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An Eco-Friendly Absorption Method of Cu^{2+}, Cd^{2+}, and Pb^{2+} Using the Shells and Chitosan Derived from *Solen vagina*

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ABSTRACT

The processing of Mollusca for food consumption generates a large amount of by-products; for instance, shells, which may contaminate and deposit in the marine coastal environment. Therefore, additional processing is needed to reduce and transform shells into valuable materials, such as chitosan or another derivate product. This study aimed to isolate and characterize the chitosan from *Solen vagina* (known as *lorjuk* shells in Indonesian) and to determine the application of chitosan. This is to be compared with *Solen vagina* shell powder that commonly functions as bio-sorbent of water pollutants Cu^{2+}, Cd^{2+}, and Pb^{2+}. The isolated chitosan was characterized based on its physicochemical properties, namely purity as tested by using X-ray fluorescence (XRF), and functional group as confirmed by Fourier transform infrared (FTIR). In addition, bio-absorbent ability was evaluated through a column method, where the chitosan products are used and compared with *Solen vagina* shell powders. In this study, the chitosan isolated from *Solen vagina* shells yielded 15.92 ± 1.78% and showed a high deacetylation degree (DD) by 85.00 ± 3.98%. FTIR and physicochemical properties analysis confirmed that the isolated chitosan is of good quality, as standardized by industry regulator; thus, it could be used as food product and bio-absorbent material. Moreover, the bio-absorbent ability of chitosan demonstrated a similar value with *S. vagina* shell powders, which can absorb more than 92% of heavy metals around second elution. In conclusion, the *S. vagina* shell powder and the isolated chitosan have the potential as natural bio-absorbent to reduce the heavy metal contents in industrial wastewater.

Keywords: *Solen vagina*; chitosan; mollusca shell; biosorbent ability; water pollution; zero waste

INTRODUCTION

The problem of heavy metal contamination in water and wastewaters has become a global issue. Increased industrialization releases large amounts of heavy metal pollutants into the aquatic ecosystem. Intensive industrial and human activities have resulted in the release of heavy metal waste into the environment (Mohiuddin *et al*., 2011). If the pollution continues, environmental degradation will be inevitable. The presence of heavy metal contamination in fresh water and coastal marine ecosystem will accumulate in the bodies of mudskipper, shrimp, crabs, shellfish, and various types of aquatic biota, which will subsequently affect the health of the consumers (Arifin *et al*., 2012; Heidarieh *et al*., 2013; Sangur *et al*., 2021). In addition, it might cause a serious environmental problem to the coastal area community, such as the *itai-itai* disease (Melgar *et al*., 2016). The health impacts may vary, as the toxicity level of heavy metal pollutants depends on many factors, such as the chemical type, doses, route of exposure, as well as the exposed individual's age, sex, genetics, and nutritional status (Tchounwou *et al.*, 2012).

There are commonly applied technologies to remove heavy metals from water and polluted aquatic environments, such as chemical precipitation, ion exchange, membrane technologies,

and electrochemical treatments. However, these techniques have been reported to be ineffective, expensive, inefficient, and associated with secondary waste generation that creates treatment problems. Considering this, the current study focused on determining an alternative method that is innovative, eco-friendly, efficient, and effective in reducing the heavy metal contamination in the water environment, such as bio-absorbent using biological materials. Various materials are reported to be used in bio-absorption processes, including microbial material (Ahemad & Kibret, 2013), garlic peel (Sun *et al.*, 2018), and the *Ficus religiosa* leaf powder (Goyal *et al.*, 2011). The removal and absorption mechanism of Cu^{2+} and Cd^{2+} from aqueous layer were discovered by using a bio-absorption extracted from activated sludge waste (Zhang *et al*., 2014). Kelly-Vargas *et al*., (2012) reported that the use of fruit waste as an absorbent such as banana peel, lemon skin, and orange skin, showed an absorption ability of heavy metals through a metabolic process; and that the ability depends on the physicochemical properties of a material.

Marine biotas, such as shellfish, shrimps, and crabs are consumed as a source of protein, but their shells are disposed of as waste that pollutes the coastal environment. According to the observation made by the authors in some coastal area in East Java, Indonesia, shell waste potentially reaches 156 tons per year; it is unused and might be deposited in the coastal area. This study aimed to seek the potential of this shellfish waste as a bio-absorbent material and as a raw material for chitosan. In this study, *S. vagina* (commonly known as *lorjuk* in Indonesian) shell waste was converted to shell powder, and extracted into chitosan, which was then used in heavy metal bio-absorbent modeling. This research aimed to determine and compare the potential of shell powder and chitosan isolated from the *S. vagina* shell as a heavy metal bio-absorbent. The chitosan produced was compared with unprocessed shell powder and tested for its ability as a bio-absorbent against heavy metals, i.e. Cu^{2+}, Cd^{2+}, and Pb^{2+} (Kelly-Vargas *et al*., 2012). This study should contribute to the literature of bio-absorbent material from *S. vagina* because, to the best of the authors' knowledge, the research on bio-absorbent of heavy metals using isolated chitosan and *S. vagina* shell powder waste remains lacking.

