IJFAC

Indonesian Journal of Fundamental and Applied Chemistry

Article http://ijfac.unsri.ac.id

Analysis of Lead (Pb) and Cadmium (Cd) in Oyster *Crassostrea gigas* and *Saccostrea cucullata* using Atomic Absorption and Ultraviolet-Visible Spectrophotometer Methods

Lelifajri Lelifajri, Salsabila Salsabila and Khairi Suhud*

Chemistry Department, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia, 23111.

*Corresponding Author: khairi@usk.ac.id

Abstract

Lead (Pb) and Cadmium (Cd) metals are highly toxic when accumulated in the body and cause serious diseases and even death. This study aimed to determine the metal content of Pb and Cd in the oyster species *Crassostrea gigas* and *Saccostrea cucullate* in the Lampulo and Krueng Cut areas at three different places. Analysis of the metals was carried out using AAS and UV-Visible spectrophotometers. Samples were prepared using concentrated HNO_3 wet destruction and the addition of dithizone. The results showed Pb measured by AAS in *Saccostrea cucullata* 2.33-8.00 ppb, and by UV-Vis 2.77-8.66 ppb. Pb within *Crassostrea gigas* by AAS method 1.77-10.30 ppb, and by UV-Vis method 2.11-10.66 ppb. The Cd metal measured by AAS in *Saccostrea cucullate* is 3.80-10.50 ppb, and by UV-Vis method is 4.29-10.77 ppb, whereas Cd in *Crassostrea gigas* measured by AAS is 12.11-13.22 ppb and by UV-Vis method is 12.77-13.66 ppb. Linearity of Pb measurements with AAS and UV-Vis was obtained R^2 =0.9979 and R^2 =0.9938 and for Cd were R^2 =0.9986 and R^2 =0.9810. Accuracy (%recorvery) of Pb measurements by AAS and UV-Vis are 80-110% and 98-113% whereas Cd showed 100-106% and 91-107% respectively. The relative value of the standard deviation (%RSD ± SD) is 0.001 indicating excellent measurement results. Based on t-test calculations, measurements of Pb and Cd levels showed no difference between the AAS method and the UV-Vis method

Keywords:Pb, Cd, Crassostrea gigas, Saccostrea cucullata, AAS and UV-Vis Spectrophotometer

Article Info

Received 5 March 2023
Received in revised 5 May 2023
Accepted 12 May 2023
Available online 27 June 2023

Abstrak (Indonesian)

Logam Pb dan Cd merupakan logam berat yang sangat beracun jika terakumulasi di dalam tubuh dapat menyebabkan penyakit yang sangat serius bahkan kematian. Penelitian ini bertujuan untuk mengetahui kandungan ion logam Pb dan Cd pada spesies tiram *Crassostrea gigas* dan *Saccostrea cucullate* di daerah Lampulo dan Krueng Cut pada tiga titik yang berbeda. Analisis Pb dan Cd dilakukan menggunakan Spektrofotometer AAS dan UV-Vis. Sampel dipreparasi secara destruksi basah dengan HNO_3 pekat dan penambahan senyawa kompleks dithizon. Hasil penelitian menunjukkan bahwa kadar Pb menggunakan SSA dan UV-Vis logam Pb (SSA) pada spesies *Saccostrea cucullata* masing-masing 2,33-8,00 ppb dan 2,77-8,66 ppb, sementara pada spesies *Crassostrea gigas* masing-masing 1,77-10,30 ppb dan 2,11-10,66 ppb. Hasil analisis logam Cd dengan metode SSA dan UV-Vis pada spesies *Saccostrea cucullata* masing-masing 3,80-10,50 ppb, dan 4,29-10,77 ppb, dan pada spesies *Crassostrea gigas* masing-masing 12,11-13,22 ppb dan 12,77-13,66 ppb. Linearitas pengukuran Pb dengan SSA dan UV-Vis diperoleh masing-masing R^2 =0,9979 dan R^2 =0,9938 serta untuk logam Cd masing-masing R^2 =0,9986 dan R^2 =0,9810. Akurasi (%recorvery) pengukuran logam Pb pada metode SSA dan UV-Vis masing-masing sebesar 80-110% dan 98-110% serta logam Cd masing-masing 100-106% dan 91-107%. Nilai relatif standar deviasi (% RSD ± SD) adalah 0,001 menunjukkan hasil pengukuran yang sangat baik. Berdasarkan perhitungan uji-t, kadar logam Pb dan Cd menunjukkan tidak ada perbedaan pengukuran dengan metode SSA dan metode UV-Vis.

