Proceedings of Science and Mathematics
Faculty of Science, Universiti Teknologi Malaysia
Vol. 14, 2022, page 1-7

Removal of Toxic Metal Arsenic in *Perna Viridis* using Natural Chelating Agents

Nur Atiqah Razali and Susilawati Toemen*
Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Johor, Malaysia
*Corresponding author: susilawatitoemen@utm.my

Abstract
Asian Green mussels, *Perna Viridis* is a local seafood and aquatic catches in Malaysia. Unfortunately, the contamination of the heavy metals in this aquatic life have been increase day to day because of the industrial waste did not disposed in proper manner. The purpose of this research is to remove the heavy metal Arsenic (As) from *Perna Viridis* by chelation technique using three types of natural chelating agents, which are red onions, garlics and lemons. The toxic metal As content in *Perna Viridis* was record below the Malaysian standard and in range by Codex standard with the initial concentration of As was 0.852 mg/kg. The *Perna Viridis* was treated by three chelating agents at two studied parameters of different volumes (1, 3 and 5mL) and treatment times (15, 30 and 45 mins). The efficiency trend of chelating agent for the removal of As was lemons (85%) > garlics (39.66%)> red onions (37.80%). Treatment using all the chelating agents were observed under optimized conditions of 3 mL of volumes of chelating agent solution and 15 minutes of treatment time. The removal capacity of As was increased by using catalytic chelation technique in the presence of Ca/Cr(10:90)/Al_2O_3 catalyst. Percentage removal of As on lemons, garlics and red onions chelating agents are 94.51%, 69.51% and 68% respectively with 1 g of catalyst. Therefore, this study successfully proven that the catalytic chelation technique has capability to enhance the removal of As in *Perna Viridis*.

Keywords: *Perna Viridis*, catalyst, chelation technique, natural agents, heavy metal removal

1. Introduction

Aquatic life such as Asian Green mussels scientifically called as *Perna Viridis* is example of the food sources that consumed regularly by humans. *Perna Viridis* is one of example shellfish that very valuable for certain person that have allergen to fleshy food. According to Precision Nutrition's Encyclopaedia of Food, *Perna Viridis* rich in nutrition it has high in protein, low in fat yet providing 680 mg of Omega-3 from Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) in one serving. Moreover, it has more Omega 3 fatty acids than any other shellfish and far more than any other popular meat choice today [1]. In addition, taking mussels as the food are very good in healthy lifestyles due to the present of many vitamins and minerals such as vitamin B12, iron, vitamin C, riboflavin, and zinc (Star Online, 18 August 2013). *Perna Viridis* is packed with many benefits, it is best known for its curative ability on arthritis and also beneficial for bone and muscle. However, this aquatic life was reported to be contaminated with heavy metals in wide range of concentration especially at Pasir Gudang area.

Johor Port and Tanjung Langsat Port are example of the port that have the most rapid industrial development at Pasir Gudang, Johor likes logistic, shipbuilding, transportation and other heavy industries. Nowadays, Pasir Gudang have been categorized as a polluted town due to the irresponsible parties from some industries that did not dispose their chemical waste according to the guideline that have been regulated by the Department of Environment. There are many rivers at Pasir Gudang have been contaminated with this chemical waste that consists of organic and inorganic materials. The example of these materials are, nitrates, carbonates, pesticides and radioactive materials. Kim-Kim River is one of the rivers that have been classified as "death-river" due to highly contamination by the chemical waste. On March 6th, 2019, several students, teachers and canteen workers from numerous

schools at Pasir Gudang had been reported having experienced nausea, dizziness, and vomiting effect of the immature of illegal tyre factory dumped the chemical waste in Kim-Kim River [1]. When heavy metals exposed to the aquatic life, the metal ions will be consumed by humans through the food chains or direct intake which can cause acute and chronic poisoning.

Many conventional methods such as chemical precipitation, membrane filtration, ion exchange, carbon adsorption, and co-precipitation have been used for the removal of the heavy metals [2]. Unfortunately, they are not suitable for the high concentration of heavy metals, profitable and also cannot be used in the food. The chelation is one of the methods that can be used to remove the heavy metal in food. Chelation is the process where the molecules or ions of ligand bind to central metal ion via coordination bond in a cyclic structure where this ligand is called chelants, chelating agents, or chelators [3].