MATERIALS AND METHODS

Materials

The shells of *Solen vagina* (commonly known as *lorjuk*) (0.2–0.9 g per shell) were collected from Karang Entang, Kwanyar, Bangkalan, East Java, Indonesia. The samples were identified by Dr Moch Affandi from Faculty of Science and Technology, Universitas Airlangga, Indonesia. Hydrochloric acid (HCl) (37%, analytical grade) was purchased from Sigma-Aldrich®, chitin standard (Agency for the Assessment and Application of Technology (BPPT) – Indonesia), chitosan standard (Sigma-Aldrich®). $CuSO_4.5H_2O$ (analytical grade), standard solutions of $Pb(NO_3)_2$ and standard solutions of $Cd(NO_3)_2$ (analytical grade), HNO_3 65% (analytical grade), NaOH (analytical grade), and KBr (analytical grade for FTIR), were purchased from Merck, whereas the demineralized water was purchased from PT – Brataco, Surabaya, Indonesia.

Isolation of chitosan from S. vagina shells

Chitosan was extracted by following the method described by Zamri *et al.* (2020) with a slight modification. The *S. vagina* shells were washed in hot (90°C) tap water, dried, then ground (mesh number 100). A 100 grams of the ground material were deproteinated with 3% NaOH 1: 6 (w/v), at 85°C, for 30 minutes; then rinsed until it reached neutral pH, filtered, then dried at 35°C for 24 hours. The sample was then demineralized with 1N HCl 1:10 (w/v), at 75°C, for 1 hour, washed until reached neutralized pH, filtered, dried at 35°C for 24 hours to produced chitin as an intermediary product. Subsequently, 60% NaOH 1:20 (w/v) was added to the sample, at 120°C for 2 hours. It was then cooled and after it reached a room temperature, it was washed until reaching neutral pH, filtered, and then dried at 80°C for 24 hours. The end-product was chitosan. The isolated chitosan was then tested with FT-IR analysis (Shimadzu IR-Tracer-100, Kyoto, Japan).

The physiochemical properties of the isolated chitosan were characterized according to the National Standardization Agency of Indonesia (2013), namely the degree of deacetylation, water content, ash content, yield, and pH. The degree of deacetylation (DD) was calculated with the formula as described by Hossain *et al*. (2015), while

the pH, moisture, ash, and As and Pb content were conducted by following AOAC (2002).

XRF analysis

The X-ray fluorescence analysis was determined using an XRF spectrometer (Axios P4400, PANalytical, Almelo, the Netherlands). The sample was ground and placed into small-aperture stainless steel sample holders using spring-loaded lids. The analysis was performed by using WD-XRF operational parameters such as the X-ray spot size characteristic line for the Ca Kα (λ =3.359 Å and 2θ = 113.086°), which follows the methods described by Babos *et al.* (2018) and Śliwiński et al. (2020).

Analytical determination for Cu^{2+}, Cd^{2+}, and Pb^{2+}

The quantitative analyses of Cu^{2+}, Cd^{2+}, and Pb^{2+} solution concentrations in the experiments were performed with atomic absorption spectrophotometry (AAS) (Analytikjena, contrAA 700, Jena, German), by using the light source from Xenon short-arc lamp and acetylene/air as the carrier gas. The burning speed for Cu^{2+} and Cd^{2+} was 50 L per hour (L/h) and for Pb^{2+} was 65 L/h, respectively. The wavelengths used for Cu^{2+}, Cd^{2+}, and Pb^{2+} were 324.7540 nm, 228.8018 nm, and 217.0005 nm, respectively. A standard calibration curve for each metal was prepared with a concentration of 0.2–1 mg/L. The verification method of atomic absorption spectrophotometry for the Cu^{2+}, Cd^{2+}, and Pb^{2+} assay that yields the parameters for this study consist of linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) (The United States Pharmacopoei, 2018).

Analysis of Cu^{2+}, Cd^{2+}, and Pb^{2+} content in Solen vagina shell powder and isolated chitosan

The AAS analysis was conducted to determine the purity (from Cu^{2+}, Cd^{2+}, and Pb^{2+}) of the shell powder and chitosan before they were used as bio-absorbent. Each sample (2 gram of shell powder and chitosan), mixed with 2.5 mL of H_2SO_4 concentration and 5 mL of 30% H_2O_2, was slowly processed and heated until a clear solution was obtained. After that, it was cooled at a room temperature, then demineralized water was added. Afterwards, the level of Cu^{2+}, Cd^{2+}, and Pb^{2+}, in 10.0 mL of each prepared sample was analyzed by using AAS (Bakkali *et al.*, 2012).