Kata Kunci: Pb, Cd, Crassostrea gigas dan Saccostrea cucullata, Spektrofotometer AAS dan UV-Vis.

INTRODUCTION

The aquatic environment is one of the major impact problems faced by society in the current industrial era [1, 2]. Almost all regions in Aceh Province have experienced rivers pollution, especially in the capital city, one of which is the city of Banda Aceh [3]. The waters of Banda Aceh city have enormous potential, especially in the marine and fisheries sector with 805 natural resources and cultivators, and 440 fisheries business units [4]. Environmental pollution is a very urgent matter, because environmental damage can affect the region. The main cause of environmental damage is caused by human actions, such as disposing of industrial waste, household waste, agriculture and fisheries [3]. Substances that pollute the environment are harmful and can interfere with the health of the body, including substances that contain harmful heavy metals, such as Pb and Cd [5].

Heavy metals found in waters can harm the lives of aquatic organisms directly and indirectly. Furthermore, they can threaten human health through food chain contamination [6]. Heavy metals contained in aquatic biota and consumed by humans can cause harm to human health. Heavy metals can be absorbed into the human body and cannot be decomposed, the remains of these heavy metals will accumulate in the human body [7]. In general, the content of contaminants can be predicted using bioindicators, which are types of organisms that live in one place such as clams, snails, oysters, and others. Aquatic biota such as oysters are benthic macrofauna species and are one of the bioindicators to determine the level of heavy metal contamination in the surrounding environment. Oysters are included in the *mollusca phylum* and belong to the bivalve class.

Aquatic life such as oysters tend not to move far and obtain their food by filter feeding. The oysters used in the study were *Crassostrea gigas* and *Saccostrea cucullata*. Based on the above background, this study aims to analyze the content and level of Pb and Cd metals contamination in oyster biota taken in the Krueng Cut and Lampulo areas. This study will determine the levels of Pb and Cd metals in oyster samples using the Atomic Absorption Spectrophotometer (AAS) method, and the measurement results will be compared with the Ultra Violet-Visible (UV-Vis) spectrophotometer method. The parameters to be studied are linearity, limit of detection (LOD), and limit of quantification (LOQ), accuracy, precision, and t-test [8].

MATERIALS AND METHODS

Materials

The materials used in this study were oysters (*Crassostrea gigas* and *Saccostrea cucullata*), HNO_3, HCl, $Cd(NO_3)_2$, and $Pb(NO_3)_2$, dithizon, KCN, C_3H_6O, and distilled water (All chemicals used were from E. Merck).

Methods

Oysters that have been taken from the sampling location are then cleaned, the meat is taken, and rinsed using distilled water. Oyster meat was weighed as much as 15 grams, then oven-dried. Samples that have been dried are then crushed and filtered with a sieve to be easily read by an Atomic Absorption Spectrophotometer (AAS) and UV-Vis spectrophotometer. Furthermore, the sieved sample was weighed as much as 5 grams, then extracted using 5 mL of HNO_3 (concentrated), and heated using a hot plate, then added 5 mL of HCl. The metal content of Pb and Cd in the samples was then analyzed using the AAS method and UV-Vis spectrophotometer as recommended methods [1].

Data Analysis

Linearity

Determination of linearity by measuring standard solutions of Pb and Cd metals with concentrations of 1; 2; 3; 4 and 5 ppb. These concentrations will be measured using AAS and UV-Vis as recommended methods. In the AAS method, the specific wavelength of Pb metal is 283.3 nm, and Cd is 228.8 nm. While UV-Vis to determine the wavelength in the range of Pb 450-580 nm and Cd 350-490 nm. Absorbance values that have been obtained from AAS and UV-Vis calibration curve data. The following linear regression equation:

$$y = ax+b \quad (1)$$

Limit of Detection (LOD) and Limit of Quantity (LOQ)

