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, Kwanary, 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\(_2\)O (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, then dried at 35°C for 24 hours. The sample was then deacetylated with 1N HCl 1:10 (w/v), at 75°C, for 1 hour, washed until reached neutralized pH, filtered, then 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 Cu²⁺, Cd²⁺, and Pb²⁺**

The quantitative analyses of Cu²⁺, Cd²⁺, and Pb²⁺ 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 Cu²⁺ and Cd²⁺ was 50 L per hour (L/h) and for Pb²⁺ was 65 L/h, respectively. The wavelengths used for Cu²⁺, Cd²⁺, and Pb²⁺ 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 Cu²⁺, Cd²⁺, and Pb²⁺ 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 Pharmacopoeia, 2018).

**Analysis of Cu²⁺, Cd²⁺, and Pb²⁺ content in Solen vagina shell powder and isolated chitosan**

The AAS analysis was conducted to determine the purity (from Cu²⁺, Cd²⁺, and Pb²⁺) 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 H₂SO₄ concentration and 5 mL of 30% H₂O₂ 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 Cu²⁺, Cd²⁺, and Pb²⁺, 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 Cu²⁺, Cd²⁺, and Pb²⁺ 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):

\[
\text{Absorption (\%)} = \left( \frac{C₀ - Cₛ}{C₀} \right) \times 100\% 
\]  

where: \( C₀ \) 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):

\[
q = \left( \frac{V(C₀ - Cₛ)}{m} \right) 
\]

where: \( q \) was the adsorption capacity;

\( C₀ \) 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
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 us 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

| 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 viridis linnaeus (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$_2$O$_3$ | 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$_2$O$_3$); Copper (II) oxide (CuO); Strontium oxide (SrO).

Table 4. Characterization of chitosan derived from Solen vagina shells

| 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 |

* 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 \(\pm\) 0.03 \(\mu\)g/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 \(\mu\)g/L, 11.40 \(\mu\)g/L, and 10.393 \(\mu\)g/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

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

| Parameters | Cu\(^{2+}\) | Cd\(^{2+}\) | Pb\(^{2+}\) |
|------------|------------|------------|------------|
| Linearity  | 0.20 - 0.98 mg/L; \(r = 0.9989;\) V\(\text{oa} = 2.39\%\) | 0.10 - 0.80 mg/L; \(r = 0.9992;\) V\(\text{oa} = 3.02\%\) | 0.20 - 1.0 mg/L; \(r = 0.9996;\) V\(\text{oa} = 1.15\%\) |
| LOD (mg/L) | 0.0067     | 0.0288     | 0.0207     |
| LOQ (mg/L) | 0.0255     | 0.1052     | 0.0768     |
| Accuracy (%) | 88.29 \(\pm\) 3.22 | 91.40 \(\pm\) 3.09 | 95.23 \(\pm\) 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

| Tested sample | Cd\(^{2+}\) | Pb\(^{2+}\) | Cu\(^{2+}\) |
|---------------|------------|------------|------------|
| *S. vagina* shell powder | Nd*        | Nd*        | 16.68 \(\pm\) 0.03 \(\mu\)g/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).
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-adsorbent before and after adsorbing Cd\(^{2+}\) are presented in Figure 2. The bio-adsorbent 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-adsorbent 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 (Rodriguez-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