Bio-absorption process using Solen vagina shell powder and isolated chitosan

The bio-absorption experiment was conducted in a glass column (internal diameter of 1.75 cm and a length of 15 cm) by adding 1.5 gram of bio-absorbent, and 20.0 mL of a metal solution. Each metal solution contained approximately 10 µg/L of Cu^{2+}, Cd^{2+}, and Pb^{2+} as initial concentration. The *S. vagina* shell powder or chitosan derived from the shell was used as bio-absorbent. While the column tap was closed, each heavy metal solution was added and left for 30 minutes. Then, the flow in the column was opened, and the heavy metal concentration in the solution was determined by AAS. The second absorption process was conducted in the same column. All experiments were performed with three replications and followed the method described by Kelly-Vargas *et al*. (2012).

Absorption percentage is the ratio between the heavy metal that was absorbed by the bio-absorbent and the initial concentration. Absorption (%) for each shell powder or chitosan was calculated by the following Equation (1):

$$Absorption\ (\%) = \left[\left(\frac{Co - Cs}{Co}\right) \times 100\%\right] \quad (1)$$

where: Co was the initial metal concentration in solution (mg/L) and
Cs was the final metal concentration (mg/L).
Meanwhile, the adsorption capacity (q) was calculated by following Equation (2) as described in (Kelly-Vargas *et al*., 2012):

$$q = \left[\frac{(V(Co - Cs))}{m}\right] \quad (2)$$

where: q was the adsorption capacity;
Co was the initial metal concentration in solution (µg/mL);
Cs was the final metal concentration (µg/mL);
V was the total volume used in every load plus reload (L), and
m was the mass of the bio-absorbent (g).

The analysis of infrared spectrum of the bio-absorbent (KBr discs) was performed at a room temperature in the wavenumber between

4000–400 cm^{-1} with an FTIR analyzer (Shimadzu IR-Tracer-100, Kyoto, Japan).

RESULTS AND DISCUSSION

Isolation of chitosan from S. vagina shells

The yields of the isolation of chitin and chitosan after the deproteination, demineralization, and deacetylation processes can be seen in Table 1. The OH absorption showed a broadband at 3,452 cm^{-1}, whereas OH usually presents a broad absorption peak around 3,650 – 3,200 cm^{-1} when in the NH the region (Pavia *et al*., 2014). As reported by Pavia (Pavia *et al.*, 2014), the band is identified as the amine group (N-H stretching bands) absorbing IR 3,500 – 3,100 cm^{-1}, while CH adsorption uptake peak is at 2,922, the C=O stretch adsorption band peaks at 1,654, and the stretching band for C-O-C group peak at 1,082. In addition, the FTIR characterization of chitin and chitosan derived from *S. vagina*, compared with the standard, could be seen in Table 2.

Regarding the chitosan (Table 2) originating from the *S. vagina* shells, the FTIR spectrum formed comprises an absorbent band of the NH amine groups at wavenumber of 3,446 with widespread uptake that also indicates an absorption band of O-H. The C-H uptake bands were at 2,920, while absorption was at 1,654, showing a less sharp peak due to the reduced amide group at the time of deacetylation of chitin. The standard of chitosan from Sigma-Aldrich® was derived from crustaceans, then each peak of a particular functional group transmittance was compared to the literature (Palpandi *et al*., 2009; Pavia *et al*., 2014) (Table 2). In the FTIR spectrum of the chitin sample, the presence peaked at wavenumbers of 1,431 and 879, no peak was found on the standard chitin. The same patterns were obtained in the chitosan product spectrum, such as peaking at 1,479 and 871. Accordingly, there were still mineral residues, for instance $CaCO_3$, due to low demineralization ability, even though the process had been repeated three times (Mohammed *et al*., 2013). The FTIR spectra of chitin and chitosan at wave numbers of 1,483, 862; 1,473, 862 as well as in 1,428, 874; 1,468, 862 also reported in the isolate spectrum of *Crepidularia nerita* and *Achatina fulica* (Palpandi *et al.*, 2009), which were from the Mollusca family. The difference of FTIR spectra was influenced by the starting material used, method of isolation, temperature, and time of deacetylation process (Duarte *et al*., 2002). The demineralization process aims to remove inorganic salts or mineral content in the shell, which is indicated by the formation of CO_2 when the HCl solution is added to the sample. This process was conducted repeatedly for *S. vagina* shells that contain high calcium. This is in line with the study reported by (Alharbi & El-Taher, 2017) stating that mineral is the main content of Mollusca shells. The calcium content and other minerals in *S. vagina* shells was confirmed by using XRF analysis.

The XRF analysis (Table 3) showed a high calcium content compared to other minerals in

Table 1. The initial weight of chitin and chitosan produced from *Solen vagina* shells[1]

Initial weight of *S. vagina* shells (g)	Chitin Yield (%)	Chitosan Yield (%)
100.0600 ± 0.0276	30.07 ± 1.50	15.92 ± 1.78

[1] Result are mean ± Standard deviation, *n*=3.