Determination of limit of detection (LOD) and limit of quantity (LOQ) can be calculated based on the standard deviation value obtained from blank measurements using AAS and UV-Vis. LOD and LOQ values of the blank with 5 repetitions [9]. The following equation is used to find the LoD and LoQ values:

$$LoD = 3\times\frac{(Sy/x)}{slope} \quad (2)$$

$$LoQ = 10\times\frac{(Sy/x)}{slope} \quad (3)$$

Accuracy test

Determination of the accuracy value is carried out to determine the % recovery. The validation method is considered to meet the requirements of % recovery with a value range of 80-110% [10]. Determination of the accuracy of each measured concentration of Pb and Cd metals 1; 3; and 5 ppb at the concentration of the standard used, and compared with the initial concentration with the same concentration. The concentration of the standard solution was measured using AAS and UV-Vis [11]. Equation to determine the accuracy value:

$$R = \frac{X}{\mu} \times 100\% \quad (4)$$

Precision test

The determination of the precision value is carried out by measuring the instrument response to Pb and Cd metals at a measured concentration of 1; 3; and 5 ppb. Measurements were taken 3 times using AAS and UV-Vis and the average value was calculated with the Relative Standard Deviation (% RSD) equation [12]. The following equation to determine the precision value of %RSD:

$$SD = \sqrt{\frac{\Sigma(xi-\bar{x})^2}{n-1}} \quad (5)$$

The % RSD equation is shown in the following equation:

$$\%RSD = \frac{SD}{\bar{x}} \times 100\% \quad (6)$$

Comparison of t test method

Comparison of methods was carried out to determine the oyster samples in the study conducted by AAS and UV-Vis methods in determining the characterization of the samples tested in the t test [13].

RESULTS AND DISCUSSION

Sample preparation was carried out by the deconstruction method with a mixture of HNO_3 acid solution and then measured the levels of Pb and Cd metals using the AAS and UV-Vis methods. This is because HNO_3 is a strong acid, so that it can be used as an oxidizing agent and accelerate the deconstruction process [14]. The use of oxidizing agents aims to determine the effective oxidizing agent for determining heavy metal levels in samples [15]. Wet deconstruction of the sample greatly affects the metal content resulting from the AAS and UV-Vis methods [16]. Wet deconstruction is indicated by obtaining a clear solution, and indicates that all constituents have dissolved completely or the breakdown of organic compounds has been successful [17]. The sample solution will be measured for Pb and Cd metals using AAS and compared with UV-Vis.

Pb Metal Content in Samples

Determination of maximum λ of Pb standard solution

Measurement of the maximum λ of the Pb standard solution by AAS using a hollow cathode lamp that is specific for Pb metal. This hollow cathode lamp has a maximum λ on Pb metal of 283.3 nm, the hollow cathode lamp has a radiation source for measuring Pb metal levels in the sample. Light at that wavelength has enough energy to change the electronic level of Pb. The results of the maximum λ measurement on Pb metal are supported by the results of research conducted previously [18]. Furthermore, the maximum λ of Pb standard solution was measured by UV-Vis method. Measurement of the maximum λ of Pb metal was carried out on Pb which had been complexed first to produce a colored solution. Pb standard solution was reacted with dithizon complexing agent and KCN was added as a binder to bind other metals besides lead [19]. Mixing of Pb standard solution and complex compound form a reddish colored complex compound. The maximum λ is measured in the range of λ 450-580 nm (**Figure** 1).

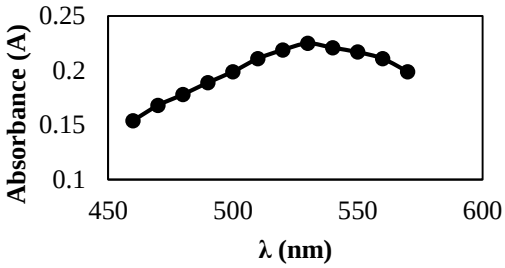

Figure 1. Wavelenght curve of Pb by UV-Vis

Calibration curve measurement

Calibration curves are used to determine heavy metal levels in samples. In this study, the determination of the Pb solution calibration curve was carried out by measuring the standard solution with a concentration of 1-5 ppb. Measurement of the calibration curve with the regression equation is y = ax + b, and the results of the measurement of the Pb calibration curve with the AAS method are shown in **Figure** 2.