### Table 7. Decreasing percentage in metal concentration using Solen vagina shells and chitosan as biosorbent\(^1\)

| Biosorbent/metal       | Cu\(^{2+}\) (%) | Cd\(^{2+}\) (%) | Pb\(^{2+}\) (%) |
|------------------------|-----------------|-----------------|-----------------|
|                        | 1\(^{st}\)elution | 2\(^{nd}\)elution | 1\(^{st}\)elution | 2\(^{nd}\)elution | 1\(^{st}\)elution | 2\(^{nd}\)elution |
| S. vagina shell powder | 92.88 ± 0.78     | 92.63 ± 1.79    | 99.21 ± 0.53    |
| Chitosan derived from S. vagina shells | 97.54 ± 0.05 | 94.10 ± 2.88 | 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) |
|------------------------|---------------------|---------------------|---------------------|
|                        | 1\(^{st}\)elution | 2\(^{nd}\)elution | 1\(^{st}\)elution | 2\(^{nd}\)elution | 1\(^{st}\)elution | 2\(^{nd}\)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, \(n=3\).
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 1. FTIR spectra of *Solen vagina* shell powder before biosorption of lead (A) and after biosorption of lead (B)
of each metal. \( \text{Cu}^{2+} \), \( \text{Cd}^{2+} \), and \( \text{Pb}^{2+} \) have a high electronegativity so that they are able to attract electrons in the formation of chemical bonds. \( \text{Cu}^{2+} \) has a smaller atomic radius compared to \( \text{Cd}^{2+} \) and \( \text{Pb}^{2+} \), 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 \( \text{Cu}^{2+} \), \( \text{Cd}^{2+} \), and \( \text{Pb}^{2+} \) in both samples, which were both shell powder and chitosan from the \textit{S. vagina} shells reached more than 92%. Statistical analysis showed that the shell powder and chitosan isolated from the \textit{S. vagina} shell had the ability to absorb \( \text{Cu}^{2+} \), \( \text{Cd}^{2+} \), and \( \text{Pb}^{2+} \), 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 \textit{S. vagina} can be applied as a bio-absorbent especially to \( \text{Cu}^{2+} \), \( \text{Cd}^{2+} \), and \( \text{Pb}^{2+} \), to maintain the preservation of the aquatic environment.

The result of the absorption \( \text{Cu}^{2+} \), \( \text{Cd}^{2+} \), and \( \text{Pb}^{2+} \) in both bio-absorbents, namely shell powder or the chitosan products from the \textit{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.

Acknowledgements

We are indebted to the Kementerian Pendidikan Tinggi and Ristek Republik Indonesia for financial support, by Contract No 122/SP2H/PTNBH/DRPM/2018.

