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Analysis of Heavy Metal Cadmium (Cd) and Lead (Pb) in Eggplant (*Solanum melongena* L.) in Pontianak City by Using Atomic Absorption Spectrophotometry (AAS)

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Abstract

In Indonesia, eggplant is a favored vegetable with substantial production. The main objective of this study is to investigate the level of safety of eggplants marketed in traditional wholesale markets in Pontianak City against possible contamination of the heavy metals cadmium (Cd) and lead (Pb), which are commonly discovered in water waste, vehicle exhaust, and excessive pesticide and fertilizer use. Alizarin Red S for Cd metal and Dithizon for Pb metal were used in qualitative assays. Cd and Pb levels were measures using an Atomic Absorption Spechtrophotometer (AAS) instrument. The analytical method has been verified for linearity, accuracy, precision, limit of detection, and limit of quantification. The qualitative test results showed that all samples had positive results for Cd and Pb. The quantitative test results revealed that the Cd and Pb metal contents in the FB (Flamboyan) sample were 0.056 mg/kg and 3.5066 mg/kg, consequently, while they were 0.0986 mg/kg and 3.957 mg/kg, consequently, in the PR (Puring) sample. In keeping with BPOM standard No. 5 of 2018, Cd metal levels in FB samples are at a safe threshold, while Cd metal levels in PR samples and Pb metal levels in FB and PR samples exceed the maximum thresholds that have been set.

Keywords: Eggplant; Cadmium (Cd); Lead (Pb); Ditizon; Alizarin; AAS.

INTRODUCTION

One of the food products that can be used as fresh food and processed food can be utilized from eggplant plants (Solanum melongena L.) (Hayati et al, 2022). In Indonesia, eggplant production has increased by 17.54%, and in West Borneo, the production reached 7,254 tons (Badan Pusat Statistik (BPS), 2021). In addition to its good health benefits, because it contains high antioxidants, several studies have found that eggplant is contaminated with heavy metals, such as cadmium (Cd) and lead (Pb) (Ahmed Youssef, 2018; Mor & Ceylan, 2008). These contaminants can come from pesticides, fertilizers, air, and water (Defarge, 2018; Lakherwal, 2014; Lugon-Moulin et al., 2006).

Agricultural activities in the use of pesticides and fertilizers according to several previous studies contain heavy metals Cd and Pb, especially in phosphate fertilizers containing very high Cd metal because the basic ingredients of its manufacture come from phosphate rock which naturally contains Cd metal (Defarge, 2018; El & Selim, 2017; Pradika et al., 2019). Meanwhile, from the air, contaminant sources can come from transportation activities that emit Cd and Pb metal exhaust emissions, while contaminant sources from water come from waste disposal due to industrial and community activities (Dong, 2017; Gautam, 2014; Suvarapu & Baek, 2017).

Lead (Pb) contamination in eggplant was determined to be 1.96 ± 0.23 mg/kg, and Cd contaminants were 0.18 ± 0.02 mg/kg (Ahmed Youssef, 2018). The safe threshold value for cadmium metal (Cd) is 0.05 mg/kg and for lead (Pb) is 0.2 mg/kg wet weight in the category of fruit and vegetable products. According to the standard regulations of BPOM No.5 of 2018 (Badan Pengawas Obat dan Makanan (BPOM), 2018) the highest tolerated limit for Cd and Pb metals entering the body within a week is 7 µg/kg and 25 µg/kg, respectively (World Health Organization, 2009). Long-term exposure to these metals can harm the neurological system and result in renal and liver problems (Mahurpawar M, 2015).

Based on the explanation above, there has been very few researches that examines the contamination of Cd and Pb metals in eggplant in traditional wholesale markets in Pontianak City, such as Flamboyan (FB) and Puring (PR) markets. Therefore, researchers are interested in measuring the levels of Cd and Pb metals in eggplant samples with standard addition quantification

techniques using Atomic Absorption Spectrophotometry (AAS) instrumentation.