Pb measurement in AAS method obtained calibration curve value with regression equation y = 0.0062x + 0.0025 with coefficient of determination R^2 = 0.9979.

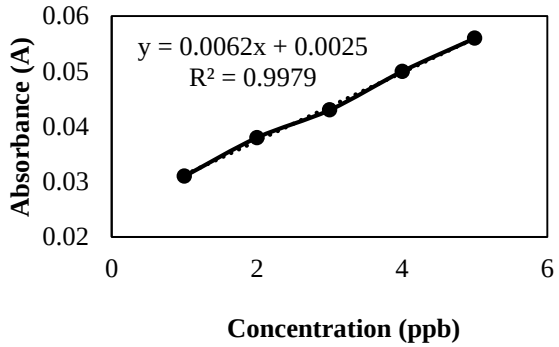

Figure 2. Calibration curve of Pb by AAS

Calibration curve of Pb standard solution in UV-Vis method with concentrations of 1-5 ppb. The calibration curve obtained is also in accordance with the Lambert-Beer law, which states that the greater the concentration of the solution, the greater the absorbance value obtained. The measurement results of the calibration curve using UV-Vis are shown in Figure 3.

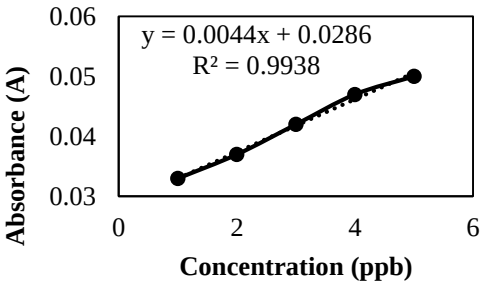

Figure 3. Calibration curve of Pb with UV-Vis

Based on the calibration curve of Pb measurement in UV-Vis method, the calibration curve value was obtained with the regression equation y = 0.0044x + 0.0286 with the coefficient of determination R^2 = 0.9938. The curve obtained shows a linear line with concentration directly proportional to the absorbance value.

Analysis of Pb content in sample

Analysis of Pb metal content in the samples that have been prepared using the wet destruction method with the addition of concentrated nitric acid, and heated at 110 ˚C as a process of breaking the bonds of organic compounds contained in the sample [20].

Measurements with the AAS method and with the UV-Vis method show the same accuracy. There is a difference in the measurement with the AAS method and the UV-Vis method. Due to the UV-Vis method in analysis, there are matrices or impurities that interfere with the analysis of samples of other metals [21].

Table 1. Comparison of Pb metal sample measurement methods

Sample	AAS	UV-Vis
	Concentration (ppb)	Concentration (ppb)
Saccostrea cucullata Lampulo (1)	4.00	4.66
Saccostrea cucullata Lampulo (2)	2.33	2.77
Saccostrea cucullata Lampulo (3)	8.00	8.66
Crassostrea gigas Krueng Cut (4)	3.55	3.90
Crassostrea gigas Krueng Cut (5)	1.77	2.11
Crassostrea gigas Krueng Cut (6)	10.30	10.66

Cd Metal Content in Samples

Determination of maximum λ of Cd standard solution

Measurement of the maximum λ of the Cd standard solution by AAS was carried out using a hollow cathode lamp specific for Cd metal. The maximum λ was measured in the range of λ 350-490 nm. The results of determining the maximum wavelength of Cd metal by UV-Vis as shown in **Figure** 4.

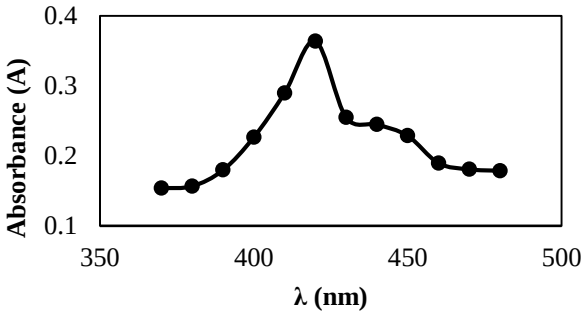

Figure 4. Wavelength curve of Cd metal using UV-Vis.

