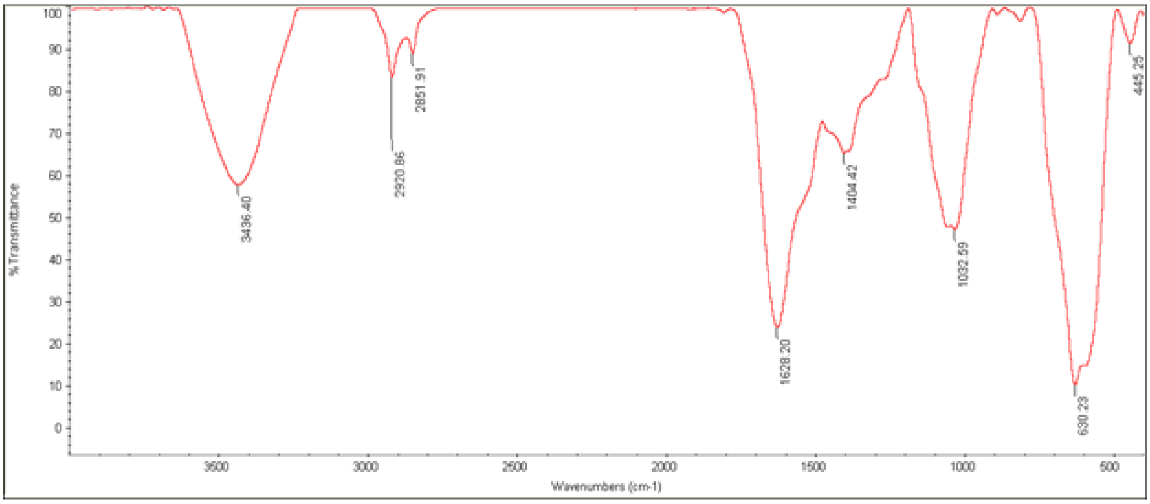

Fig. 6. FT-IR spectra for azolla@Fe_3O_4 NCs.

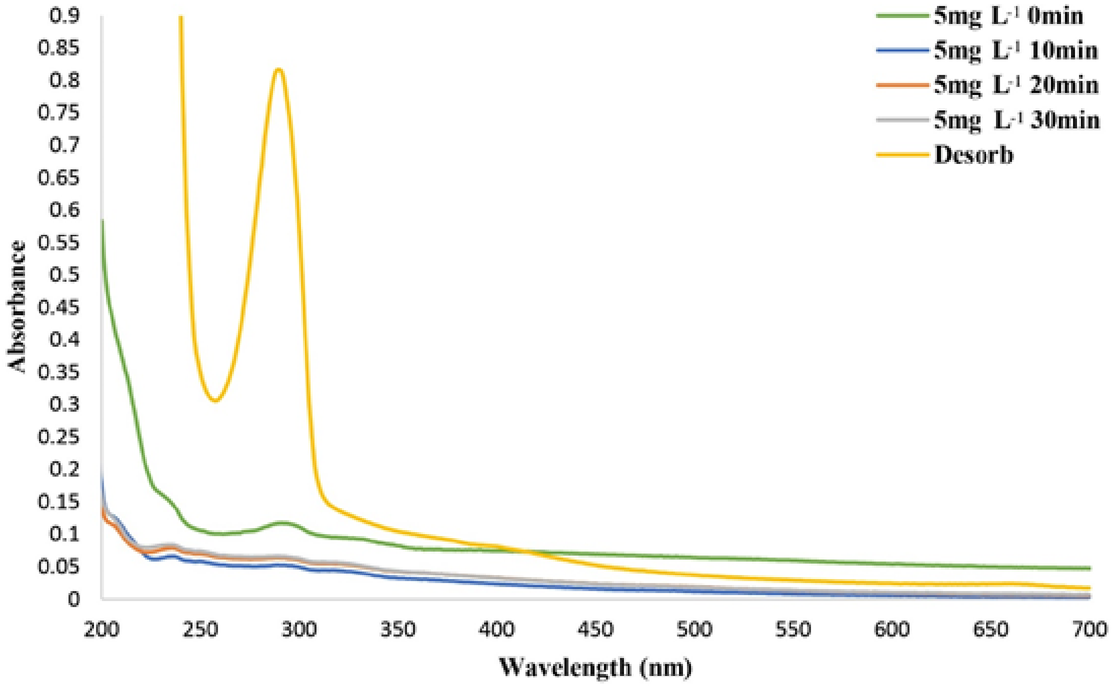

Fig. 7. UV-Vis spectra of the residual solutions after MSPE process using azolla@Fe_3O_4 NCs adsorbent during 5-30 min for 5 mg L^{-1} chlorpyrifos solutions a long with UV-Vis spectra of desorbed solution (EtOH).

3.Conclusion

In this study, we aimed to preconcentrate chlorpyrifos pesticide from aqueous solutions with MSPE method prior to UV-Vis spectroscopy using magnetite loaded azolla powder as a low cost and natural adsorbent. The azolla fern that was used as a natural adsorbent is recognized as an environmental problem and is freely available. The produce azolla@Fe_3O_4 NCs are stable during the time, therefore it is possible to synthesis these NCs once in a large volume and use them many times. The results demonstrate the potential of utilizing natural sources, such as azolla filiculoides fern, in developing eco-friendly and efficient methods for the removal of environmental pollutants. The use of magnetite modified azolla nanocomposites as a solid phase extraction material offers a simple and cost-effective approach for preconcentration and determination of trace amounts of chlorpyrifos pesticide. This method has the potential to be applied in various environmental aqueous samples, contributing to the preservation and protection of our environment.

