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Biosorption of Ni (II), Pb (II), and Cu (II) metal ions on the chitin isolated from spider species of *Drassodes lapidosus*

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Abstract: This study evaluates the biosorption ability of chitin derived from a specific spider species, namely *Drassodes lapidosus* (Walckenaer, 1802), that belongs to the family Gnaphosidae. The obtained chitin was characterized using Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), X-ray diffraction (XRD), and scanning electron microscopy (SEM) analysis. In the alpha form of the obtained chitin, its thermal stability was observed to be 356.9 °C and, the CrI value was 69.16%. In addition, this chitin was used as a bioadsorbent to remove heavy metal ions such as Ni (II), Cu (II), and Pb (II) from the aqueous medium by solid phase extraction (SPE) method using mini adsorption columns. The SPE method was optimized by changing one variable at a time. Main parameters affecting the SPE technique such as solution pH, sample volume, and interfering ions were optimized in model metal solutions containing Pb (II), Cu (II), and Ni (II) at certain concentrations. Metal concentrations were determined using a flame atomic absorption spectrophotometer (FAAS). The results revealed that Pb (II), Cu (II), and Ni (II) ions could be quantitatively removed from their environment at pH 5 through adsorption onto the surface of the chitin, even in the presence of possible interfering anions and cations at high concentrations. Pb (II), Cu (II), and Ni (II) could be separated quantitatively from aqueous solutions by using *Drassodes lapidosus* chitin as an adsorbent.

Key words: *Drassodes lapidosus*, chitin, solid phase extraction, heavy metal removal

1. Introduction

Biosorption is the sorption of analytes on the biologically derived materials through their physical and/or chemical binding abilities. The process of biosorption can be considered an environmentally friendly, economical, simple, and green alternative for removal of pollutants, especially in aquatic media. The materials used in the process are derived from biomass, which consists of chemical compounds originating from biological materials with varying degrees of transformation (Torres, 2020).

Many biological samples have been investigated and identified as biosorbents for removing various pollutants from water. Some of the most common biosorbents include agricultural waste materials (Taki et al., 2019), plant-derived samples (Medhi et al., 2020) and, biopolymers (Zhang et al., 2020).

Biopolymers are polymeric materials produced by living organisms. Their chemical structures consist of long, chain-like compounds containing repeating units of environmentally degradable monomers (Yaashikaa et al., 2022).

Chitin is the polysaccharide called N-acetyl-D-glucosamine, which is the most abundant in nature after cellulose and is connected by β-1,4-glycosidic bonds. Chitin is also a biocompatible and biodegradable nontoxic material. It has unique features such as enhanced chemical resistance and mechanical strength, low cost, and is both renewable and eco-friendly (Lv et al., 2023).

Chitin is found in the cell walls of fungi, the exoskeletons of insects, the shells of crustaceans, the teeth of mollusks, and the beaks of cephalopods. The main function of chitin is to provide protection and strength to fragile bodies of these organisms. The biosynthesis of chitin involves several catalytic reactions mediated by the enzyme chitin synthase. Insects and arthropods secrete chitin to form a hard exoskeleton. Industrially, chitin is produced by chemical and biological extraction from arthropods (Liu et al., 2019).

Because of these valuable properties, researchers focused on its adsorption abilities as a low-cost biosorbent for removal of pollutants from aqueous samples. Studies have primarily focused on chitin derived from crabs,

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shrimp shells, fungi, and insects. (Ahmed et al., 2020). However, the potential of the Araneae, including spiders, as an alternative source of chitin has been observed (Machałowski et al., 2019). There are approximately 55,000 spider species worldwide[1], yet only a few studies have explored spider chitin content and its physicochemical properties (Demir and Seyyar, 2020; Kaya et al., 2014). The spider *Drassodes lapidosus* belongs to the family Gnaphosidae, commonly known as ground spiders. Members of this species can be found all over the world. Their ease of collection from the field and abundant population make them a valuable material for studies on alternative chitin sources.

This study aims to separate and characterize the chemical features of chitin acquired from the spider species of *Drassodes lapidosus* for the first time. It explores the optimal sorption conditions for separating, preconcentrating, and removing Pb (II), Cu (II), and Ni (II) metal ions from aqueous samples using chitin derived from *Drassodes lapidosus*.

2. Materials and methods

2.1. Materials

2.1.1. Spider collection

Drassodes lapidosus (Walckenaer, 1802) is a common ground spider belonging to the family Gnaphosidae. It is spread out in Europe, Türkiye, Russia (from Europe to the Far East), the Caucasus, Israel, Central Asia, Iran, China, Japan, and Korea. This species is also widespread in Türkiye. It is found in stony and steppe areas. Specimens of *D. lapidosus* were obtained from steppe areas in the Central Anatolia by hand collection. Nearly 30 samples of *D. lapidosus* were collected from Niğde Province, and these samples were identified under a stereomicroscope according to the available literature. The general habitus of the species is shown in Figure 1.