Calibration curve measurement

The calibration curve is made based on the Lambert-Beer Law, with the calculation of linear regression, y = ax + b getting a straight line [22]. The measurement results of the Pb calibration curve with the AAS method are shown in **Figure** 5.

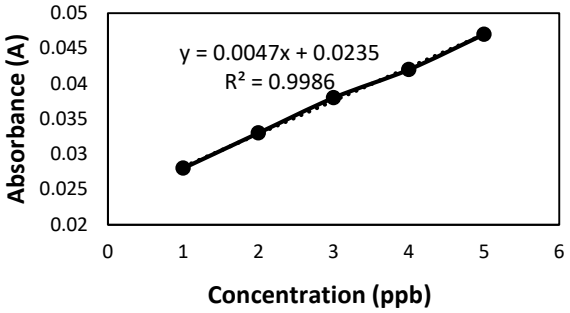

Figure 5. Calibration curve of Cd with AAS at λ 228.8 nm.

Based on **Figure** 5, it can be seen that the calibration curve of the measurement results of the Cd standard solution with the AAS method is obtained with the regress Cd ion equation y = 0.0047x + 0.0235 with the coefficient of determination R^2 = 0.9986. The calibration curve shows a linear line, directly proportional to the absorbance value, and in accordance with the Lambert-Beer Law which states that the higher the concentration, the higher the absorbance value obtained (**Figure** 6).

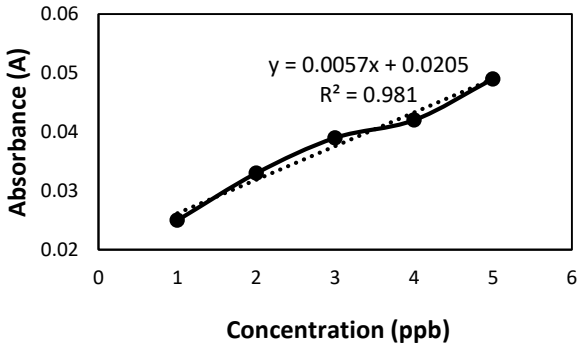

Figure 6. Calibration curve Cd with UV-Vis at λ 420 nm.

A calibration curve was obtained with the regression equation y = 0.0057x + 0.0205 with a coefficient of determination R^2 =0.981. The curve obtained shows a linear line, so it is directly proportional to the absorbance value.

Analysis of Cd content in the sample

Analysis of Cd metal content in the samples that have been prepared using the wet dextruction method with the addition of concentrated nitric acid, and heated at 110 ˚C as a process of breaking the bonds of organic compounds contained in the sample [20]. The results of the measurement of Cd metal content with both AAS and UV-Vis methods are shown in **Table** 2.

Table 2. Comparison of Cd metal sample measurement methods

Sample	AAS Concentration (ppb)	UV-*Vis* Concentration (ppb)
Saccostrea cucullata Lampulo (1)	10.50	10.70
Saccostrea cucullata Lampulo (2)	4.33	4.77
Saccostrea cucullata Lampulo (3)	3.80	4.20
Crassostrea gigas Krueng Cut (4)	12.66	12.77
*Crassostre gigas*Krueng Cut (5)	13.22	13.66
Crassostrea gigas Krueng Cut (6)	12.11	12.80

The measurement results of Cd metal levels in both methods do not have a significant difference. This shows that measurements with AAS and UV-Vis are equally accurate. Measurements with the AAS method and with the UV-Vis method there are differences, because in the UV-Vis method in an analysis there are matrices or impurities that interfere when analyzing samples of other metals [21].

Validation Methods

Linearity

Linearity testing is a change in response to the AAS method with changes in analyte concentration with a linear curve shape, with the line equation y = ax + b and the coefficient value R^2 close to 1. Cd metal against the AAS method with linearity parameter values on Cd metal at concentrations of 1-5 ppb with a value of R^2 = 0.9986, showing linear results.

Limit of Detection (LoD) and Limit of Quantity (LoQ)

The LOD value is determined to determine the number of analytes that give a significant response to the method. The determination of the LOQ value serves to determine the smallest level or the smallest concentration of Pb for determining the accuracy and precision of a sample (**Table** 3).