MATERIALS AND METHODS

Samples and Materials

Samples of *Solanum melongena* L. (purple eggplant variety) were collected on FB and PR traditional wholesale markets of Pontianak City (Figure 1). The materials used in this research were HNO3 (65%), H2O2 (30%) p.a Merck (*Germany*), Alizarin Red S (Cd) and Ditizon (Pb) Merck (*Germany*), standard metal Cd and Pb concentrations of 1000 µg/mL Merck (*Germany*). The tools used in this study were Atomic Absorption Spectrophotometry (SSA) Instrumentation (Shimadzu ASC-2000®, *Kyoto, Japan*). Glassware (Pyrex®), analytical balance (Ohaous®), hot plate (Jeio Tech), Whatman No.42 filter paper, 60 mesh sieve (*Pharma Lab*), and micropipette (*TopPette Pipettor*).

Figure 1. *Solanum melongena L.* (purple eggplant variety) found by author on FB and PR wholesale markets of Pontianak City (personal documentation).

Sample Collecting and Processing

Samples were purchased from several traders selling purple eggplant at FB and PR Markets in Pontianak City. After that, the samples were washed, cleaned, and chopped to minimize the surface area of the samples. The samples were dried in an oven for around 12 hours at a temperature of 105º C. The surface area of the sample was reduced with a blender and equalized in size using a mesh 60 sifter.

Verification of Analysis Method

Linearity

The linearity test of Cd and Pb metals was carried out using the addition standard curve of each sample. The prepared 1.5 mL sample was put into six of 5 mL volumetric flasks. Then, each volumetric flask was added 0; 0.4; 0.6; 0.8; 1; and 1.2 mL of 10 µg/mL Cd standard concentration and added 0; 0.5; 0.7; 0.9; 1.1; and 1.3 mL of 350 µg/mL Pb standard concentration. Each volumetric flask was then set to the limit mark with aquabidest in a 5 mL volumetric flask.

Accuracy and Precision

The standard addition method is the one utilized to determine the accuracy test. The purple eggplant sample of 5 grams was dissolved with 24 mL of HNO3 (65%) using a 250 mL beaker (Pyrex®) and 5 mL of Cd and Pb standard solution per concentration level was added, namely C1 (25 µg/mL Cd and 600 µg/mL Pb), C2 (30 µg/mL Cd and 700 µg/mL Pb) and C3 (35 µg/mL Cd and 800 µg/mL Pb). The mixture was deconstructed using a hot plate (Jeio Tech, Korea) at 120ºC for 50 minutes in a fume hood. At the 30th minute, the mixture was added with 6 mL of H2O2 (30%) while shaking gently. After the deconstruction process was complete, the volume of the deconstruction was measured and filtered using Whatman No.42 paper. Furthermore, the sample was pipetted in the amount of 1.5 mL, and determined the volume with aquabidest in a 5 mL volumetric flask which will then be measured absorbance with an AAS instrument. Measurements were carried out 3 times for replication of each concentration.

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

Determination of the limit of detection (LOD) and limit of quantification (LOQ) of Cd and Pb metals using linearity data from the addition standard curve of each sample with the linearity regression equation $y = bx + a$.

Sample Preparation

The oxidizing agent used to deconstruct samples with the wet deconstruction method is a mixture of nitric acid and peroxide acid in a ratio of 4:1. The stages carried out are the same as the accuracy test method that is not added to the standard. Next, the volume of the deconstruction was measured in a measuring cup (Pyrex®) and then filtered using Whatman No.42 filter paper. The sample was stored in a glass vial bottle and sealed.

Qualitative Analysis

A qualitative test of Cd metal was carried out with Alizarin *Red* S (ARS) complexing reagent, namely into a test tube added 2 mL of sample. Furthermore, H_2SO_4

0.05 M and ARS 750 µg/mL are added a few drops, and observe the color changes that occur. A qualitative test of Pb metal was carried out with Ditizon complexing reagent, namely into the test tube added 2 mL. Furthermore, 1 N NH_4OH and 0.005% (b/v) Ditizon solution were added to a few drops, and observe the color changes that occur.