2.2. Methods

2.2.1. Reagents and chemicals

All stock solutions were prepared by using purified water. All reagents and chemicals utilized were bought from Sigma-Aldrich and Merck. The glass equipment was stored in HNO_3 10 % (v/v) for 12 h, washed with distilled water, and dried at 50 °C before use. Standard solutions of Pb (II), Ni (II), and Co (II) were prepared using their respective nitrate salts, each at a concentration of 1000 mg L^{-1}. Model metal solutions were prepared by diluting the stock metal solutions during the optimization procedure of the column solid-phase extraction experiments.

2.2.2. Apparatus

A Shimadzu AA-7000 FAAS equipped with an air-acetylene burner was used to determine metal concentrations. A WTW level 1 model pH meter was utilized for pH measurements. Chitin drying was carried out using a Heraeus D-6450 model oven. An analytical balance was used for mass measurements. Lastly, 10 cm long glass mini adsorption columns with a diameter of 0.8 cm were used in solid phase extraction applications.

2.2.3. The extraction stages of chitin

Thirty *D. lapidosus* samples were washed, dried, and milled. The milled materials were then processed with solutions (100 mL of 2 M HCl) to eliminate the inorganic content at 60–65 °C for 2 h. Subsequently, the solutions were filtrated and the raw extracts were treated several times with pure water. After that, the isolated materials were placed in 50 mL of 1.0 M NaOH solutions to eliminate proteins. This stage lasted 16 h at 130–135 °C. The solutions were refiltered and rewashed with purified water. The extracts were treated in a mixture of methanol, chloroform, and water (2:1:4 ratio) for 1 h at ambient temperature. This stage caused discoloration and lipid elimination. Lastly, the process was completed by drying the washed chitin materials at 60 °C for 24 h (Figure 2).

2.2.4. FT-IR analysis

The FTIR peaks of chitin samples extracted from *Drassodes lapidosus* were analyzed using a Bruker Vertex 70 FT-IR spectrometer, covering a frequency range from 625 to 4000 cm^{-1}.

2.2.5. SEM analysis

The surface images of *D. lapidosus* chitin was comparatively analyzed with scanning electron microscopy (Carl Zeiss, Evo LS 10). The surfaces were coated with Au by sputter coating system (SCS) before SEM analysis.

2.2.6. TGA analysis

The chitin samples were analyzed using a TGA machine (STA PT1600) with a heating rate of 10 °C/min, ranging from 25 to 650 °C.

2.2.7. XRD analysis

The chitin samples were examined with a Rigaku D max 2000 at 2θ in the range of 5–45°. The value of crystalline index (CrI) was calculated using the following formula: $CrI110 = [(I110 - Iam)/I110] \times 100$. I110 = the maximum intensity at 2θ = 20°. Iam = the intensity of amorphous diffraction at 2θ = 16° (Sajomsang and Gonil, 2010).

2.2.8. General procedure for the column method

Co (II), Ni (II), and Pb (II) containing model solutions were prepared by adjusting total mass of each metal ion to 5.0 µg, 5.0 µg, and 10 µg, respectively in a 100 mL beaker. The pH solution was calibrated to 5.0 with the addition of 5 mL of acetate buffer solution. The obtained solution was diluted to 100 mL by using purified water. Five milliliters of buffer solution was used for conditioning the mini adsorption

[1]World Spider Catalog (2023). World Spider Catalog, version 24.5 [Online]. Website https://wsc.nmbe.ch/ [21 March 2024]

Figure 1. General appearance of *Drassodes lapidosus*.

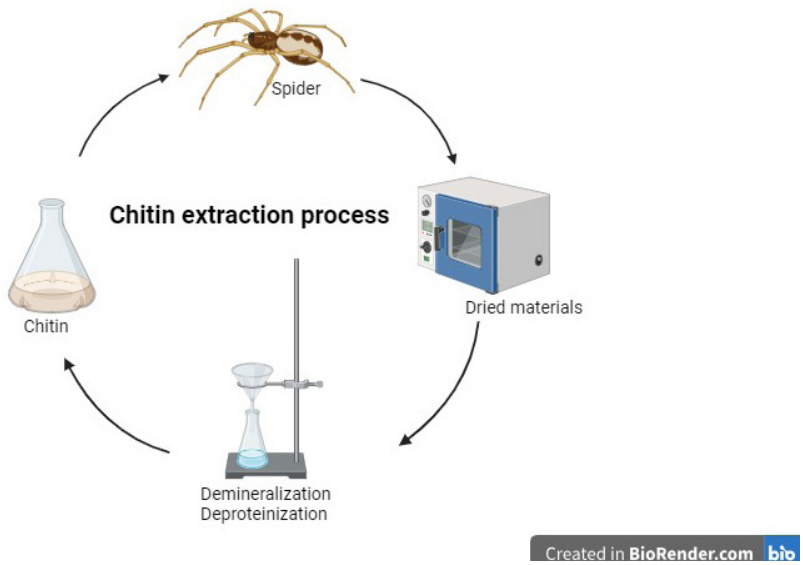

Figure 2. Scheme of chitin extraction process from the spider species, *D. lapidosus*.

column loaded with the chitin bioadsorbent. Next, the model metal solutions were passed through the column at a 1 mL min^{-1} flow rate. The bioadsorbent was eluted with 1.0 M HNO3 in methanol to 5 mL of total volume. Absorbance values of metal ions in the final solution were measured under air-acetylene flame of FAAS.