Table 3. LOD & LOQ values of Pb metal using AAS and UV-Vis method

Validation method	Pb AAS (ppb)	Pb UV-Vis (ppb)
LoD	0.002	0.042
LoQ	0.009	0.142

The smaller the LoD and LoQ values indicate that the method used is more thorough, because it is able to measure the smallest amount of analyte [23]. The LoD and LoQ values of Cd metal can be seen in **Table** 4.

Table 4. LOD & LOQ values of Cd metal using AAS and UV-Vis methods

Validation Methods	Cd AAS (ppb)	Cd UV-Vis (ppb)
LoD	0.006	0.061
LoQ	0.023	0.200

Based on the smaller LoD and LoQ values, it shows that the method has a high value for a number of tested components.

Accuracy

Determination of the accuracy value is done as a recovery value in the form of percent (% recovery). The concentration values used were 1; 3; and 5 ppb. Accuracy values were obtained using AAS and UV-Vis methods (**Table** 5).

Table 5. The % recovery value of Pb metal using AAS and UV-Vis methods (n=3)

Concentration (µg/L)	Measurement Concentration (ppb)		% *Recovery*	
	Pb (AAS)	Pb (UV-Vis	Pb (AAS)	Pb (UV-Vis)
1	0.80	0.98	80	98.6
3	3.30	3.25	110	108.3
5	4.80	4.69	96	93.8

Based on **Table** 5, it can be seen that the percentage of recovery and repeatability of concentrations of 1; 3; and 5 ppb of %recovery for Pb metal using the AAS method is in the range of 80-110%, while for the UV-Vis method in the range of 93.8-108%. The %recovery results obtained have met the criteria, where the measurement of Pb metal levels is in the range of 80-110% [11].

Recovery for Cd metal using AAS method is in the range of 100-106%, while for UV-Vis method in the range of 91.8-107.3%. The % recovery results obtained have met the criteria, where the measurement of Cd metal levels is in the range of 80-110% [11].

Table 6. Cd metal recovery % values using AAS and UV-Vis methods (n=3)

Concentration (ppb)	Measurement Concentration (ppb)		% *Recovery*	
	Cd (AAS)	Cd (UV-Vis)	Cd (AAS)	Cd (UV-Vis)
1	1.00	0.91	100.0	91.8
3	3.10	3.05	103.3	101.8
5	5.30	5.36	106.2	107.3

Precision

Precision tests were carried out on Pb and Cd by measuring concentrations for 3 repetitions. Determination of the precision value is done as a relative value of standard deviation (%RSD). The precision value is used to see the closeness of the value in the measurement repetition process (**Table** 7).

Table 7. %RSD values of Pb metal using AAS and UV-Vis methods

Concentration (ppb)	Repetition	%RSD		SD	
		Pb (AAS)	Pb (UV-Vis)	Pb (AAS)	Pb (UV-Vis)
1	1 2 3	0.961	1.369	0.001	0.001
3	1 2 3	0.847	1.379	0.001	0.002
5	1 2 3	0.314	0.281	0.001	0.001

The standard deviation value indicates that the measurement has a high level of accuracy. The standard deviation and %RSD values obtained from Pb metal (AAS) are 0.961%; 0.847%; 0.314%, while the calculation of %RSD using the UV-Vis method on Pb metal obtained a standard deviation value of about 0.001-0.002. The standard deviation value has very good accuracy. The standard deviation value and %RSD value obtained from Pb metal (UV-Vis) are 1.369%; 1.379%; 0.281%.

The standard deviation and %RSD values obtained from Cd metal (AAS) are 0.844%; 1.488%; and 0.214%, while the calculation of %RSD using the UV-Vis method on Cd metal obtained a standard deviation value of about 0.001-0.002. The standard deviation value has very good accuracy. The standard deviation value and %RSD value obtained from Cd metal (UV-Vis) are 0.763%; 1.498%; and 0.295%. The results obtained show that the %RSD value obtained has met the good precision value of <2%. [22].

