

# C3. Muti'ah

*by Muti'ah Muti'ah*

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# The Efficiency of Heavy Metal Analysis Method in Marine Fish Samples by Atomic Absorption Spectrophotometry

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**Abstract:** This research studies the efficient method for heavy metal analysis in marine fish samples by atomic absorption spectrophotometry. The dissolution of the samples used the wet destruction technique in 2 ways. The first method uses a mixture of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HClO<sub>4</sub> (1:2:1), and the second method uses a mixture of HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> (1:1). Tuna (*Euthynnus affinis*) was taken as Marine fish samples. The results showed that the first method took 180 minutes with a % recovery of 98.79% - 99.55%. In contrast, the second method took 240 minutes and a % recovery of 98.08% - 98.45%. The results of measurements by atomic absorption spectrophotometry (AAS) using standard calibration curve and standard addition techniques are as follows: using standard regression curve technique obtained Pb (0.664 ± 0.067) mg/kg, Cu (2.780 ± 0.01) mg/kg, Cd (0.192 ± 0.044) mg/kg, and Zn (1.824 ± 0.075) mg/kg, while the standard addition method obtained Pb (0.612 ± 0.016) mg/kg, Cu (2.364 ± 0.016) mg/kg, Cd (0.148 ± 0.029) mg/kg and Zn (1.692 ± 0.016) mg/kg. The calculation of the level of accuracy of the analysis showed that the standard addition method RSD gave a precision value of 0.97% - 3.76% and the standard curve method RSD with a precision of 5.76% - 31.77%. In conclusion, (1) Heavy metal content in tuna (*Euthynnus affinis*) is still within the permissible limits. (2) wet digestion technique using a mixture of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HClO<sub>4</sub> (1:2:1) can be more efficient. (3) The measurement results of the standard addition method give more precise results.

**Keywords:** Heavy Metals; Atomic absorption spectrophotometry; Double standard addition

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## Introduction

Heavy metals Pb, Cu, Cd, and Zn, are classified as toxic elements that are dangerous if they enter the body either directly or through food (Maslowska, 1993; Sarojam, 2009). The poisonous effects of Pb, Cd, and Hg impaired kidney function and liver. Pb and Hg decreased cognitive function, Cd and Pb impaired reproductive capacity, hypertension (Cd), changes in neurological (Hg, Pb), teratogenic (Hg), and cancer effects (Cd) (Lee et al., 2011; Mattia et al., 2004; Weerasinghe & Kaumal, 2018). The presence of heavy metals in marine fish is caused by environmental contamination, such as water and sediment (Fernandes et al., 2007; Goyer, 1997; Kabata-Pandias & Zmudzky,

1992; Papagiannis et al., 2004). Therefore, the presence of heavy metals in marine fish needs to be known precisely.

Several researchers have reported heavy metal content in marine fish, such as *Gadus morhua* (Hellou et al., 1992), *Anarhichas*, and *Raja fyllae* (Zauke et al., 1999), *Dicentrarchus labrax* (Romeo et al., 2000) and types of *Sparus auratus* and *Atherina hepsetus* (Canli & Atli, 2003). Meanwhile, other researchers reported heavy metals in Cod (Hendry et al., 2004) and *Saurida undosquamis* and *Sparus aurata* (Turkmen et al., 2005).

Sample dissolution and measurement are crucial stages of analysis. The process of dissolving samples in chemical analysis is called destruction, which changes the shape of a solid sample into a solution ready to be

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measured on measurements using AAS, spectrophotometry, or other methods.

The main problem in the analysis of heavy metals in marine fish samples is the method of destruction and measurement. Methods are known as digestion dry digestion (Maslowska, 1993). The digestion is based on an ashing process followed by dissolution with inorganic acids. This method takes a long time, and there can be a loss of analyte during the washing process. The wet digestion method is based on a reaction with a mixture of inorganic acids at high temperatures, for example, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub> + H<sub>2</sub>SO<sub>4</sub> (1 : 1), H<sub>2</sub>SO<sub>4</sub> + HNO<sub>3</sub> + HClO<sub>4</sub> (1 : 2 : 1 : 1). (Ranasinghe et al., 2016) This method destruction has problems, namely the difficulty of dissolving caused by the presence of fat in the sample, which affects the quality of both the process and analysis.