Table 2. Specific vibration modes corresponding to chitosan from *Solen vagina* by compared with literatures

Functional groups	Chitin derived from *S. vagina*	Chitin based on BPPT standard	Chitosan comes from *S. vagina*	Chitosan standard (Sigma-Aldrich)	Chitosan derived from *Mytulus virdis linneaus* (Mohammed et al., 2013)	Literature (Palpandi et al., 2009)
OH	3452	3431	3446	3433	3445; 3471	3200-3400
-NH (Amine)	3452	3431	3446	3433	3445; 3471	3300-3500
C-H	2850; 2983; 2922	2922	2852; 2920	2922	2927	2850-3000
C=O (Amide I)	1654	1631	-	1654	-	1630-1680
CH_2	1479	-	1483	1427	1420	1466
C-O-C	1082	1070; 1149	1082	1072	1021; 1089	1083

S. vagina shells. This result affected the peak of wave numbers in chitin spectra and isolated chitosan, which is around 1,483 and 871, due to incomplete elimination process of calcium. The characteristic parameter of chitosan from *S. vagina* shells was compared with the National Standardization Agency of Indonesia (2013) as described in Table 4.

In this study, the result of DD of chitosan isolate (85%) is higher compared with the study reported by Mursida *et al.* (2018), when the chitosan isolated from green shell and snail shell has DD around 83%. Mursida *et al*. (2018) also suggested that NaOH could yield the chitosan with high DD percentage compared with KOH and $Ca(OH)_2$. Chitin deacetylation yielded rapidly in 50% NaOH (w/v) at 100°C during the first hour of the treatment, but the extended reaction time produced more hydrolysis chains than significant deacetylation. Abdel-Rahman *et al*. (2015) found that DD chitosan was of 95% with 50% NaOH at a temperature of 90°C in 3.5 hours, but it should be noted that long deacetylation can cause degradation of the chitosan molecular structure (Abdou *et al.*, 2008). On the basis of this finding, 60% NaOH and 2 hours deacetylation time were applied, then the chitosan was obtained with DD of 85.00% and chitosan yield was around 15%.

The results of the determination of water content, pH, and deacetylation degree (DD) of the isolated chitosan from *S. vagina* shells also did not detect the As and Pb presence, so it met the requirement of Indonesian National Standard (SNI), while the ash content in the chitosan from shells was 14%. That result exceeds the SNI standard, which was a maximum of 5%. XRF analysis data (Table 3) showed a high calcium content (CaO) of 98.64% and other inorganic compounds in *S. vagina* shells. This caused high ash content in the isolated chitosan from shells. Paridah *et al*. (2018) and El Knidri *et al*. (2018) reported that mussel shells show proximate compositions of chitosan sources that have high mineral content, which are 9.99% protein, 23.25% chitin, and 23.25% ash. A similar result was obtained in this study, as the chitosan was isolated from the Mollusca family organism.

The result of the verification method of the analysis is presented at Table 5. The acceptance criteria for correlation coefficient (r) > 0.999 and Vxo < 5%, which is an acceptance requirement by AOAC (2002). Those results revealed that each analysis showed a linear response between concentration and absorbance, as well as the accuracy and precision for Cu^{2+}, Cd^{2+}, and Pb^{2+} met the requirement of AOAC (2002), in which the recovery percentage should be 80 – 110% and relative standard deviation (RSD) should be ≤ 11% for sample concentration ≤ 1 ppm.

Table 3. The XRF data showed that the *Solen vagina* shells as material content source

Compound as	Concentration (%)	Compound as	Concentration (%)
Sulfur	0.16	SO_3	0.33
Calcium	98.54	CaO	98.64
Iron	0.15	Fe_2O_3	0.15
Copper	0.095	CuO	0.076
Strontium	1.06	SrO	0.812

* Sulfur trioxide (SO_3); Calcium oxide (CaO); Iron (III) oxide (Fe_2O_3); Copper (II) oxide (CuO); Strontium oxide (SrO).

Table 4. Characterization of chitosan derived from *Solen vagina* shells[1]

Parameter Characteristic	Chitosan Isolate	The National Standardization Agency of Indonesia (2013)
Degree of deacetylation (%)	85.00 ± 3.98	min 75
Water content (%)	0.37 ± 0.00	max 12
Ash content (%)	14.10 ± 0.04	max 5
pH	7.4 ± 0.1	7-8
Arsenic (As)	Nd*	max 5 mg/kg
Lead (Pb)	Nd*	max 5 mg/kg

[1] Result are mean ± standard deviation, *n*=3.

* Not detected; As (Limit of Detection: 0.1525 µg/L; Limit of quantitation: 0.5770 µg/L) and Pb (Limit of detection: 0.0207 mg/L; Limit of quantitation: 0.0768 mg/L).