3. Results and discussion

3.1. Chitin characterization from Drassodes lapidosus

3.1.1. Chitin content of D. lapidosus

The chitin rate of crustaceans like crab and shrimp is about 20% by dry weight (Kucukgulmez et al., 2011; Wang et al., 2013). In insects, this rate is between 15% and 20% (Gonil and Sajomsang, 2012; Zhang et al., 2000). In this study, we reached the following findings. *D. lapidosus*'s dry weight chitin content was nearly 7%. This ratio is similar to that of other arachnids (Demir and Seyyar, 2020; Kaya et al., 2014; Seyyar and Demir, 2020). Since the abdomen is softer in spiders than in other arthropods, the amount of chitin is lower than that of crustaceans and insects. Although we might assume that this spider has soft opisthosoma, their chitin amount indicates that they are an important source of chitin.

3.1.2. FT-IR

In several studies, the structure of chitin analyzed by FT-IR spectroscopy, revealing characteristic peaks for α–chitin (Cho et al., 2000; Gonil and Sajomsang, 2012; Hu et al., 2007; Ifuku and Saimoto, 2012; Wang et al., 2013; Yen et al., 2009; Zhang et al., 2000). These bands are: 3259 (N-H stretching), 1650 (Amide I), 1621 (Amide I), and 1550 cm^{-1} (Amide II). In this research, chitin extracted from the *D. lapidosus* was analyzed by FTIR. Two peaks around 1650 and 1621 cm^{-1} were observed, consistent with former studies (Figure 3). Additional peaks are listed in Table 1. These findings indicate that the chitin extracted from *D. lapidosus* is in the α-form.

3.1.3. XRD

The XRD findings of chitin were scanned at 2θ angles between 5 and 45°, showing peaks (Figure 4). As depicted in the figure, isolated chitins exhibit two sharp peaks around 9° and 19°. Similar peak patterns were observed in XRD results from shrimp, crab, insects, and krill, consistent with those reported in this study (Liu et al., 2012; Sajomsang and Gonil, 2010; Wang et al., 2013; Yen et al., 2009). The crystalline index value (CrI) of *D. lapidosus* chitin is 69.16%. Liu et al. (2012) reported crystalline index (CrI) values for chitin extracted from an insect and a shrimp as 89.05% and 89.17%, respectively. In contrast, CrI values for silkworm pupa and larvae cuticles of B.

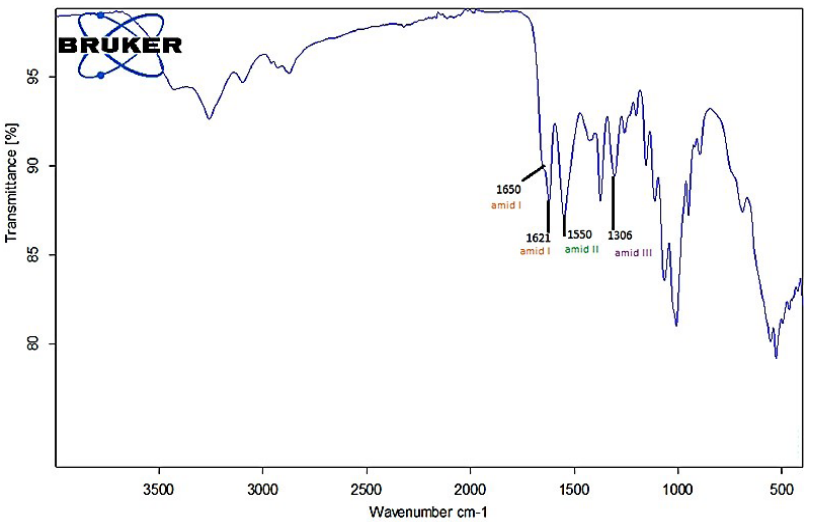

Figure 3. FTIR bands of chitin samples isolated from *D. lapidosus*.

Table 1. FTIR bands of the chitin isolated from *D. lapidosus*.

Functional group and vibration modes	Classification	*D. lapidosus*
O-H stretching	-	-
N-H stretching		3259.47
CH_3 sym. stretch and CH_2 asym. stretch	Aliphatic compounds	3098.15
CH_3 sym. stretch	Aliphatic compounds	2875.57
C=O secondary amide stretch	Amide I	1650.20
C=O secondary amide stretch	Amide I	1621.20
N-H bend, C-N stretch	Amide II	1550.54
CH_2 ending and CH_3 deformation	-	1428.49
CH_3 sym. deformation	-	1374.19
CH_2 wagging	Amid III, components of protein	1306.27
Asymmetric bridge oxygen stretching	-	1154.7
Asymmetric in phase ring stretching mode	-	1112.59
C-O-C asym. stretch in phase ring	Saccharide rings	1065.21
C-O asym. stretch in phase ring	-	1008.83
CH_3 wagging	Along chain	951.11
CH ring stretching	Saccharide rings	894.81