The difference in the matrix between the sample solution and the standard solution is a measurement problem using AAS. We need a method to reduce or avoid the interference matrix to overcome this. The standard addition method is an analytical technique that aims to equate the sample solution matrix with the standard. (Harvey, 2002; Jasim et al., 2020; Suwarsa & Nurdin, 1986). The efficiency of the destruction process shows the quality of the analytical process aspect. In contrast, the quality of the analysis results aspect can be shown by the accuracy (precision) of the measurement results and the value of a high level of confidence

In marine fish samples, the specific objectives are finding the destructive substance's time and accuracy (recovery). The accuracy and confidence level of the analysis was performed using the standard regression method and the double standard addition method heavy metal content in tuna (*Euthynnus affinis*) samples.

The benefit of this research is that it can be used as a basis for heavy metal analysis in fish tissue by finding an effective analytical method.

## Method

### Samples

The samples used in this study were marine fish obtained from the waters of Lombok from the type of tuna (*Euthynnus affinis*).

### Working

Procedures The work procedures in this study refer to previous researchers with the following steps: (Hendry et al., 2004; Ranasinghe et al., 2016).

#### Mineralization/Destruction

In this study, the mixture of acids used in wet digestion was HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HClO<sub>4</sub> (1 : 2 : 1), and a mixture of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> (1 : 1). The parameter studied was the time required for the destruction to form a clear solution. Five grams of dry and delicate samples were

put into a 250 ml Kjeldahl flask and added 20 ml of a mixture of (1) HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, and HClO<sub>4</sub> (1 : 2 : 1) and a mixture of (2) HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> (1 : 1). Then each is heated until completely dissolved. 5 ml of HNO<sub>3</sub> was added to dissolve completely. After completely dissolved (almost dry solution), add 20 ml of HNO<sub>3</sub> (1) and reheat until the solution turns clear (Weerasinghe & Kaumal, 2018). Each treatment was determined the time required so that the fish tissue turned into a solution.

The solution resulting from digestion was diluted and filtered with Whatman 42 filter paper into a 100 ml volumetric flask and added aqua to 100 ml.

#### Measurements with AAS Atomic Absorption Spectrophotometry

Heavy metal concentrations were measured with AAS at a specific wavelength: Pb 217 nm; Cu 324.7 nm; Cd 228.8 nm; and Zn. 213, 9 nm (Sarojam, 2009). The concentration of heavy metals in the sample solution was calculated based on the standard curve equation Y = bx + a, which is the curve of the relationship between the absorbance of the standard solution and the standard concentration. (Harvey, 2002).

#### Calculation of Heavy Metal Concentration in Samples

$$mgkg^{-1} = \frac{C \times V}{w} \times 1000 \dots\dots\dots (1)$$

where:

C = sample solution concentration (mgL<sup>-1</sup>) calculated from the standard curve equation

C = (Y - a)/b where a is the intercept and b is the standard curve constant. As for the standard addition curve, the concentration of the sample solution is C = a/b.

V = volume of sample solution in liters

w = sample weight

1000 = conversion from kg to grams

#### Data Analysis

To determine the time and percent recovery of the results of the analysis of heavy metal content from both methods of destruction and the level of confidence, then analyzed descriptively (Endah & Nofriyaldi, 2020; Miller & Miller, 1991).

## Result and Discussion

### Process Destruction Sample

In analyzing heavy metals in fish tissue, the initial process that needs to be carried out is the destruction or dissolution of the sample. In this study, the mixture of acids used in wet digestion was HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HClO<sub>4</sub> (1:2:1) and a mixture of (2) HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> (1:1). The parameter studied was the time required for the destruction to form a clear solution. Validation of the method is carried out to determine the destruction,

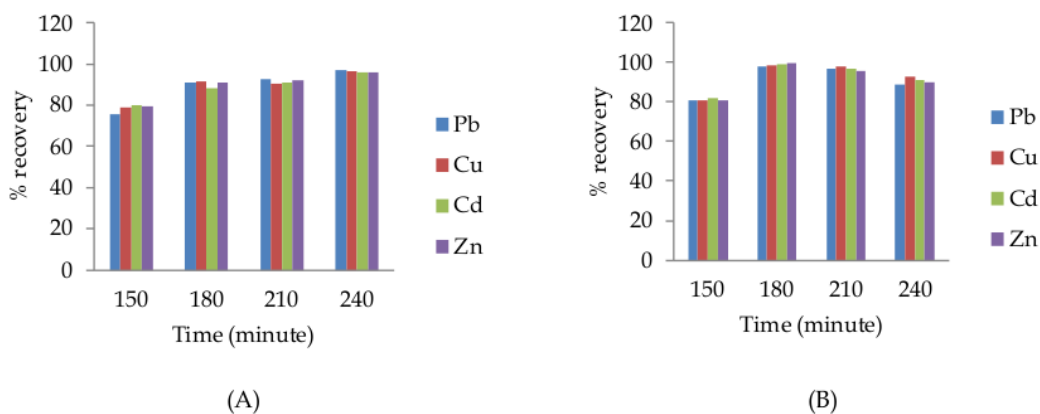
namely accuracy testing, by adding a solution to the digestion process to calculate the percent recovery – the measurement of the destruction time and % recovery of each heavy metal area listed in Table 1.