Chitosan and the shell powder sample were screened before being used in this research to ensure the bio-absorbent material used did not contain Cu^{2+}, Cd^{2+}, and Pb^{2+}. The results are presented in Table 6. From the shell powder of *S. vagina*, Cu^{2+} of 16.68 ± 0.03 µg/g was obtained, whereas Cd^{2+}, and Pb^{2+} were not detected. In the chitosan isolated from *S. vagina*, Cu^{2+} was not detected, but Cd^{2+}, and Pb^{2+} were present. The existence of Cu^{2+} on *S. vagina* shells is natural, for example copper, is an essential metal that functions as a co-factor and catalyst for various enzyme systems in the cells of living organisms (Gaetke & Chow, 2003), including shellfish. This was supported by the data from XRF analysis of *S. vagina* shells, showing that the CuO present was as much as 0.076%. Meanwhile, in chitosan products, Cu^{2+} was not detected because in chitosan isolated from the *S. vagina* shell, the demineralization process was carried out with 1N HCl 1:10 (w/v), at 75°C, 1 hour, and repeated three times. Therefore, Cu^{2+} reacted with HCl to become $CuCl_2$, which was dissolved and lost during the sample washing and neutralizing.

Meanwhile, Cu^{2+} that entered the waters and accumulated in the *S. vagina* shells could originate from rock erosion or rainwater. Human activities such as industrial activities, copper mining, and shipyard industry were some of the causes of increased copper content in the water (Sudirman *et al*., 2013). The heavy metal threshold value of Cu^{2+}, which was considered as pollutants in waters, was 0.05 mg/L (Ministry of Environment and Forestry of Republic of Indonesia, 2004)

Bio-absorption process

The initial concentration of Cu^{2+}, Cd^{2+}, and Pb^{2+} solutions before being used in the bio-absorption process were 9.533 µg/L, 11.40 µg/L, and 10.393 µg/L, respectively. The solutions showed a decrease in the heavy metal content of Cu^{2+}, Cd^{2+}, and Pb^{2+} after passing the column contained shell powder and chitosan. The decreasing percentage of Cu^{2+} that was passed by powder from *S. vagina* shells was 92.38% and chitosan was 97.54% in the first elution. In the second elution, decreasing percentage at shell powder was 97.02% and chitosan was equal to 97.48%.

The decreasing percentage of Cd^{2+} which was passed through shell powder was 92.88% and chitosan was 99.27% in the first elution. In the second elution, Cd^{2+} decreasing percentage in shell powder was 92.63% and chitosan was 94.10%. Meanwhile, the decreasing percentage of Pb^{2+} by shell powder was 99.98% and chitosan was 100% in the first elution. In the second elution, decreasing percentage in the shell powder was 99.21% and chitosan was 100% (Table 7–8).

FTIR spectroscopy of the *S. vagina* shell powder was performed (Figure 1) to study the mechanism of metal removal and the main functional groups responsible for Pb^{2+} binding. The board peak at 3,420 cm^{-1} indicates hydroxyl (-OH) and

Table 5. Result of verification method of Cu^{2+}, Cd^{2+}, and Pb^{2+} analysis[1]

Parameters	Cu^{2+}	Cd^{2+}	Pb^{2+}
Linearity	0.20 - 0.98 mg/L; r =0.9989; Vxo = 2.39%	0.10 - 0.80 mg/L; r = 0.9992; Vxo = 3.02%	0.20 - 1.0 mg/L; r = 0.9996; Vxo = 1.15%
LOD (mg/L)	0.0067	0.0288	0.0207
LOQ (mg/L)	0.0255	0.1052	0.0768
Accuration (%)	88.29 ± 3.22	91.40 ± 3.09	95.23 ± 9.95
RSD (%)	3.65	3.38	10.45

[1] Limit of Detection (LOD); Limit of quantitation (LOQ); Relative standard deviation (RSD).

Table 6. Cu^{2+}, Cd^{2+}, and Pb^{2+} contents in *Solen vagina* shells and chitosan isolate from shells before used as biosorbent[1]

Tested sample	Cd^{2+}	Pb^{2+}	Cu^{2+}
S. vagina shell powder	Nd*	Nd*	16.68 ± 0.03 µg/g
Chitosan isolates from *S. vagina*	Nd*	Nd*	Nd*
LOD (mg/L)	0.0288	0.0207	0.0067
LOQ (mg/L)	0.1052	0.0768	0.0255

[1] Limit of Detection (LOD); Limit of quantitation (LOQ); Not detected (Nd).

Table 7. Decreasing percentage in metal concentration using *Solen vagina* shells and chitosan as biosorbent[1]

Biosorbent/metal	Cu^{2+} (%)		Cd^{2+} (%)		Pb^{2+} (%)	
	1st elution	2nd elution	1st elution	2nd elution	1st elution	2nd elution
S. vagina shell powder	92.38± 0.47	97.02 ± 0.72	92.88 ± 0.78	92.63 ± 1.79	99.98 ± 0.03	99.21 ± 0.53
Chitosan derived from *S. vagina* shells	97.54 ± 0.05	97.48 ± 0.18	99.27 ± 0.33	94.10 ± 2.88	100 ± 0	100 ± 0

[1] Result are mean ± standard deviation, n=3.