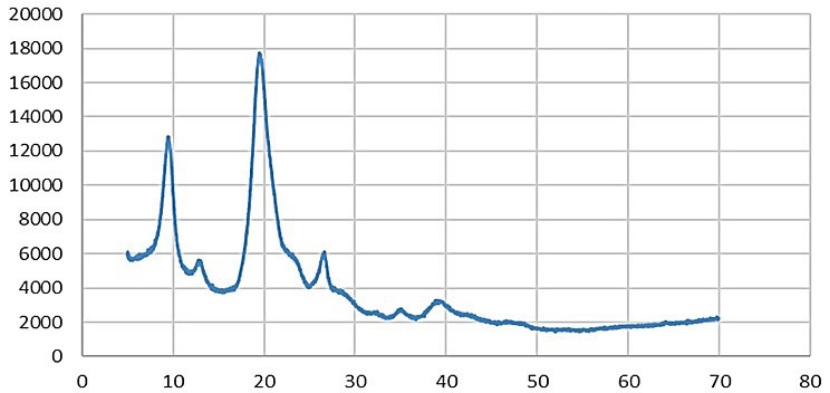

Figure 4. X-ray diffraction patterns of α-chitins from *D. lapidosus*.

mori ranged between 54% and 58% (Zhang et al., 2000). Additionally, the crystalline index (CrI) values for chitin from spiders and opilionids were recorded as 70.1% and 69.6%, respectively (Seyyar and Demir, 2020; Yürtmen and Seyyar, 2019). Comparatively, the CrI value of *D. lapidosus* was found to be similar to other arachnid groups.

3.1.4. TGA

In previous studies, the findings of TGA analysis of chitins isolated from living organisms such as crabs, shrimps, and insects revealed that mass losses take place in two different stages (Abdou et al., 2008; Al Sagheer et al., 2009; Juárez-de La Rosa et al., 2012). The first mass loss is from water evaporation in the chitin, and the second is from decomposition of the chitin structure (Wang et al., 2013). In this study, the mass loss in the chitin in *D. lapidosus* occurred in two different steps, similar to former studies (Figure 5). For *D. lapidosus*, the first mass loss was 8% and the second mass loss was 72%. The mass loss occurred at 0–150 °C, as a result of the removal of H_2O-molecules in the first stage, while the second mass loss occurred at 150–400 °C due to the deterioration of chitin compounds. The highest deterioration temperature of chitin (DTGmax) observed for *D. lapidosus* was 356.9 °C. The TGA values of spider, crab, shrimp, krill, and insect chitin showed a two-stage mass loss similar to that in our work.

3.1.5. SEM

The surface image is one of the most important properties that significantly influences the use of chitin and its derivatives (Aranaz et al., 2009). The optimal utilization field for chitin can be determined based on its surface image. The morphology of chitin obtained from arachnid and crustaceans such as shrimp, krill and crab reveals a nanofibre structure with pores (Seyyar and Demir, 2020; Yürtmen and Seyyar, 2019). In the current study, the morphology of the chitin extracted from the *D. lapidosus* was investigated and a different type of surface morphology was observed. In this type of chitin, the surface is composed of long and wide nanofibers, but nanopores are not observed (Figure 6). When compared with the chitin morphology of the previously studied *Hogna radiata* and *Geolycosa vultuosa* spiders (Kaya et al., 2014), it was observed that *D. lapidosus* chitin has a different surface morphology than the other two species with its lack of pores. Further studies are needed to learn more about the biological or ecological reasons for these differences in chitin morphology of spider species.

3.2. pH effect

The adsorption of metal ions onto a solid phase strongly depends on solution pH. In our experiments, we investigated the influence of solution pH on the recovery of Ni(II), Co(II), and Pb(II) using chitin bio-adsorbent over a pH range of 2–8. The pH adjustments of the model solutions were made by various buffer solutions with 0.25 M ionic strength. The obtained results are presented in Figure 7.

According to Figure 1, Ni (II), Co (II), and Pb (II) recoveries were pH-dependent and quantitatively recovered at pH 5. This can be explained by chemical and/or physical interactions between bioadsorbent surface and metal ions that preferentially and favorably occurred at pH 5 because of surface binding characteristics. Further adsorption studies were performed at pH 5.

3.3. Effect of eluent type

Desorption experiments are important for preconcentration and determination studies. The solution should break the physical and/or chemical bonds between the metal ions studied and the bioadsorbent. To achieve this, 1 mol L^{-1} HNO_3 and HCl solutions were prepared using three different solvents (acetone, ethanol, and water). The model solutions were passed through the column, and 5 mL of eluent was added on the bioadsorbent. The obtained results are given in Table 2.

The recovery values for all studied metal ions were quantitative in 1 mol L^{-1} HNO_3 in ethanol; thus, this solution was chosen as the optimum eluent solution.