The chelating agents can be classified into two type which are synthetic and natural. Ethylenediaminetetraacetic acid (EDTA) ($C_{10}H_{16}N_2O_8$), Pentetic acid or diethylenetriaminepentaacetic acid (DTPA) ($C_{14}H_{23}N_3O_{10}$), Ethylenediamine-N, N′-bis (2-hydroxyphenylacetic acid) (EDDHA) ($C_{18}H_2ON_2O_6$) are examples of synthetic chelating agents. Meanwhile, example of natural chelants are amino acid and organic acid likes acetic acid, citric acid, ascorbic acid, lactic acid [4]. Lemon juice is one of example that contain to 8% of citric acid [5]. Moreover, according to Symonowicz and Kolanek [6] flavonoids also can acts as chelating agents due its specific chemical structure which can undergo metal ion chelation process. Flavonoids are any of a large class of plant pigments having a structure based on or similar to that of flavone. It can be found in a variety of fruits and vegetables like red onion and garlic. Flavonoid is known as important natural antioxidants. Chelate formation can be formed from the interaction of the flavonoid and metal ions. Therefore, this research focused on the removal of heavy metal arsenic from *Perna Viridis* three natural chelating agents of lemons, garlics and red onions.

2. Methodology

2.1. Chemicals and Sample Collection

The chemicals that used were nitric acid, HNO_3 (analytical grade, Merck 65%), calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$), aluminium oxide (Al_2O_3) (Sigma Aldrich), chromium salt and 29 elements Standard 3 by Merck Germany. All reagents that used in the form of analytical grades and no further purification required. Then, the deionized water was obtained from the NANO pure water system. The natural chelators used were red onion, garlics, and lemons purchased from the local market at Taman University, Skudai.

Samples of *Perna Viridis* were obtained from a Kampung Pasir Puteh, Pasir Gudang area. The samples were kept in clean zip log bags and will be transported to the laboratory at Universiti Teknologi Malaysia (UTM Skudai). It is necessary to use with almost similar weight to obtain accurate result for the initial concentration and for the removal heavy metals. The collected *Perna Viridis* average sizes 6-8 cm horizontal of shell size and weight around 16-20 g. All the samples were washed and cleaned first before further step of chelation technique and acid digestion was done. The samples were packed and kept in refrigerator prior to the treatment. Before treatment, the *Perna Viridis* were defreeze for 1 hour.

2.2. Preparation of Ca/Cr(10:90)/Al_2O_3 catalyst

Wetness impregnation method was used for the preparation of the catalyst. Firstly, the sapodilla leaves was cleaned with appropriate amount of deionized water and dried under sunlight for 2 weeks. The dried sapodilla leaves was blended until it becomes powder. 1.0 g of sapodilla leaves powder was transfer in 250 mL conical flask and it was dissolved with 100 mL of distilled water, then boiled for approximately 3 hours and later left at room temperature until the solution cool down. At the same time, 5.0 g of $Ca(NO_3)_2.4H_2O$ and 0.7 g of $Cr(NO_3)_3.9H_2O$ catalyst was dissolved separately using distilled water in two separate 100 mL beaker. The amount of distilled water used to dissolve both substances must be 5 mL in total. The catalyst solutions were then mixed together with the 5 ml of leaves extraction and stirred for about 30 minutes in room temperature. Then, 5.0 g of alumina bead, γ-Al_2O_3 was dipped into catalyst solution for 1 hour. After that, the alumina supported catalyst was transferred onto filter paper and aged in an oven at temperature 90^{o}C for 24 hours to remove moisture and allowed good coating of catalyst on the surface of alumina. The catalyst was then calcined at temperature of 700^{o}C

using a ramp rate of 5°C/min for 5 hours to remove all metal precursor impurities and water in the catalyst.

2.3. Removal of As in Perna Viridis

Three types of natural chelating agents that used in this research were red onions, garlics and lemons. 215 g of red onion were blended with 50 mL of distilled water and filtered to produce red onion chelating agent solution. After that, 1 mL of red onion solution, 16-20 g of *Perna Viridis*, and one tablespoon of salt were added to the 500 mL of distilled water in a beaker. Bubble technique were applied by using the bubble aerator aquarium. Figure 1 shows the procedure for removal toxic metal using natural chelating agents. The *Perna Viridis* samples were treated by immersing them in natural chelating agent solution based on the volume of chelating solutions (1-5 mL) and treatment times (15-45 minutes). The quantitative analysis was conducted to determine the concentration of Arsenic in ppb level by using Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS).