Analysis of Heavy Metal Content of Cd and Pb by Standard Addition Technique

Samples of 1.5 mL were added to six of 5 mL volumetric flasks. Each volumetric flask was then filled with 0; 0.4; 0.6; 0.8; 1; and 1.2 mL of 10 µg/mL Cd standard concentration and 0; 0.5; 0.7; 0.9; 1.1; and 1.3 mL of 350 µg/mL Pb standard concentration. Determined the volume until the limit line of the 5 mL volumetric flask with aquabidest.

RESULTS AND DISCUSSION

Sample Processing

Samples were collected from the traditional wholesale markets of Flamboyan (FB) and Puring (PR) in Pontianak City. One kilogram of wet material is processed into 78.805 g (FB) and 78.765 g (PR) of dry powder. The yield and moisture content produced in the FB market was 7.88% and 5.49%, while in the PR market, they were 7.87% and 5.41%. The moisture content of <10% indicates that the sample is dry and minimal microbial growth is possible.

Verification of Analysis Method

Linearity

Based on the results of linearity testing of Cd and Pb metals on the addition standard curve of each sample, there is a linear relationship between the absorbance value (y) and concentration (x) of each metal. This can be seen in **Figure 2** which shows that the correlation coefficient values of Cd and Pb metals in FB samples are 0.998 and 0.995, respectively. While the correlation coefficient values of Cd and Pb metals in the PR sample shown in **Figure 3** are 0.998 and 0.997, respectively. The correlation coefficient (r) of Cd and Pb metals in each sample as a parameter of the linearity test meets the requirements set by SNI, namely the value of r ≥ 0.995 (Habibi, 2020).

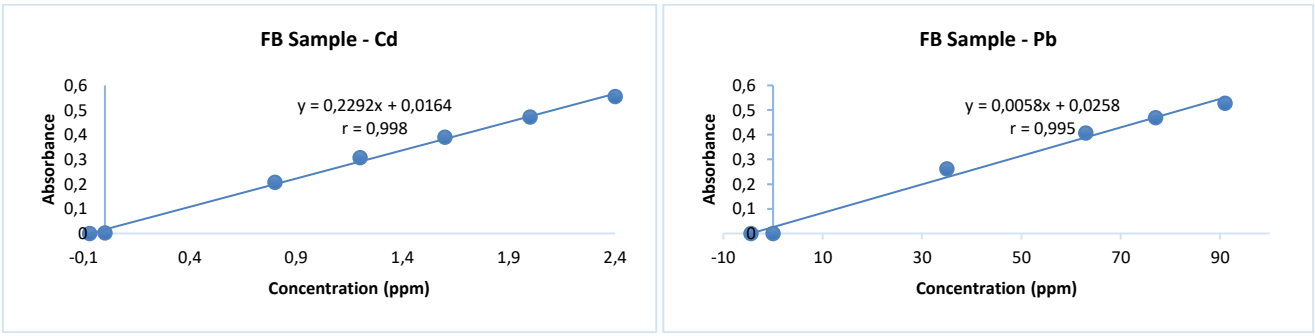

Figure 2. Linearity Curve of Cd and Pb Metals in (FB) Market using Standard Addition Technique.