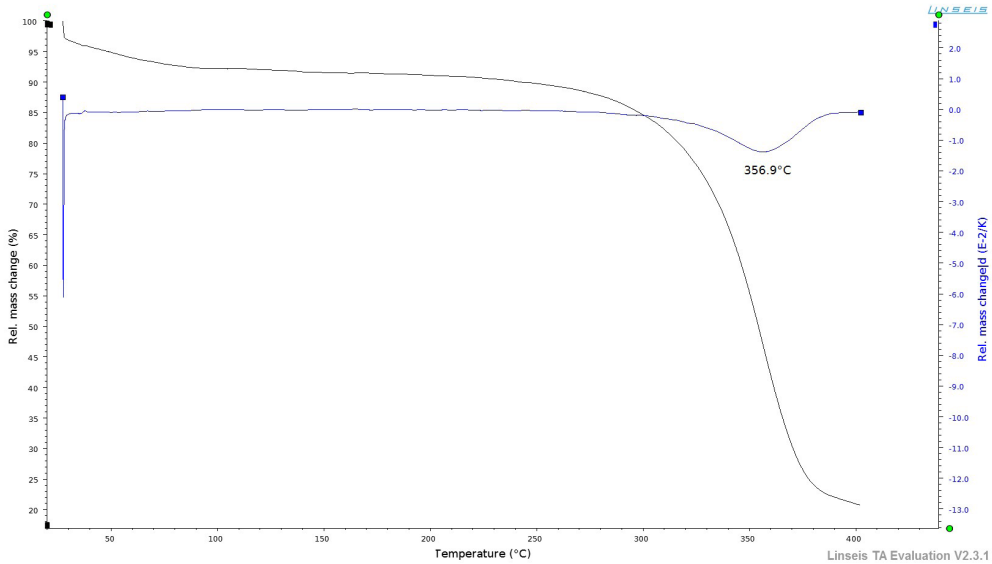

Figure 5. TGA curves for chitin from *D. lapidosus*.

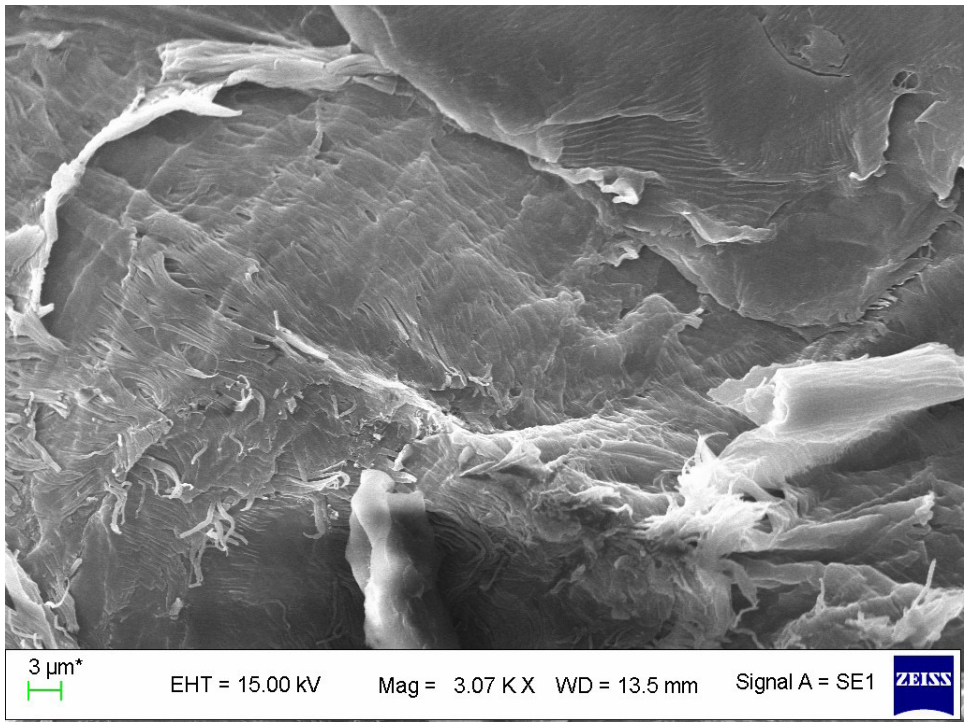

Figure 6. SEM photographs of chitin from *D. lapidosus*.

3.4. Effect of sample volume

The sample volume is a key parameter for achieving satisfactory preconcentration factors in column solid phase extraction studies. The effect of sample volume on the recovery of studied metal ions on the bioadsorbent chitin was investigated over a range of 25–500 mL. The results are presented in Table 3.

Quantitative recoveries of Cu and Pb were observed up to a sample volume of 250 mL, whereas Ni was quantitatively recovered up to a sample volume of 100 mL. The preconcentration factor (PF) values were calculated as 50 for Cu and Pb, and 20 for Ni, based on the ratio of the maximum sample volume to the final elution volume.