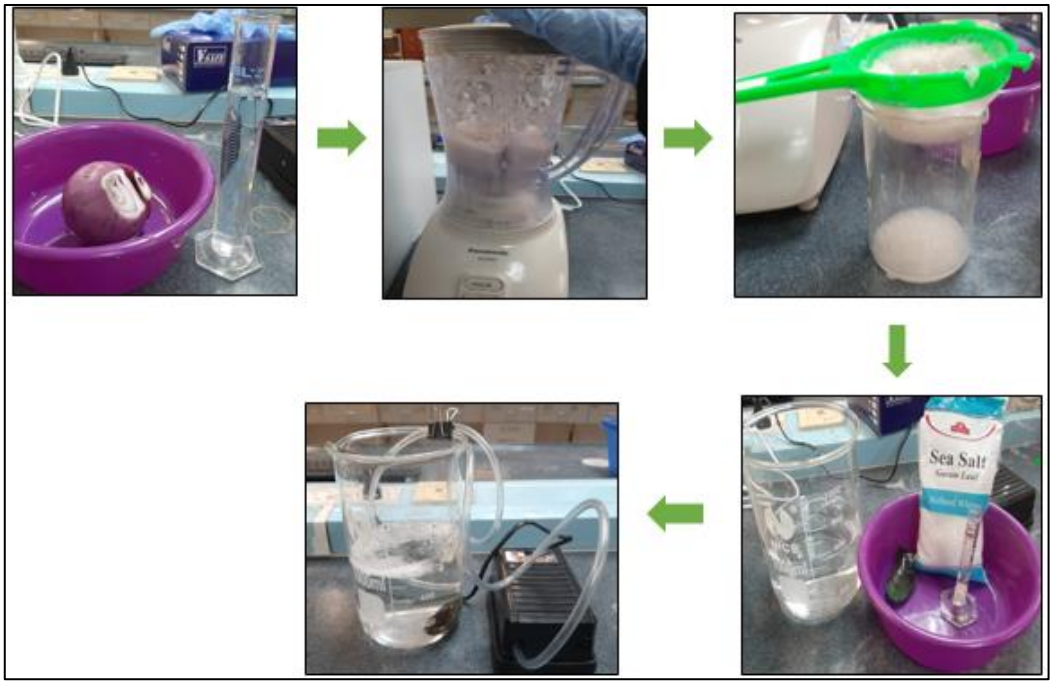

Figure 1. Procedure for removal of toxic metal As using natural chelating agents

3. Results and Discussion

In this research, the initial concentration of toxic metals As in *Perna Viridis* was analysed using Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS) before it undergo the chelation treatment. The concentration of As at ambient temperature (T= 27.0 – 28.0 ºC) for *Perna Viridis* with the permissible limit of Malaysia standard and Codex standard are showed in Table 1. It is important to determine the concentration of toxic metal in commercial seafood in order to evaluate the possible risk of seafood consumption for human health.

Table 1. Concentration of As in *Perna Viridis* and the permissible limit of Malaysia and Codex Standard

Toxic Metal	**As**
Initial Concentration (mg/kg)	0.852
Permissible limit (mg/kg):	
Malaysia Standard	1.0
Codex Standard	0.1-1.0

The data of the initial concentration of toxic metal As in *Perna Viridis* was compared with the permissible limit of Malaysia standard and Codex standard. Initial concentration for As was 0.852 mg/kg, which is lower than the permissible limit by Malaysia standard which was 1.00 mg/kg and for Codex standard was 0.1-1.0 mg/kg which is still can be considered as hazardous for large consumption but based on the research by [7], the initial concentration of As in *Perna Viridis* at Pasir Gudang was 1.58±0.23 µg/g which higher than permitted level of Malaysia and Codex standard. Due to the difference result, season is one of the factor that affected percentage removal of As in *Perna Viridis*. Besides that, the difference was also because of the significant difference of physical-chemical factors such as salinity, conductivity, temperature, and river flow speed between dry season and rainy season [8].

This means that the *Perna Viridis* in Malaysia is safe to consume according to Malaysia standard but must be careful to consume as referring Codex standard. Although, the level of As has not exceed the admissibility limit by Malaysia standard, the content of Arsenic in *Perna Viridis* should be reduced. Therefore, this study is still can be used to recognise the best chelating agents to remove the heavy metal As from the Asian green mussels at the safe level to be consumed by human.

3.1. Effect of Volume of Chelating Agents

The results of the treatments are shown in bar plot in Figure 2. The chelating agents with varying volume of 1-5 mL and soaking time at 15 minutes was studied to reduce the level of As content in *Perna Viridis*. Based on Figure 2 it showed that those chelating agents used have an ability to remove As in *Perna Viridis* and each volume has great impact on the removal process.