REFERENCES

1. Abdel-Rahman, R. M., Hrdina, R., Abdel-Mohsen, A. M., Fouda, M. M. G., Soliman, A. Y., Mohamed, F. K., Mohsin, K., Pinto, T. D. 2015. Chitin and chitosan from Brazilian Atlantic Coast: Isolation, characterization and antibacterial activity. International Journal of Biological Macromolecules, 80, 107–120.
2. Abdou, E. S., Nagy, K. S. A., Elsabee, M. Z. 2008. Extraction and characterization of chitin and chitosan from local sources. Bioresource Technology, 99(5), 1359–1367.
3. Ahemad, M., Kibret, M. 2013. Recent Trends in Microbial Biosorption of Heavy Metals: A Review. Biochemistry & Molecular Biology, 1(1), 19.
4. Alharbi, W. R., El-Taher, A. 2017. Environmental geochemistry and mineralogy of molluscan shells as related to their ecology. Arabian Journal of Geoscience, 10(24), 533.
5. AOAC. 2002. Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals. https://members.aoac.org/AOAC_Docs/StandardsDevelopment/SLV_Guidelines_Dietary_Supplements.pdf
6. Arifin, Z., Puspitasari, R., Miyazaki, N. 2012. Heavy metal contamination in Indonesian coastal marine ecosystems: A historical perspective. Coastal Marine Science, 35(1), 227–233.
7. Babos, D. V., Costa, V. C., Sperança, M. A., Pereira-Filho, E. R. 2018. Direct determination of calcium and phosphorus in mineral supplements for cattle by wavelength dispersive X-ray fluorescence (WD-XRF). Microchemical Journal, 137, 272–276.
8. Bakkali, K., Martos, N. R., Souhail, B., Ballesteros, E. 2012. Determination of Heavy Metal Content in Vegetables and Oils From Spain and Morocco by Inductively Coupled Plasma Mass Spectrometry. Analytical Letters, 45(8), 907–919.
9. Barakat, M. A. 2011. New trends in removing heavy metals from industrial wastewater. Arabian Journal of Chemistry, 4(4), 361–377.
10. Duarte, M. L., Ferreira, M. C., Marvão, M. R., Rocha, J. 2002. An optimised method to determine the degree of acetylation of chitin and chitosan by FTIR spectroscopy. International Journal of Biological Macromolecules, 31(1–3), 1–8.
11. El Knidri, H., Belaared, R., Addaou, A., Laajeb, A., Lahsini, A. 2018. Extraction, chemical modification and characterization of chitin and chitosan. International Journal of Biological Macromolecules, 120, 1181–1189.
12. Gaetke, L. M., Chow, C. K. 2003. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology, 189(1–2), 147–163.
13. Gerente, C., Lee, V. K. C., Le Cloirec, P., McKay, G. 2007. Application of chitosan for the removal of metals from wastewaters by adsorption – Mechanisms and models review. Critical Reviews in Environmental Science and Technology, 37(1), 41–127.
14. Goyal, P., Piara, S., Srivastava, S. 2011. A Novel Eco-friendly Biomaterial Ficus religiosa Leaf Powder (FRLP) for the Removal of Ni (II) Ion from Water Bodies. Asian Journal of Water, Environment and Pollution, 8, 77–82.
15. Heidarieh, M., Maragheh, M. G., Shamami, M. A., Behgar, M., Ziae, F., Akbari, Z. 2013. Evaluate of heavy metal concentration in shrimp (Penaeus semisulcatus) and crab (Portunus pelagicus) with INAA method. SpringerPlus, 2(1), 1–5.
16. Hossain, A., Aditya, G. 2013. Cadmium biosorption potential of shell dust of the fresh water invasive snail Physa acuta. Journal of Environmental Chemical Engineering, 1(3), 574–580.
17. Hossain, A., Bhattacharyya, S. R., Aditya, G. 2015. Biosorption of cadmium by waste shell dust of fresh water mussel Lamellidens marginalis: Implications for metal bioremediation. ACS Sustainable Chemistry and Engineering, 3(1), 1–8.
18. Kelly-Vargas, K., Cerro-Lopez, M., Reyna-Tellez, S., Bandala, E. R., Sanchez-Salas, J. L. 2012. Biosorption of heavy metals in polluted water, using different waste fruit cortex. Physics and Chemistry of the Earth, 37–39, 26–29.
19. Melgar, M. J., Alonso, J., García, M. A. 2016. Cadmium in edible mushrooms from NW Spain: Bioconcentration factors and consumer health implications. Food and Chemical Toxicology, 88, 13–20.
20. Ministry of Environment and Forestry of The Republic of Indonesia, (2004). Keputusan Menteri Negara Lingkungan Hidup No 51 Tahun 2004 Tentang Baku Mutu Air Laut, Pedoman Penetapan Baku Mutu Lingkungan, Menteri Negara Kependudukan dan Lingkungan Hidup (in Indonesian), Jakarta. https://onlimo.bppt.go.id/Regulasi/km512004.htm