Table 8. Biosorption capacity of *S. vagina* shells and chitosan as biosorbent[1]

Biosorbent/metal	Cu^{2+} (µg/g)		Cd^{2+} (µg/g)		Pb^{2+} (µg/g)	
	1st elution	2nd elution	1st elution	2nd elution	1st elution	2nd elution
S. vagina shell powder	117.4 ± 0.6	123.3 ± 0.9	141.2 ± 1.1	140.8 ± 2.7	138.6 ± 0.1	137.5 ± 0.7
Chitosan derived from *S. vagina* shells	124.0 ± 0.1	123.9 ± 0.2	151.1 ± 0.7	143.0 ± 4.4	138.6 ± 0.1	138.6 ± 0.0

[1] Result are mean ± standard deviation, n=3.

amino (-NH) groups. The FTIR of metal loaded by *S. vagina* shell powder showed a distinct shift of some bands and a charge in intensity indicated *S. vagina* shell powder. Some peaks shifted and became weak, and the broad intense peak of 3,420 shifted to 3,426. The band at 2,920 cm^{-1} was due to the CH stretching frequency and the peak at 1,787 cm^{-1} was the characteristic for C=O stretching mode of the primary and secondary amides. The bands at 1,473 and 862 were attributed to the $CaCO_3$ mineral content in that starting material, as shown by the XRF data and the band at 1,082 was due to the C-O stretching of the alcoholic groups, which was possibly linked to the degradation of the glycosylated proteins of the shell matrix (Hossain *et al*., 2015; Zhang *et al.*, 2014). The FTIR of metal loaded by *S. vagina* shell powder showed that a distinct shift of hydroxyl (-OH) and amino (-NH) groups, which was to 3,426; while 2,921 cm^{-1} due to the CH stretching and the band at 1,471 indicates mineral content; a change in intensity indicates the ion-exchange behavior of *S. vagina* shell powder.

The FTIR spectra of the chitosan as bio-absorbent before and after adsorbing Cd^{2+} are presented in Figure 2. The bio-absorbent chitosan displayed a broad stretching intense peak around 3,448 wave number was the characteristic for hydroxyl and amino groups. The weak peak at 2,900/cm was known as the indicator of alkyl CH stretch, while a peak at 1,794 was the characteristic of the carbonyl C=O group. The absorption peaks at around 1,400, indicating the amide groups ($CONH_2$). The absorption peak at around 1,000 – 1,100 cm^{-1} is known to be the characteristic for all sugar derivatives (C-O-C). The absorption peak below 1,000 cm^{-1} means it contains majorly inorganic material. After the chitosan bio-absorbent adsorbing the metal ions, the absorption peaks changed, some peaks became weak and shifted, and other peaks disappeared. The peak around 3,300 cm^{-1} shifted to 3,442. The weak peak at around 2,900 shifted to 2,923 and the peak at 1,794 cm shifted to 1,796. For Cd^{2+} adsorption, the peak at 1,485 shifted to 1,428, and the peak at 1,082 changed to lower intensity. The functional groups responsible for the Cd^{2+} adsorption in chitosan mainly composed of –OH, –NH, COOH, and $CONH_2$. Therefore, the ion exchange interaction between adsorption sites with the functional groups and the metal ions was the major mechanism responsible for the adsorption process of chitosan derived from *S. vagina* shells (Rodríguez-Tirado *et al.*, 2012; Wang *et al.*, 2011).

Conch shells contain many minerals, namely calcium and protein (El Knidri *et al.*, 2018), also contain chitin (Abdel-Rahman *et al.*, 2015). Chitosan is a biopolymer of alkaline N-deacetylation processed from chitin and both have the adsorption ability to remove heavy metal ions (L. Zhang *et al.*, 2016). Chitosan is a poly-glucosamine, which is an excellent chelating agent and interacts very efficiently in transition metal ions (Barakat, 2011). The absorption of metal ions by chitosan is also caused by chelation and the formation of chitosan-metal ion complexes. Some studies support the theory that two or more amino groups from one chain bind to the same metal ions and hydroxyl groups may be involved in coordination as demonstrated by proton release (Gerente *et al.*, 2007; Wu *et al*., 2010). This was also proven by Panggalo *et al*. (2016), who isolated chitosan