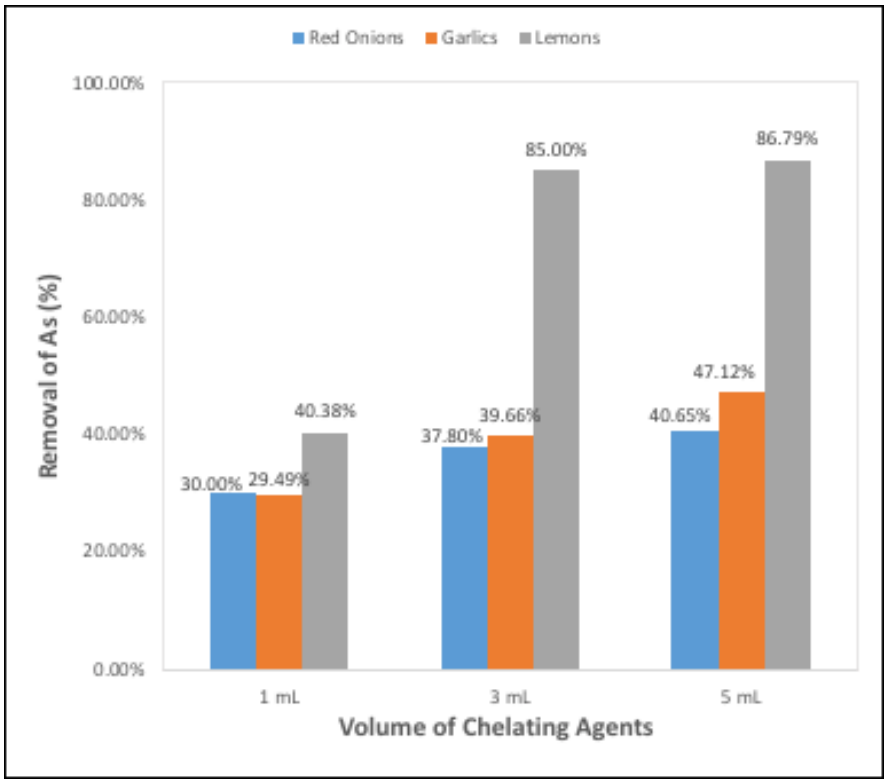

Figure 2. Percentage removal of As with different volume of chelating agents at 15 minutes treatment time

The findings showed lemons recorded the highest percentage of As removal as compared to red onions and garlics. At 15 minutes treatment, the removal of As achieved 40.38% for 1 mL of lemons used during the treatment. The As removal abruptly increased to 85% when 3 mL of lemon was used, and the removal consider constant even after increasing the volume of lemons solution to 5 mL. From the results it was evidently proved that lemons could chelate the highest amount of As in *Perna Viridis.* It able to make a bond with heavy metal ions to form complex compound and thus metal the ions can be excreted out from the samples as suggested by [9]. Besides that, citric acid in the lemons can change

the metal ion ability. It consists of three donor atoms of oxygen. The donor atoms of citric acid (O) contain unshared electron pair, which can coordinate a metal ion [10].

When using red onions as chelating agents, it showed that the result for removal As at 1 mL, 3 mL and 5 mL were 30.00 %, 37.80% and 40.65%. Meanwhile, the result for garlics were 29.49% (1 mL), 39.66% (3 mL) and 47.12% (5 mL). The trend of type of chelating agents used to remove As was lemons > garlics > red onions. The volume of 3 mL was used for further study.

3.2. Effect of Treatment Times

Treatment time for removing heavy metals also play an important role as it will affect the percentage of heavy metals excretion. The removal of As were measured up to 45 minutes contact time with 15 minutes time interval. Figure 3 shows the efficiency of chelating agent for As removal as a function of contact time. As general, the treatment time gave significant effect on each sample.