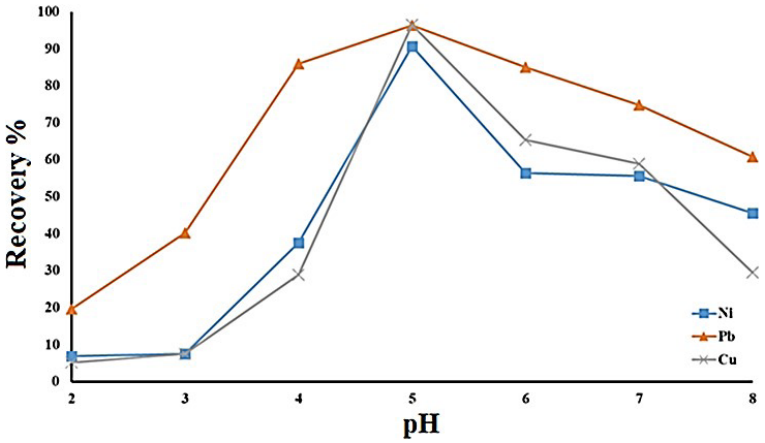

Figure 7. Effect of pH.

Table 2. Effect of the eluent type.

Eluent type	Recovery		
	Ni^{2+}	Co^{2+}	Pb^{2+}
1 mol L^{-1} HNO_3 in acetone	91	92	91
1 mol L^{-1} HNO_3 in ethanol	97	95	100
1 mol L^{-1} HCl in acetone	90	92	94
1 mol L^{-1} HCl in ethanol	94	95	95
1 mol L^{-1} HNO_3	27	35	40
1 mol L^{-1} HCl	20	41	55

Table 3. Effect of the sample volume on the preconcentration of Cu^{2+}, Pb^{2+}, and Ni^{2+} ions.

Sample volume (mL)	Recovery (%)		
	Cu (II)	Ni (II)	Pb (II)
25	99	96	98
50	97	94	96
100	95	90	95
250	94	73	90
500	59	52	41

3.5. Interfering ion effect

The influence of major cations and anions on the analytical signal of Cu^{2+}, Pb^{2+} and Ni^{2+} ions of the proposed technique was investigated under optimized conditions. The experiments were conducted at ambient temperature and the tolerable concentrations of interfering ions are presented in Table 4.

The results of the interference experiments showed that the proposed method was not affected by the presence of the studied anions and cations up to the limits given in Table 4.

3.6. Stability and reusability of the bioadsorbent

The stability and reusability of the chitin bioadsorbent were studied under optimal conditions by repeating the experiments up to 25 cycles on the same chitin bioadsorbent, and the obtained results are presented in Figure 8.

The stability of the chitin bioadsorbent was evaluated based on the recovery values of Cu^{2+}, Pb^{2+}, and Ni^{2+} ions adsorbed onto the chitin surface. According to Figure 8, the recovery values remained stable and quantitative for Pb and Cu ions, while the recovery of the Ni ions remained above 90% even after 25 cycles.

3.7. Greenness evaluation

The analytical greenness of the proposed solid phase extraction method was evaluated using AGREE (Analytical GREEnness), a novel software designed to assess the environmental sustainability of analytical techniques (Pena-Pereira et al., 2020). The obtained results are presented in Figure 9.

Table 4. Effect of interfering ions on the preconcentration of Cu^{2+}, Pb^{2+}, and Ni^{2+} ions.

Interfering ion	Added as	Concentration (ppm)	Recovery (%)		
			Cu^{2+}	Ni^{2+}	Pb^{2+}
Cd^{2+}	$Cd(NO_3)_2.6H_2O$	25	94	98	94
Co^{2+}	$Co(NO_3)_2$	25	94	95	94
Cr^{3+}	$Cr(NO_3)_3.3H_2O$	25	97	97	92
Al^{3+}	$Al(NO3)_3.9H_2O$	20	96	92	98
Na^{+}	$NaNO_3$	1000	97	98	95
K^{+}	KNO_3	500	94	99	93
Ca^{2+}	$CaCl_2$	100	92	96	95
Mg^{2+}	$Mg(NO_3)_2.6H_2O$	100	91	93	92
Cl^{-}	NaCl	100	96	95	100
NO_2^{-}	$NaNO_2$	500	95	94	97
NO_3^{-}	$NaNO_3$	1000	97	98	95
SO_4^{2-}	Na_2SO_4	100	95	95	95

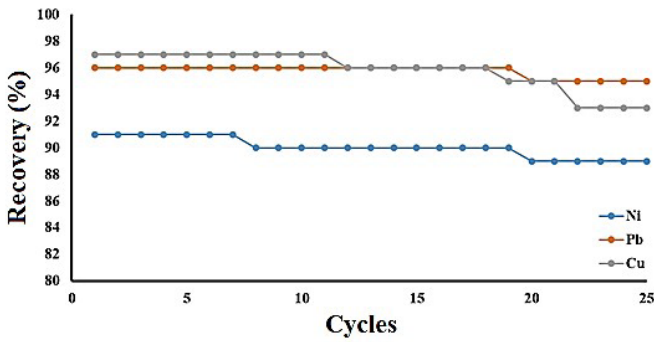

Figure 8. Stability and reusability of the bioadsorbent.