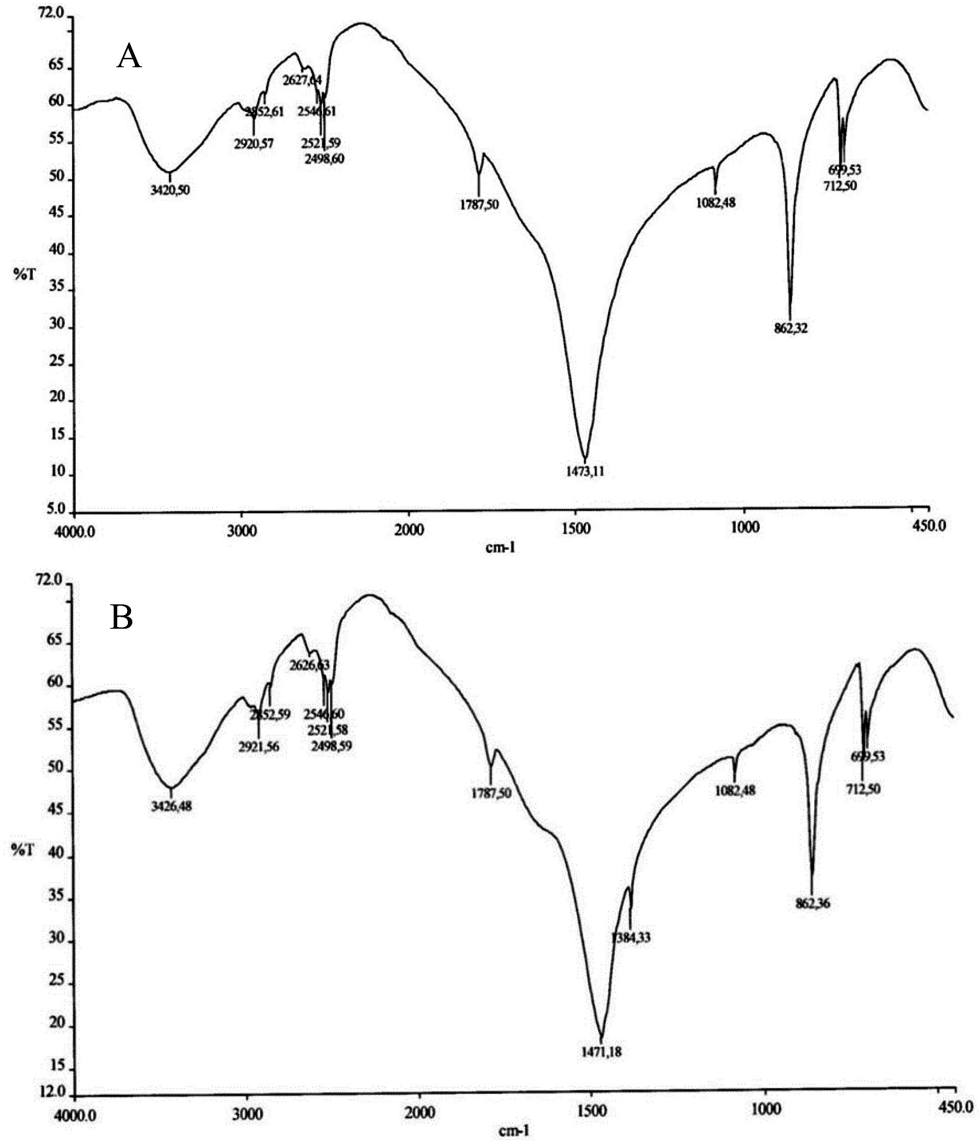

Figure 1. FTIR spectra of *Solen vagina* shell powder before biosorption of lead (A) and after biosorption of lead (B)

from the *Telescopium* sp. shell, including Mollusca with DD of 64% as a lead metal ion binder (Pb^{2+}) with a percentage of absorption of 98.27%. Hossain *et al.* (2015) suggested that mussel shell dust (*Lamellidens marginalis*) could be used as a Cd^{2+} adsorbent. It shows that conch shells can be used as heavy metal adsorbents by binding mechanism or chelating metal ions in the presence of functional groups such as –OH, –C=O, and –C=C. Hossain & Aditya (2013) suggested that $CaCO_3$, $Ca_3(PO_4)_2$, and the possibility of silica in the shell can absorb metals with an ion-exchange mechanism.

The analysis of research data required using a non-parametric statistical test; for instance, the Kruskal Wallis test, because the data obtained were not homogeneous and not normally distributed. The statistical test results on the percentage adsorption levels of Cu^{2+}, Cd^{2+}, and Pb^{2+} from the *S. vagina* was compared from the first to second elution, which obtained significant values of 0.100 and 0.223 (p> 0.05). This shows no difference in the adsorption of Cu^{2+}, Cd^{2+}, and Pb^{2+} between the shell powder and chitosan and there was no difference between the first and second elution. When each metal is compared, a significant value obtained was 0,000 (p<0.05), showing a difference in the percentage of powder or chitosan adsorption on each metal. The difference in the adsorption ability of each metal is due to the influence of the radius of the atom and the electronegativity