21. Mohammed, M. H., Williams, P. A., Tverezovskaya, O. 2013. Extraction of chitin from prawn shells and conversion to low molecular mass chitosan. Food Hydrocolloids, 31(2), 166–171.
22. Mohiuddin, K. M., Ogawa, Y., Zakir, H. M., Otomo, K., & Shikazono, N. 2011. Heavy metals contamination in water and sediments of an urban river in a developing country. International Journal of Environmental Science & Technology, 8(4), 723–736.
23. Mursida, Tasir, Sahariwati. 2018. Efektifitas Larutan Alkali pada Proses Deasetilasi (in Indonesian). JPHPI, 21(2), 356–366.
24. National Standardization Agency of Indonesia. (2013). Kitosan Syarat Mutu dan Pengolahan (in Indonesian) SNI 7949: 2013. BSN. Jakarta, 8.
25. Palpandi, C., Shanmugam, V., Shanmugam, A. 2009. Extraction of chitin and chitosan from shell and operculum of mangrove gastropod Nerita (Dosta) crepidularia Lamarck. International Journal of Medicine and Medical Sciences, 1(5), 198–205.
26. Panggalo, D., Bahri, S., Sumarni, N. K. 2016. Pemanfaatan Kitosan Cangkang Keong Bakau (Telescopium sp.) Sebagai Pengikat Ion Logam Timbal (Pb) Dalam Larutan (in Indonesian). Kovalen, 2(1), 14–21.
27. Paridah, M., Hamzah, A., Rizal, S., Yap, S., Tye, Y., Nurul Fazita, M., Lai, T., Chong, E., Abdul Khalil, H. 2018. A review of extractions of seaweed hydocolloids: Properties and applications. J, 12(4).
28. Pavia, D. L., Lampman, G. M., Kriz, G. S., Vvyyan, J. A. 2014. Introduction to spectroscopy. Nelson Education.
29. Perrone, J., Foureast, B., Giffaut, E. 2001. Sorption of nickel on carbonate fluoroapatites. Journal of Colloid and Interface Science, 239(2), 303–313.
30. Rodriguez-Tirado, V., Green-Ruiz, C., Gómez-Gil, B. 2012. Cu and Pb biosorption on Bacillus thio-params strain U3 in aqueous solution: Kinetic and equilibrium studies. Chemical Engineering Journal, 181–182, 352–359.
31. Sangur, K., Leiwakabessy, F., Tuaputty, H., Tuwankotta, L. V., Samloy, S. V., Rattla, C., Salakory, O. B., Matulessy, C., Rumahlatu, D. 2021. Mudskipper as an Indicator Species for Lead, Cadmium and Cuprum Heavy Metal Pollution in the Mangrove, Ambon, Indonesia. Journal of Ecological Engineering, 22(4), 1–19.
32. Śliwiński, M. G., Latty, C. J., Spaleta, K. J., Taylor, R. J., Severin, K. P. 2020. Rapid, non-destructive analysis of calcium and strontium in eggshells by WD-XRF. Chemosphere, 251.
33. Sudirman, N., Husrin, S., Ruswahyuni, R. (2013). Water Quality Standards For Port Area And Water Pollution Index In Fisheries Port Kejawanan, Cirebon. Saintek Perikanan : Indonesian Journal of Fisheries Science and Technology, 9(1).
34. Sun, J., Li, X., Ai, X., Liu, J., Yin, Y., Huang, Y., Zhou, H., Huang, K. 2018. Efficient removal of cadmium from soil-washing effluents by garlic peel biosorbent. Environmental Science and Pollution Research, 25(19), 19001–19011.
35. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., Sutton, D. J. 2012. Molecular, clinical and environmental toxicicology (3): Environmental Toxicology. In Molecular, Clinical and Environmental Toxicology (101).
36. The United States Pharmacopoeia. 2018. United States Pharmacopoeia 41, United States Pharmacopoeial Convention, Rockville, MD.
37. Wang, X., Liang, X., Wang, Y., Wang, X., Liu, M., Yin, D., Xia, S., Zhao, J., Zhang, Y. 2011. Adsorption of Copper (II) onto activated carbons from sewage sludge by microwave-induced phosphoric acid and zinc chloride activation. Desalination, 278(1–3), 231–237.
38. Wu, F. C., Tseng, R. L., Juang, R. S. 2010. A review and experimental verification of using chitosan and its derivatives as adsorbs for selected heavy metals. Journal of Environmental Management, 91(4), 798–806.
39. Zamri, A. I., Latiff, N. F., Abdullah, Q. H., Ahmad, F. 2020. Extraction and optimization of chitosan from razor clam (Ensis arcuatus) shells by using response surface methodology (RSM). Food Research, 4(3), 674–678.
40. Zhang, L., Zeng, Y., Cheng, Z. 2016. Removal of heavy metal ions using chitosan and modified chitosan: A review. Journal of Molecular Liquids, 214, 175–191.
41. Zhang, Z., Wang, P., Zhang, J., Xia, S. 2014. Removal and mechanism of Cu (II) and Cd (II) from aqueous single-metal solutions by a novel biosorbent from waste-activated sludge. Environmental Science and Pollution Research, 21(18), 10823–10829.