References

Alizadeh N, Shariati Sh, Besharati N (2017) “Adsorption of Crystal Violet and Methylene Blue on Azolla and Fig Leaves Modified with Magnetite Iron Oxide Nanoparticles” Int. J. Environ. Res. 11: 197-206.

Alloway B, and Ayres D C (1997) Chemical Principles of Environmental Pollution. Taylor & Francis, London.

Bagheri A.R, Ghaedi M, Asfaram A, Bazrafshan A.A, Jannesar R (2017) “Comparative Study on Ultrasonic Assisted Adsorption of Dyes from Single System onto Fe3O4 Magnetite Nanoparticles Loaded on Activated Carbon: Experimental Design Methodology.” Ultrason Sonochem. 34: 294–304.

Besharati N, Alizadeh N, Shariati Sh (2021) “Removal of Tetracycline from Aqueous Solution by Azolla, Fig Leaves, Eggshell and Egg Membrane Modified with Magnetite Nanoparticles.” Desalin. Water Treat. 225: 214–224.

Kohler M, Fritzsche W (2004) “Nanotechnology. An Introduction to Nanostructuring Techniques. By Michael Köhler and Wolfgang Fritzsche.” Wiley 43(43): 5723.

Darvishi M, Shariati Sh, Safa F, Islamnezhad A (2021) “Surface Blocking of Azolla Modified Copper Electrode for Trace Determination of Phthalic Acid Esters as the Molecular Barricades by Differential Pulse Voltammetry: Response Surface Modelling Optimized Biosensor.” RSC Adv. 11(52): 32630–32646.

Darvishi M, Shariati Sh, Safa F, Islamnezhad A (2023) “Magnetite Azolla Impedimetric Nanobiosensor for Phthalic Acid Esters Quantification.” Anal. Methods 15: 1985–1997.

Faraji M, Yamini Y, Saleh A, Rezaee M, Ghambarian M, Hassani R (2010) “A Nanoparticle-Based Solid-Phase Extraction Procedure Followed by Flow Injection Inductively Coupled Plasma-Optical Emission Spectrometry to Determine Some Heavy Metal Ions in Water Samples.” Anal. Chim. Acta 659(1–2): 172–177.

Golzary A, Tavakoli O, Rezaei Y, Karbassi A.R., (2018) “Wastewater Treatment by Azolla Filiculoides (A Study on Color, Odor, COD, Nitrate, and Phosphate Removal).” Pollution 4(1): 69-76.

Hamadeen H.M, Elkhatib E.A, Badawy M.E.I, Abdelgaleil S.A.M (2021) “Novel Low Cost Nanoparticles for Enhanced Removal of Chlorpyrifos from Wastewater: Sorption Kinetics, and Mechanistic Studies.” Arab. J. Chem. 14(3): 102981.

Hui T.J, Ariffin M.M, Tahir N.M (2010) “Hydrolysis of Chlorpyrifos in Aqueous Solutions at Different Temperatures and pH.” Malaysian J. Anal. Sci. 14(2): 50–55.

Lin Sh.H, Lai S.L, Leu H.G (2000) “Removal of Heavy Metals from Aqueous Solution by Chelating Resin in a Multistage Adsorption Process.” J. Hazard. Mater. 76(1): 139–153.

Lumpkin T.A, Plucknett D,L (1980) “Azolla: Botany, Physiology, and Use as a Green Manure.” Econ. Bot. 34(2): 111–153.

Matsumura F (1985) Toxicology of Insecticides. Springer US. Boston, 111–202.

Mitra S, Brukh R (2003) “Sample Preparation: An Analytical Perspective.” In Sample Preparation Techniques in Analytical Chemistry, Wiley. Canada. https://doi.org/10.1002/0471457817.ch1.

Rahnama Sa, Shariati Sh, Divsar F (2021) “Selective Aptamer Conjugation to Silver-Coated Magnetite Nanoparticles for Magnetic Solid-Phase Extraction of Trace Amounts of Pb^{2+} ions.” RSC Adv. 11(9): 4971–4982.

Roberts A.E, Boylen Ch.W, Nierzwicki-Bauer S.A (2014) “Effects of Lead Accumulation on the Azolla Caroliniana-Anabaena Association.” Ecotoxicol. Environ. Saf. 102(1): 100–104.

Shariati Sh, M Bostani, F Shariati, S Rahnama (2020) “Application of Magnetite Nanoparticle Modified Azolla as an Adsorbent for Removal of Brilliant Blue Dye.” J. Color Sci. Tech. 15(2): 131–141.

Shariati F, Shariati Sh, Amiri Moghaddam MA. (2021) “Application of Magnetite Nanoparticles Modified Azolla as an Adsorbent for Removal of Reactive Yellow Dye from Aqueous Solutions.” Desalin. Water Treat. 212: 323–332.

Toutounchi S, Shariati Sh, Mahanpoor K (2021) “Application of Magnetic Ordered Mesoporous Carbon Nanocomposite for the Removal of Ponceau 4R Using Factorial Experimental Design.” Silicon 13(5): 1561–1573.

Vafaei F, Khataee A.R, Movafeghi A, Salehi Lisar S.Y, Zarei M (2012) “Bioremoval of an Azo Dye by Azolla Filiculoides: Study of Growth, Photosynthetic Pigments and Antioxidant Enzymes Status.” Int. Biodeterior. Biodegrad. 75: 194–200.