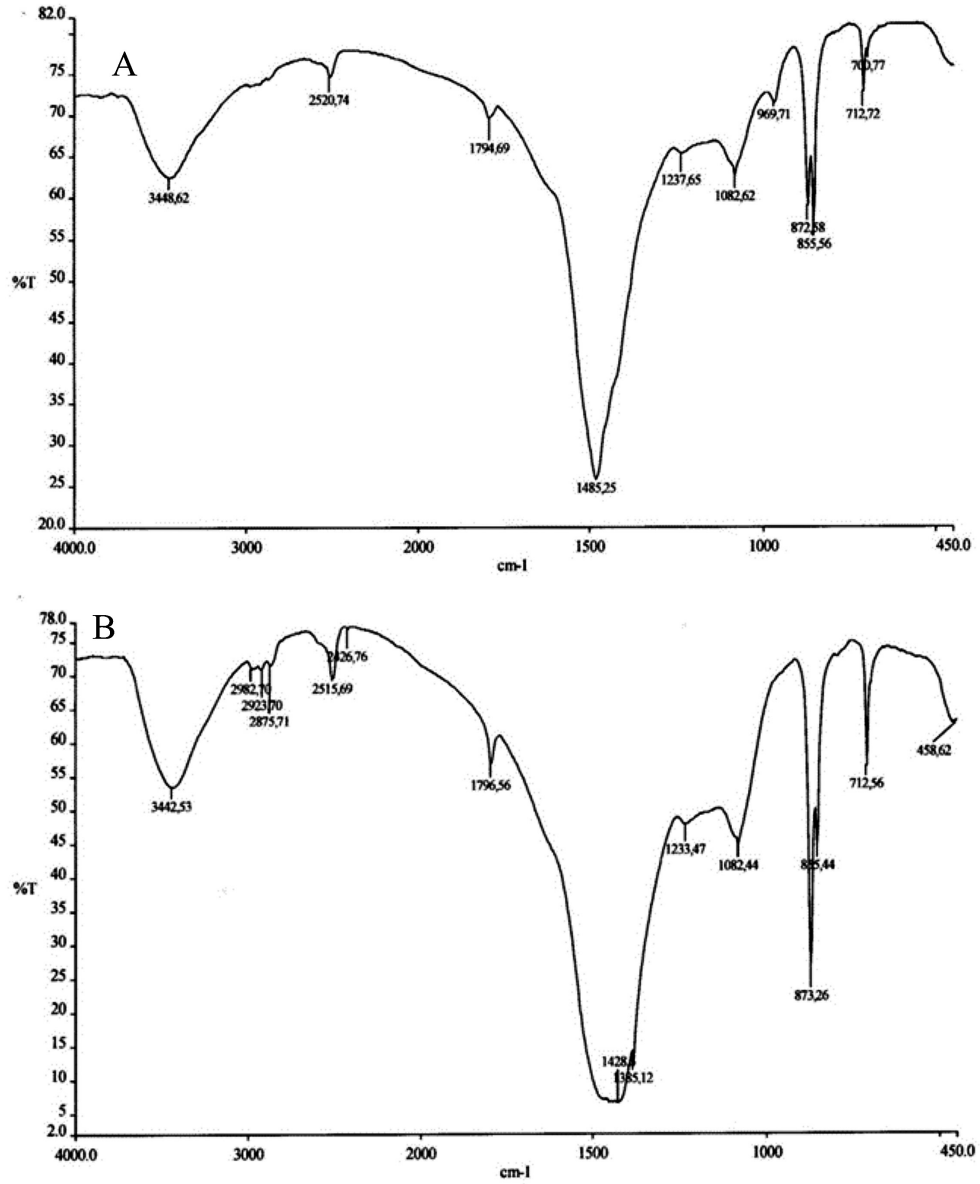

Figure. 2. FTIR spectra of chitosan derived from *Solen vagina* shells before (A) and after biosorption of cadmium (B)

of each metal. Cu^{2+}, Cd^{2+}, and Pb^{2+} have a high electronegativity so that they are able to attract electrons in the formation of chemical bonds. Cu^{2+} has a smaller atomic radius compared to Cd^{2+} and Pb^{2+}, so that the attraction with the nucleus is stronger and it reduces the ability to attract electrons (Perrone *et al*., 2001).

The column method used to measure the metal absorption ability follows the research by Kelly-Vargas *et al*. (2012). The absorption process of Cu^{2+}, Cd^{2+}, and Pb^{2+} in both samples, which were both shell powder and chitosan from the *S. vagina* shells reached more than 92%. Statistical analysis showed that the shell powder and chitosan isolated from the *S. vagina* shell had the ability to absorb Cu^{2+}, Cd^{2+}, and Pb^{2+}, and could be used repeatedly. In this research, the absorption process was conducted until the second iteration. Thus, it could be suggested that the shell waste of *S. vagina* can be applied as a bio-absorbent especially to Cu^{2+}, Cd^{2+}, and Pb^{2+}, to maintain the preservation of the aquatic environment.

The result of the absorption Cu^{2}, Cd^{2+}, and Pb^{2+} in both bio-absorbents, namely shell powder or the chitosan products from the *S. vagina* shells, reached more than 92%, indicating that both had the ability as a bio-absorbent and could be used repeatedly. In this case, the absorption process was carried out until the second iteration.

CONCLUSIONS

Chitosan could be isolated from the *S. vagina* shells. The shell powder and isolated chitosan from the *S, vagina* shells have the potential as a bio-absorbent that can reduce the heavy metal contents of Cu^{2+}, Cd^{2+}, and Pb^{2+} with the chelating and ion exchange mechanisms. Furthermore, the bio-absorbent technique from this study could be easily adopted and is a promising solution to shell waste problem. This also gives value-added material, especially among the smallholder fishermen located in the coastal area.

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