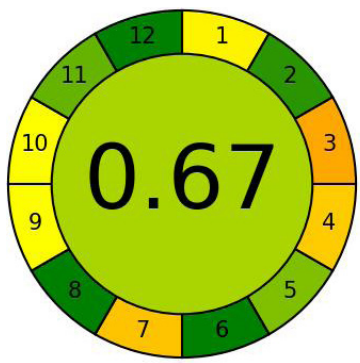

Figure 9. AGREE pictogram of the proposed SPE technique.

AGREE pictograms use green, yellow, and red colors to indicate 12 parameters of the method, scoring them from 0 to 1. A score closer to 1 indicates a greener method, while closer to 0 suggests that the method does not fully meet greenness conditions. Yellow and red colors indicate degrees of deviation from greenness, moving further away from it. After AGREE evaluation, a value displayed in the center of the pictogram (color-coded according to the score) indicates the method's level of greenness (Salamat and Soylak, 2024). In the evaluation of our experiments, the average AGREE score of the SPE method was 0.67 and the average color was green. According to this score, the proposed SPE technique has a low environmental impact and can be regarded as a green method.

4. Conclusion

In this study, the chitin structure of *D. lapidosus* has been isolated and characterized for the first time. *D. lapidosus* contains less chitin compared to crustaceans and insects, yet it is comparable to arachnids. The chitin isolated from *D. lapidosus* was found to exhibit identical FTIR bands, water content as evaluated by TGA, percentage mass losses recorded in the second step, and XRD peaks. On the other hand, this species has distinct DTGmax values and SEM surface morphology. The chitin separated from this species for this study had a lower DTGmax value than that isolated from other living things, including shrimp, crabs, and insects (Abdou et al., 2008; Al Sagheer et al., 2009; Kaya et al., 2014; Sajomsang and Gonil, 2010; Wang et al., 2013). The SEM surface view of *D. lapidosus* chitin exhibits a notable departure from earlier studies, with clear nanofibers lacking nanopores. For spider chitin, this surface appearance is rare. Consequently, many biotechnological objectives can be evaluated using the distinct chitin without nanopores found in this study. Apart from the possibility of exploring novel uses for chitin, this chitin structure can be utilized in more research. This work has demonstrated that *D. lapidosus* chitin, which has a long and broad nanofiber surface shape, has dramatically increased heavy metal adsorption capabilities. The research showed that chitin obtained from the *D. lapidosus* species could efficiently extract lead, nickel, and copper metal ions from an aqueous solution, with an extraction efficiency above 95%. Other heavy metal ions can be removed with this simple, effective, and economical heavy metal removal approach that has been used on copper, lead, and nickel ions. Thus, it can be considered that *D. lapidosus*-derived chitin can be used as a heavy metal adsorbent.

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References

Abdou ES, Nagy KSA, Elsabee MZ (2008). Extraction and characterization of chitin and chitosan from local sources. Bioresource Technology 99 (5): 1359-1367. https://doi.org/10.1016/j.biortech.2007.01.051

Ahmed MJ, Hameed BH, Hummadi EH (2020). Review on recent progress in chitosan/chitin-carbonaceous material composites for the adsorption of water pollutants. Carbohydrate Polymers 247: 116690. https://doi.org/10.1016/j.carbpol.2020.116690

Al Sagheer FA, Al-Sughayer MA, Muslim S, Elsabee MZ (2009). Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. Carbohydrate Polymers 77 (2): 410-419. https://doi.org/10.1016/j.carbpol.2009.01.032

Aranaz I, Mengíbar M, Harris R, Paños I, Miralles B et al. (2009). Functional characterization of chitin and chitosan. Current Chemical Biology 3 (2): 203-230. https://doi.org/10.2174/187231309788166415

Cho YW, Jang J, Park CR, Ko SW (2000). Preparation and solubility in acid and water of partially deacetylated chitins. Biomacromolecules 1 (4): 609-614. https://doi.org/10.1021/bm000036j

Demir Z, Seyyar O (2020). Isolation and physicochemical characterization of chitin and chitosan from *Aculepeira ceropegi*a (Walckenaer, 1802). Acta Biologica Turcica 33 (4): 252-257.

Gonil P, Sajomsang W (2012). Applications of magnetic resonance spectroscopy to chitin from insect cuticles. International Journal of Biological Macromolecules 51 (4): 514-522. https://doi.org/10.1016/j.ijbiomac.2012.06.025

Hu X, Du Y, Tang Y, Wang Q, Feng T et al. (2007). Solubility and property of chitin in NaOH/urea aqueous solution. Carbohydrate Polymers 70 (4): 451-458. https://doi.org/10.1016/j.carbpol.2007.05.002

Ifuku S, Saimoto H (2012). Chitin nanofibers: preparations, modifications, and applications. Nanoscale 4 (11): 3308-3318. https://doi.org/10.1039/C2NR30383C

Juárez-de La Rosa BA, Quintana P, Ardisson P-L, Yáñez-Limón JM, Alvarado-Gil JJ (2012). Effects of thermal treatments on the structure of two black coral species chitinous exoskeleton. Journal of Materials Science 47: 990-998. https://doi.org/10.1007/s10853-011-5878-9