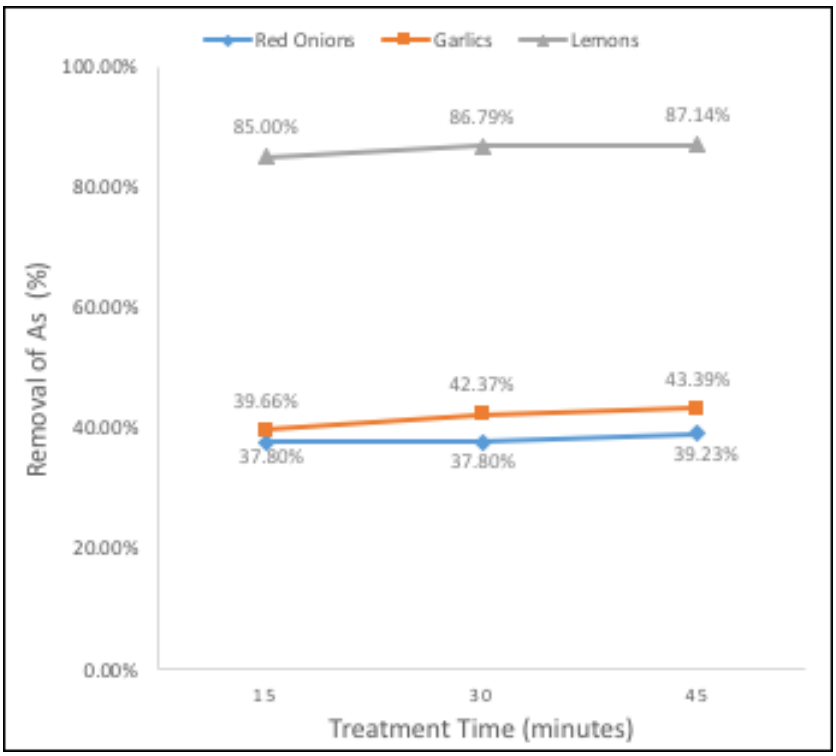

Figure 3. The efficiency of chelating agent in Arsenic removal with treatment time (minutes) at constant volume (3 mL)

From the result, it can be seen that increasing the treatment time would also increase the percentage As removal. This is because the chelating agent has enough time to penetrate throughout the mussel's tissue and reacted with the heavy metal to be removed. However, the significant removal was not observed after 15 minutes of treatment (only 1-2% difference). Therefore, it can be concluded that the best treatment time is 15 minutes as it can removed the As up to 85.00%, 36.66% and 37.80% for lemons, garlics and red onions as chelating agents.

3.3. Catalytic Chelation Method

One of the suitable catalyst for treatment catalytic chelation technique was Ca/Cr(10:90)/Al_2O_3 due to their enhancement of the formation of irreversible reaction by catalyst to produce such as anion (citrate) in chelating agents (lemons) which react with the As that contaminated the *Perna Viridis*. The best condition parameters (3 mL of chelant and 15 minute of treatment time) of chelating agents obtained from Sections 3.2 and 3.3 were used during the treatment. The catalytic chelation data is presented in Figure 4. It was proven that catalyst has ability to increase the removal of heavy metal from *Perna Viridis*.

Based on the figure, the addition of 1 g Ca/Cr(10:90)/Al_2O_3 catalyst beads into the treatment with lemons solution, increased up the removal to 94.51%. Meanwhile, for the garlics and red onions, around 27 -30 % increment for the removal of As after the catalyst was added to it. The result shows that with presence of Ca/Cr (10:90)/Al_2O_3 catalyst increased the percentage of removal of As. It was because the catalyst can speed up the reaction and lowering the energy barrier. Therefore, the catalytic chelation method was more effective since it can remove higher percentage of toxic and heavy metals compared with chelation technique only.