Table 1 shows that the highest % recovery was achieved for the destruction, 180 minutes (3 hours) with a mixture of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HClO<sub>4</sub> (1:2:1) with % recovery between 98.79% - 99.55%. At a destruction time of 240 minutes (4 hours) with a mixture of HNO<sub>3</sub> H<sub>2</sub>SO<sub>4</sub> (1 : 1 ) with recovery between 98.08 % - 98.45%. The thing that causes the time difference is the addition of the oxidizing agent, perchloric acid HClO<sub>4</sub>, a strong oxidizing agent. However, the weakness of using

perchloric acid is cost because the price of perchloric acid is relatively much higher than sulfuric acid and nitric acid. It can be seen from percent recovery with criteria between 98% to 102% to determine the effectiveness of the destruction process. A mixture of destroyers with a mixture of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HClO<sub>4</sub> (1 : 2 : 1) seems to give a better recovery value and is more effective than the destroyer of a mixture of HNO<sub>3</sub> H<sub>2</sub>SO<sub>4</sub> (1 : 1 ) at the same time. The shorter time, however, the % recovery value for both materials is still within the permissible criteria – the effect of the destruction time on the % recovery as shown in the diagram in Figure 1.

**Table 1.** Table of destruction and % recovery time

	150 (minutes)		180 (minutes)		210 (minutes)		240 (minutes)	
	HNO <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub>	HNO <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub> + HClO <sub>4</sub>	HNO <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub>	HNO <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub> + HClO <sub>4</sub>	HNO <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub>	HNO <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub> + HClO <sub>4</sub>	HNO <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub>	HNO <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub> + HClO <sub>4</sub>
Pb	75.56	80.67	90.86	98.98	92.90	97.08	89.05	98.08
Cu	78.98	81.08	91.45	98.79	90.78	97.98	98.45	92.55
Cd	80.05	82.05	88.35	99.05	91.07	97.05	98.28	90.85
Zn	79.79	80.89	90.96	99.55	92.08	95.75	98.15	89.98



**Figure 1.** Effect of destruction time on % recovery of the destruction materials  
A: HNO<sub>3</sub> + H<sub>2</sub>SO<sub>4</sub> and B: HNO<sub>3</sub> + H<sub>2</sub>SO<sub>4</sub> + HClO<sub>4</sub>

In Figure A the maximum % recovery is at 240 minutes, while for Figure B, % recovery is achieved in 180 minutes. The diagram above also shows that the longer the destruction time, the greater the % recovery. In Figure B, the % recovery at 150 and 180 minutes tends to be constant and decreases at 240 minutes. It happens because, at the beginning of the sample destruction, there has not been a complete dissolution, so increasing the digestion time will increase the concentration of the solution to the maximum. At the same time, the decrease in % recovery occurs due to the destruction of the completely dissolved sample.

*Measurement by Standard Regression and Standard Addition*

The determination of the heavy metal content of Pb, Cu, Cd, and Zn from the destruction was carried out

using the AAS atomic absorption spectrophotometry method with the standard regression curve technique and the standard addition regression curve. The technique of determining levels with ordinary standard solutions (single standard or double standard/standard solution calibration curve) has limitations, namely the presence of matrix disturbances. Matrix interference is a disturbance in the analyte signal due to other substances accompanying the measurement. One of the efforts used is to add a standard solution to the sample either singly or in multiples (Harvey, 2002). In this research, double standard addition is used or called standard addition. The principle of the standard addition method is to add a series of standard solutions with a certain concentration to the digested. The standard solution concentration is added following the concentration used



in the usual standard method. The standard addition regression difference lies mainly in the absorbance value when the standard concentration is 0 ppm. In contrast, for the standard curve method, zero is obtained because there is relatively no heavy metal. In contrast, for the standard addition curve, the value is not equal to zero due to the presence of heavy metals from the sample (Harvey, 2002).