Kaya M, Seyyar O, Baran T, Erdoğan S, Kar M (2014). A physicochemical characterization of fully acetylated chitin structure isolated from two spider species: with new surface morphology. International Journal of Biological Macromolecules 65: 553-558. https://doi.org/10.1016/j.ijbiomac.2014.02.010

Kucukgulmez A, Celik M, Yanar Y, Sen D, Polat H et al. (2011). Physicochemical characterization of chitosan extracted from *Metapenaeus stebbing*i shells. Food Chemistry 126 (3): 1144-1148. https://doi.org/10.1016/j.foodchem.2010.11.148

Liu S, Sun J, Yu L, Zhang C, Bi J et al. (2012). Extraction and characterization of chitin from the beetle *Holotrichia parallela* Motschulsky. Molecules 17 (4): 4604-4611. https://doi.org/10.3390/molecules17044604

Liu X, Zhang J, Zhu KY. Chitin in Arthropods: Biosynthesis, Modification, and Metabolism. Advances in Experimental Medicine and Biology 2019; 1142: 169-207. https://doi.org/10.1007/978-981-13-7318-3_9

Lv J, Lv X, Ma M, Oh D-H, Jiang Z et al. (2023). Chitin and chitin-based biomaterials: a review of advances in processing and food applications. Carbohydrate Polymers 299: 120142. https://doi.org/10.1016/j.carbpol.2022.120142

Machałowski T, Wysokowski M, Tsurkan MV, Galli R, Schimpf C et al. (2019). Spider chitin: an ultrafast microwave-assisted method for chitin isolation from *Caribena versicolor* spider molt cuticle. Molecules 24 (20): 3736-3755. https://doi.org/10.3390/molecules24203736

Medhi H, Chowdhury PR, Baruah PD, Bhattacharyya KG (2020). Kinetics of aqueous Cu (II) biosorption onto *Thevetia peruviana* leaf powder. ACS Omega 5 (23): 13489-13502. https://doi.org/10.1021/acsomega.9b04032

Pena-Pereira F, Wojnowski W, Tobiszewski M (2020). AGREE—Analytical GREEnness metric approach and software. Analytical Chemistry 92 (14): 10076-10082. https://doi.org/10.1021/acs.analchem.0c01887

Sajomsang W, Gonil P (2010). Preparation and characterization of α-chitin from cicada sloughs. Materials Science and Engineering: C 30 (3): 357-363. https://doi.org/10.1016/j.msec.2009.11.014

Salamat Q, Soylak M (2024). Magnetic covalent organic frameworks-based adsorbents in solid phase extraction of trace analytes in environmental samples. Trends in Environmental Analytical Chemistry 41: e00222. https://doi.org/10.1016/j.teac.2023.e00222

Seyyar F, Demir H (2020). Extraction and physicochemical characterization of chitin from *Phalangium opilio* Linnaeus, 1758 (Arachnida: Opiliones). Acta Biologica Turcica 33 (4): 258-263.

Taki K, Gogoi A, Mazumder P, Bhattacharya SS, Kumar M (2019). Efficacy of vermitechnology integration with Upflow Anaerobic Sludge Blanket (UASB) and activated sludge for metal stabilization: a compliance study on fractionation and biosorption. Journal of Environmental Management 236: 603-612. https://doi.org/10.1016/j.jenvman.2019.01.006

Torres E (2020). Biosorption: a review of the latest advances. Processes 8 (12): 1584-1599. https://doi.org/10.3390/pr8121584

Wang Y, Chang Y, Yu L, Zhang C, Xu X et al. (2013). Crystalline structure and thermal property characterization of chitin from Antarctic krill (*Euphausia superba*). Carbohydrate Polymers 92 (1): 90-97. https://doi.org/10.1016/j.carbpol.2012.09.084

Yaashikaa PR, Kumar PS, Karishma SJER (2022). Review on biopolymers and composites–Evolving material as adsorbents in removal of environmental pollutants. Environmental Research 212: 113114. https://doi.org/10.1016/j.envres.2022.113114

Yen MT, Yang JH, Mau JL (2009). Physicochemical characterization of chitin and chitosan from crab shells. Carbohydrate Polymers 75 (1): 15-21. https://doi.org/10.1016/j.carbpol.2008.06.006

Yürtmen H, Seyyar O (2019). *Chaetopelma olivaceum* (C.L. Koch 1841) (Araneae: Theraphosidae) türünün kitin yapısının karakterizasyonu. International Turkic World Congress on Science and Engineering (UTUFEM, 2019) 1: 140-145.

Zhang J, Wang P, Zhang Z, Xiang P, Xia S (2020). Biosorption characteristics of Hg (II) from aqueous solution by the biopolymer from waste activated sludge. International Journal of Environmental Research and Public Health 17 (5): 1488-1501. https://doi.org/10.3390/ijerph17051488

Zhang M, Haga A, Sekiguchi H, Hirano S (2000). Structure of insect chitin isolated from beetle larva cuticle and silkworm (*Bombyx mori*) pupa exuvia. International Journal of Biological Macromolecules 27 (1): 99-105. https://doi.org/10.1016/S0141-8130(99)00123-3