Table 8. %RSD values of Cd metal using AAS and UV-Vis methods (n=3)

Concentration (µg/L)	Repetition	% RSD		SD	
		Cd (AAS)	Cd (UV-Vis)	Cd (AAS)	Cd (UV-Vis)
1	1 2 3	0.844	0.763	0.001	0.001
3	1 2 3	1.488	1.498	0.002	0.002
5	1 2 3	0.214	0.295	0.007	0.001

Comparison of t-test methods

The determination of the t-test is done to see the comparison between the AAS and UV-Vis methods, whether both have similar test results. The hypothesis of this research are:

H_0: No difference in the measurement of Pb and Cd metals by AAS and UV-Vis methods

H_1: There are differences in the measurement of Pb and Cd metals by AAS and UV-Vis methods.

The results of comparison of sample concentrations by AAS and UV-Vis methods are shown in **Table**. 9

Table 9. Pb and Cd metal t-test by AAS and UV-Vis methods

Heavy Metal	t_{test}	t_{table} 8 ; 95%
Pb	1.3	2.31
Cd	1.6	

The determination of the t test is carried out to see if the two methods have a significant difference. The calculation of the t test is carried out with two directions with a 95% confidence interval and 8 free degrees and has a t_{table} value = 2.31. Based on the table above, it can be seen that the value of $t_{statistic} < t_{table}$, and it can be concluded that there is no difference in the measurement of Pb and Cd metals by AAS and UV-Vis methods [13].

CONCLUSION

Pb and Cd metal levels in *Crassostrea gigas* and *Saccostrea cucullata* oysters are still below the threshold set by The Indonesian Food and Drug Authority (BPOM), and are suitable for consumption.

REFERENCES

[1] Emersida, "Kandungan logam berat Pb, Cu dan Zn pada sedimen dan tiram (*Crassostrea Cucullata Born*) di muara sungai los kala kecamatan muara satu kota lhokseumawe provinsi aceh," *Jurnal Sains, Teknologi, Ekonomi, Sosial dan Budaya*,. vol, 5, no. 3, pp. 1-5, 2021.

[2] Muslim, B, "Analisis kadar logam berat timbal (pb) pada air dan tiram (crassostrea sp) di pantai mangara bombang kecamatan tallo makassar," Skiripsi sarjana, Universitas UIN Alauddin Makassar, Makassar, 2018.

[3] Rahmadi, D., Machdar, I., & Syaubari, "Analysis of water quality and quality status in aceh rivers based on environmental pollution index" *J. Environ. Chem. Eng*., vol. 17, no. 2, pp. 171-181, 2020.

[4] Angraini. N, "Penggunaan spektrofotometer Uv-Vis untuk analisis nutrien fosfat pada sedimen dalam rangka pengembangan modul praktikum oseanografi kimia," *Jurnal Penelitian Sains*, vol. 23 no. 2, pp. 78-83, 2021.

[5] Pratiwi, D. Y., "Dampak pencemaran logam berat terhadap sumber daya perikanan dan kesehatan manusia," *Jurnal Akuatek*, vol. 1 no. 1, pp. 59-65, 2020.

[6] Azizah, R., Malau, R., Susanto, A.B., Santosa, G.W., Hartati, R., Irwani, I. & Suryono, S., "Kandungan timbal pada air, sedimen, dan rumput laut sargassum sp. di perairan jepara, indonesia," *Jurnal Kelautan Tropis*, vol. 21 no. 2, pp. 155-166, 2018.

[7] Perangin-Angin, M, S., "Analisis kadar logam Pb, cd, dan cu pada ikan lele (clarias gariepinus linn) yang dibudidayakan secara spektrofometer serapan atom" Skripsi Sarjana, Universitas Sumatera Utara, Medan, 2019.

[8] Irfani, D. S., "Hubungan keracunan timbal (Pb) dengan morfologi sel darah merah pada tukang cat mobil di kota padang, Skripsi Sarjana, Sekolah Tinggi Ilmu Kesehatan Perintis Padang, Padang, 2020.

[9] Muadifah, A., & Ngibad, K., "Analisis Merkuri dan hidrokuinon pada krim pemutih yang beredar di blitar" *Jurnal Pendidikan Kimia dan Ilmu Kimia*, vol. 3, no. 2, pp. 1-9, 2020.