The regression equation obtained on both the regression curve and the standard addition curve is used

to calculate the concentration of heavy metals in the sample solution.

The measured sample concentration values are then used as the basis for calculating the concentration of heavy metals in fish samples with units of mg of heavy metals per 1000 grams of fish samples.

Comparison of the standard regression (SR) with the standard addition (SA) for each heavy metal is shown in Figure 2.

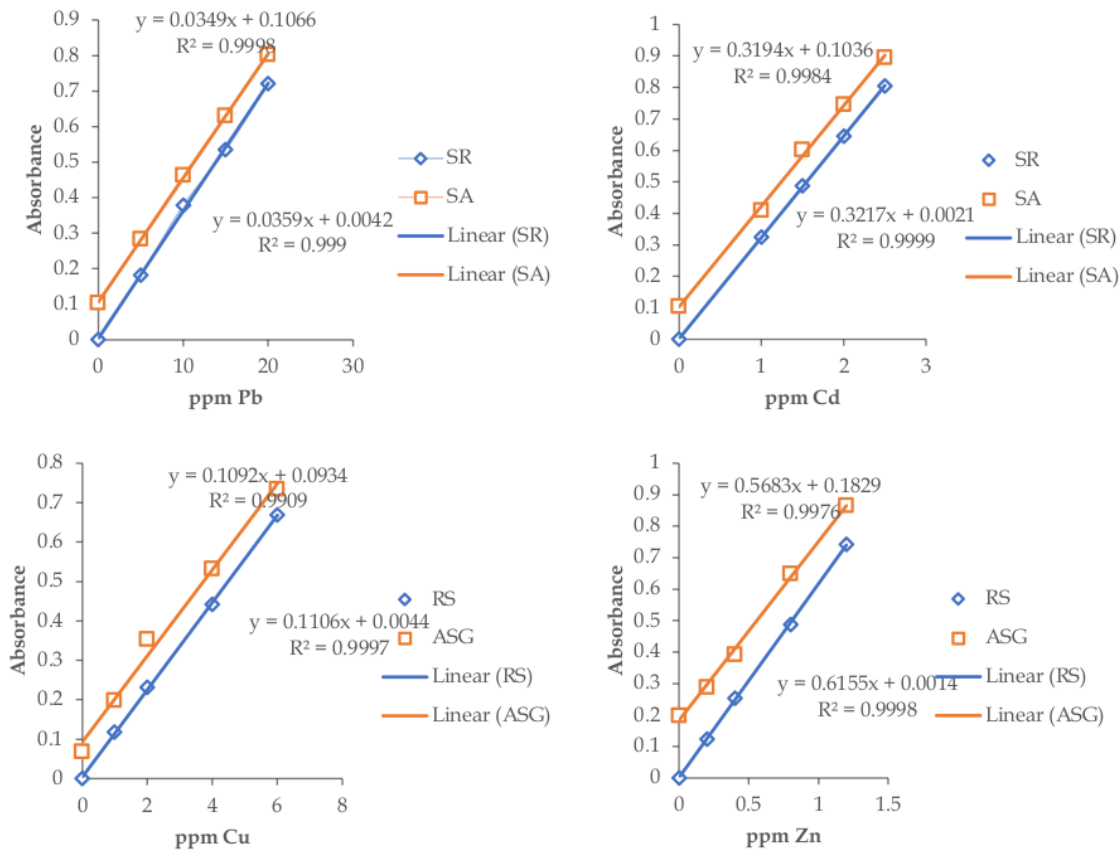


Figure 2. Standard Regression (SR) and Standard Addition (SA)

Based on Figure 2, it appears that the standard curve shows the correlation coefficient (R<sup>2</sup>) ranged from 0.9997 - 0.999. Meanwhile, on the double standard addition curve, the correlation coefficient R<sup>2</sup> is 0.9909 - 0.999. In general, the influence of matrix elements can result in self-quenching, self-absorption, and ionization processes, resulting in deviations from the analysis results. It causes the relationship of light intensity (fluorescence) emitted by atoms to be directly proportional to the concentration of elements in the sample to be non-linear.