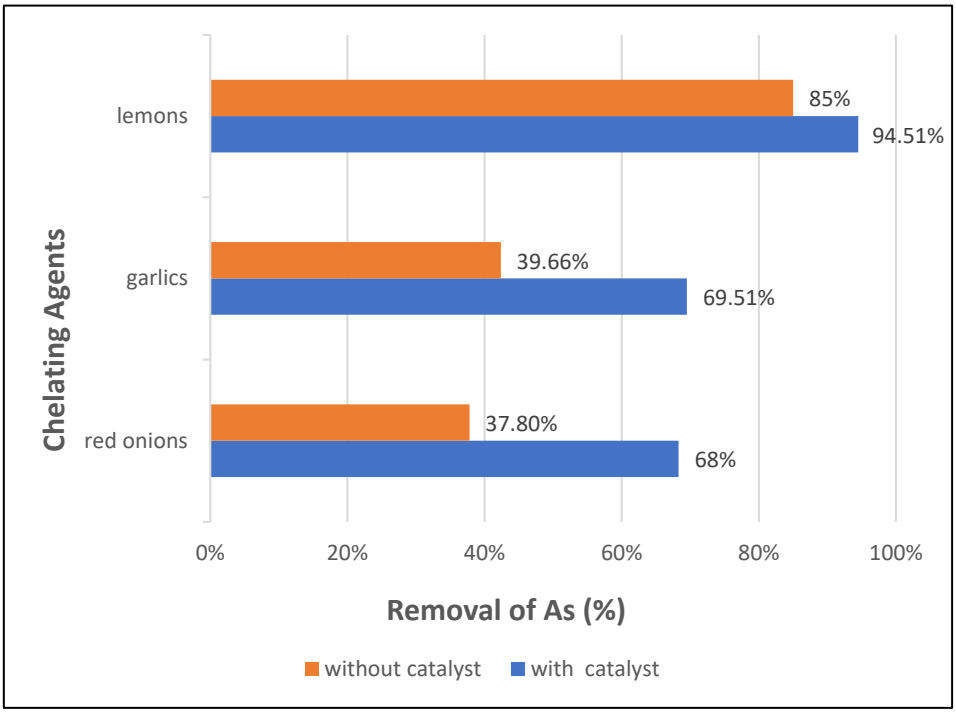

Figure 4. The percentage of As removal in *Perna Viridis* under catalytic chelation treatment with catalyst.

Conclusion

The chelation method was found to be a potential technique for the removal of arsenic (As) studied in *Perna Viridis*. The removal of As from *Perna Viridis* was carried out using natural chelating agents namely red onions, garlics and lemons. The toxic metal As in *Perna Viridis* was recorded below the permissible limit set by Malaysian standard and still in range by Codex standard with the initial concentration of 0.852 mg/kg. Present investigation illustrates the efficacy of the studied chelating agents is in the order of lemons (85%) > garlics (39.66%)> red onions (37.80%) at optimum conditions of 3 mL of volume and 15 minutes treatment time. The catalytic chelation technique in the presence of Ca/Cr(10:90)/Al_2O_3 catalyst successfully increased the As removal in *Perna Viridis* up to 94.51%, 69.51% and 68% , respectively. Therefore, it is suggested that any housewife who want to cook *Perna Viridis* should soak it first with lemons for 15 to 30 minutes to reduce the heavy metals content in *Perna Viridis*.

Acknowledgement

The researcher would like to thank all people who have supported the research, and Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia which has funded this research.

References

[1] Yap, C., Peng, S., and Chee Seng, L., 2019. Contamination in Pasir Gudang Area, Peninsular Malaysia: What can we learn from Kim Kim River chemical waste contamination? *Journal of Humanities and Education Development,* 1, 80- 87.

[2] Bisht, R., Agarwal, M., & singh, k. (2017). Methodologies for removal of heavy metal ions from wastewater: an overview. *interdisciplinary enviornmental review,* 18, 139- 124.

[3] Kim, J.-J., Kim, Y.-S., and Kumar, V., 2019. Heavy Metal Toxicity: An Update of Chelating Therapeutic Strategies. *Journal of Trace Elements in Medicine and Biology,* 54, 218-231.

[4] Kaushik, P., 2015. Use of natural organic and synthetic chelating agents for efficient phytoremediation. International Journal Enhanced Research Science and Technology Engginnering, 4, 87-101.

[5] Rodrigues, A. S., Almeida, D. P., Simal-Gándara, J., & Pérez-Gregorio, M. R. (2017). Onions: a source of flavonoids. Flavonoids: From Biosynthesis to Human Health, 9, 420-439.

[6] Symonowicz, M., & Kolanek, M. (2012). Flavonoids and their properties to form chelate complexes. Toxicity, 9, 40-50.

[7] Azelee, I. W., Ismail, R., Ali, R., & Bakar, W. (2014). Chelation technique for the removal of heavy metals (As, Pb, Cd and Ni) from green mussel, Perna viridis *Journal of Chemistry, 43,* 90-100.

[8] Suprapti, N. H., Bambang, A. N., Swastawati, F., & Kurniasih, R. A. (2016). Removal of Heavy Metals from a Contaminated Green Mussel [Perna Viridis (Linneaus, 1758)] Using Acetic Acid as Chelating Agents. *Aquatic Procedia, 7*, 154-159.

[9] Samsudin, N. A., Ismail. R., Wan Abu Bakar, W. A. and Muhammad, R. (2012). *Removal of Heavy Metals in Green-Lipped Mussels (Perna Virdis) Utilizing Edible Chelating Agents,* 30, 50-55.

[10] Al-Qahtani, K. M. A. (2017). Extraction Heavy Metals from Contaminated, Water using Chelating Agents. *Oriental Journal of Chemistry*, *33*, 1698-1704.