[10] Rona, M, N, "Validasi metode penentuan mn dalam oli lubrikan dengan metode pengenceran langsung menggunakan spektrofotometer serapan atom," *Indo. J. Chem. Res*, vol. 6, no. 2, pp. 94-100, 2019.

[11] Sujana, D., & Nurul, "Validation method for determination of niclosamide monohidrate in veterinary medicine using UV–Vis spectrophotometry" *Jurnal Ilmiah Farmako Bahari*, vol. 11, no. 2, pp. 153-160, 2020.

[12] Amalia, V, "Optimasi digesti asam pada analisis merkuri (Hg) dalam sedimen dengan menggunakan teknik Vapor Generation Accessory-Atomic Absorption Spectrophotometer (VGA-AAS)," *Al-Kimiya*, vol. 7, no. 2, pp. 67-74, 2020.

[13] Umaningrum, D., Radna, N., & Maria, D.A., "Perbandingan metode potensiometri dan spektrofotometri pada penentuan formalin," *Jurnal Sains dan Terapan Kimia*, vol. 15, no.1, pp. 31-36, 2021.

[14] Rusnawati., Yusuf, B., & Alimuddin, "Perbandingan metode destruksi basah dan destruksi kering terhadap analisis logam berat timbal (Pb) pada tanaman rumput bebek (Lemna minor)," *Prosiding Seminar Kimia*, pp. 73-76, 2019.

[15] Farida, M., "Analisis Logam Timbal (Pb) dalam eye shadow menggunakan atomic absorption spectrophotometry (AAS)" *Serambi Konstruktivis*. vol. 4, no. 2, pp. 383-387, 2022.

[16] Habibi, Y., "Validasi metoda destruksi basah dan destruksi kering pada penentuan logam timbal (Pb) dan kadmium (Cd) dalam tanaman rumput. *Integrated Lab Journal*, vol. 8, no. 1, pp. 25-31, 2020.

[17] Faqihuddin, "Perbandingan metode destruksi kering dan destruksi basah instrumen spektrofotometri serapan atom (SSA) untuk analisis logam" *Prosiding Seminar Nasional Hasil Riset dan Pengabdian*, pp. 121-127, 2021.

[18] Fajriah, N., Zulfadli., & Nasir, M., "Analisis Kadar Logam timbal (Pb) dan kadmium (Cd) pada tanaman kangkung (ipomoea aquatica) menggunakan spektrofotometer serapan atom (SSA)" *Jurnal Ilmiah Mahasiswa Pendidikan Kimia*, vol. 2, no. 3, pp. 162-171, 2017.

[19] Rahman, U., "Dithizon: Agen pengkompleks untuk analisis logam menggunakan spektrofotometer Uv-Vis, *Farmaka*, vol. 14, no. 2, pp. 308-317.

[20] Yugatama, A., Mawarni, A. K., Fadillah, H., & Zulaikha, S. N., "Analisis kandungan timbal dalam beberapa sediaan kosmetik yang beredar di kota Surakarta," *Journal of Pharmaceutical Science and Clinical Research*, vol. 4, no. 1, pp. 52-59, 2019.

[21] Hariawan, T., "Penentuan Konsentrasi Dithizon dan pH Optimum dalam Pembuatan Test Kit Timbal" Skripsi sarjana Universitas Brawijaya, Malang, 2018.

[22] Nur Y, Rohaeti E, Darusman, Latifah K., "Optical sensor for the determination of Pb^{2+} based on immobilization of dithizone onto chitosan silica membrane," *Indonesian J. Chem*., vol. 17, no. 1, pp. 7-14, 2017.

[23] Panggabean, A. S, Pasaribu, S. P., Kristiana, F., "The utilization of nitrogen gas a carrier gas in determination of Hg ions using cold vapor-atomic absorption spectrophotometer (CV-AAS)," *Indonesian J. Chem*., vol. 18, no. 2, pp. 279-285, 2018.

[24] Rohmah, S.A.A., Muadifah, A., and Rahma Diyan Martha, R.M, "Validasi metode penetapan kadar pengawet natrium benzoat pada sari kedelai di beberapa kecamatan di kabupaten tulungagung menggunakan spektrofotometer Uv-Vis," J*urnal Sains dan Kesehatan* (JSK), vol 3. no 2. pp. 120-127, 2021.