The data on the average content of heavy metals in fish samples measured by two methods, namely the SR standard regression curve and the SA standard addition curve, can be seen in Table 2. Table 2 shows that, in general, the average heavy metal content and the standard deviation of measurements as measured by the regression curve method is greater than that measured by the standard addition method. Due to the matrix disturbance in the measurement with the ordinary regression curve (matrix disturbances that have not been eliminated), the matrix interferes with the analyte/heavy metal by increasing the absorbance of the

sample being measured. The criteria for determining the method are determined by the proximity of the measurement results (precision) expressed as standard deviation or Relative Standard Deviation (% RSD) with a requirement of not more than 2%. (Endah & Nofriyaldi, 2020). The percentage of RSD from heavy metal analysis using SR for each metal was greater than 2% while % RSD using the SA method for Pb = 3.76%, Cu = 0.973%, Cd = 2.77% and Zn = 1.359%. The RSD for Pb and Cd was still greater than 2%. Due to the lower

presence of Pb and Cd in the sample compared to Zn and Cu, the reduction of matrix disturbances was not perfect.

The merits and demerits of the two methods can also be seen from the confidence value of the analysis. The confidence value of the research is the limit value of the range resulting from the measurement results. The lower the confidence, the better the measurement results. The data for the analysis confidence level in heavy metals in fish samples are listed in Table 3.

**Table 2.** Data on Metal Concentrations of Pb, Cu, Cd, and Zn in Fish Tissue

	Pb mg/kg		Cu mg/kg		Cd mg/kg		Zn mg/kg	
	SR	SA	SR	SA	SR	SA	SR	SA
Average	0.664	2.364	2.780	2.364	0.192	0.148	1.692	1.692
SD	0.093	0.023	0.363	0.023	0.061	0.0041	0.105	0.023
%RSD	14.006	3.76	13.057	0.973	31.77	2.770	5.76	1.359

**Table 3.** Data of Confidence Level of Heavy Metals Analysis

Metals	Value of confidence level of analysis	
	Standard Regression	standard addition method
Lead - Pb	(0.664 ± 0.067) mg/kg	(0.612 ± 0.016) mg/kg
Copper - Cu	(2.780 ± ) mg /kg	(2.364 ± 0.016) mg/kg
Cadmium - Cd	(0.192 ± 0.044) mg/kg	(0.148 ± 0.029) mg/kg
Zinc - Zn	(1.824 ± 0.075) mg/kg	(1.692 ± 0.016) mg/kg

Based on data Table 3, it can be shown that the value of the confidence limit in the analysis of heavy metals with the usual regression curve is greater than that of the standard addition method. It shows that the standard addition method gives more precise results.

From the results of this study, it can be informed that the tuna under study was found to contain heavy metals. However, based on the decision of the Directorate General of Drug and Food Control number: 03725/B/SK/VII/89, (BSN, 2009), the heavy metal content is still within the permissible limits, namely Pb of 2 mg/kg, Cu 20 mg/kg and Zn 100 mg/kg. When associated with the results of previous studies, the results of this study have an equivalent value. For example, heavy metals in fish species *Saurida undosquamis*, *Sparus aurata*, *Mullus barbatus* in Mediterranean Sea waters show heavy metal content of Cd 0.01 - 4.16; Fe 0.82 - 27.35; Pb 0.09 - 6.95; Zn 0.6 - 11.57; Cu 0.04 - 5.43; Mn 0.05 - 4.64; Ni 0.11 - 12.88; Cr 0.07 - 6.46; Co 0.03 - 5.61; and Al 0.02 - 5.41 mg kg<sup>-1</sup> (Turkmen et al., 2005).

## Conclusion

The conclusions obtained from the results of this study are the method digestion with a mixed technique of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HClO<sub>4</sub> (1 : 2 : 1) takes 180 minutes with % recovery in the range of 98.79% - 99.55% and a mixture of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> (1 : 1) destruction time is 240 minutes with % recovery between 98,08% to 98.45%. The value of the analytical confidence level and the heavy

metal content in the tuna samples were as follows: using standard regression curve technique, Pb (0.664 ± 0.067) mg/kg, Cu (2.780 ± ) mg/kg, Cd (0.192 ± 0.044) mg/kg, and Zn (1.824 ± 0.075) mg/kg, while the standard addition method obtained Pb (0.612 ± 0.016) mg/kg, Cu (2.364 ± 0.016) mg/kg, Cd (0.148 ± 0.029) mg/kg and Zn (1.692 ± 0.016) mg/kg. Determining the precision value for the measurement method with standard addition was better. Namely, 0.97% - 3.76%, and the precision value for the regression curve method was 5.76% - 31,77%.

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