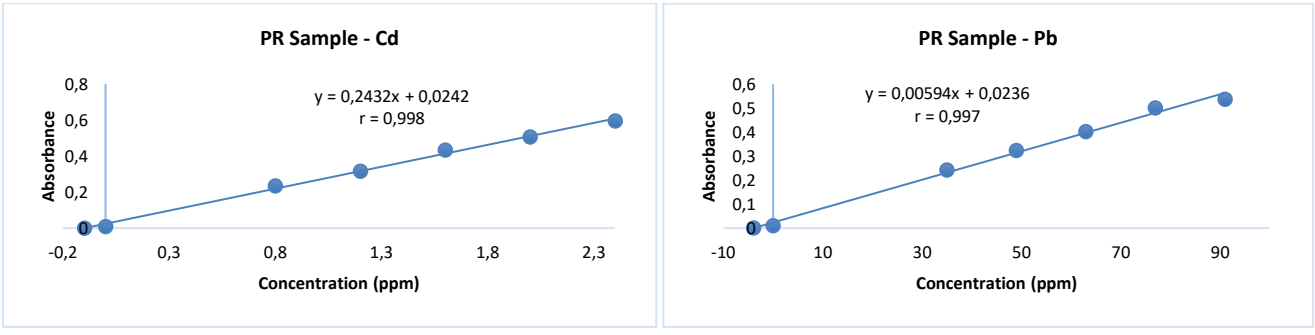

Figure 3. Linearity Curve of Cd and Pb Metals in (PR) Market using Standard Addition Technique.

Accuracy (% Recovery)

To evaluate the accuracy of an analytical method used can be assessed from the percent recovery of the accuracy test results. The percentage recovery that is closer to 100% can be said that the method used is accurate and can guarantee that the concentration of the analyte obtained is correct (Rezeki et al., 2019). The percent value of analyte recovery in **Table 1** is in the range of 81.7 - 84.98% (for Cd metal) and 98.35 - 103.22% (for Pb metal) which means the method used for sample preparation is an accurate method because the % recovery of Cd and Pb metals produced meets the

requirements according to AOAC standards, namely with criteria of 80-110% (for Cd metal) and 90-107% (for Pb metal).

There are three ways to evaluate accuracy methods, namely with Certified Reference Material (CRM), spiked-placebo, and addition standard. In this study, the method used was the addition of standards with three different concentrations with three repetitions because it is more simple, affordable, and easy to do than the CRM and spiked-placebo methods which require certified comparator compounds (CRM) and pharmaceutical preparation carrier materials without active substances (placebo) (Harmita, 2004).

Table 1. Percent (%) Recoveries of Cd and Pb Metals.

	Ca	Cs	Ct Average	*recovery* (%) Average
Cd	25	1,7046	1,448	84,98
	30	1,8	1,47	81,7
	35	2,143	1,8057	84,27
Pb	600	40,91	42,22	103,22
	700	42	42,23	100,55
	800	48,98	48,17	98,35

Note :
Ca = Concentration added (µg/mL)
Cs = True concentration (µg/mL)
Ct = Measured concentration (µg/mL)

Precision

How closely the analytical results from repeated measurements of the same concentration series are derived is how precision is defined. The precision model performed is a repeatability model because it is under the same conditions, same laboratory, analyst, and equipment (Harmita, 2004). The precision test is referred to as the percent relative standard deviation (% RSD). **Table 2** shows the % RSD values generated by Pb and Cd metals.

The % RSD values of Cd and Pb metals shown in **Table II** meet the AOAC criteria, namely % RSD ≤ 7.3% for Cd metal and % RSD ≤ 5.3% for Pb metal so that the measurement method carried out repeatedly on the same concentration series has a minimal random error, both from the methods, researcher and instrumental aspects (Gandjar G & Rohman A, 2007).

Table 2. Percent (%) RSD of Cd and Pb Metals

	Cs	Ct Average	SD	RSD (%)
Cd	1,7046	1,448	0,009	0,62
	1,8	1,47	0,01	0,68
	2,143	1,8057	0,06	3,323
Pb	40,91	42,22	1,402	3,32
	42	42,23	0,603	1,43
	48,98	48,17	0,3956	0,82

Note :
Cs = True concentration (µg/mL)
Ct = Measured concentration (µg/mL)

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

The results of the LOD and LOQ measurements in **Table 3** using the addition standard curve confirm that the instrument is still capable of detecting and quantifying Cd and Pb metal analytes at the lowest concentrations.

Table 3. LOD and LOQ.

Samples		LOD (µg/mL)	LOQ (µg/mL)
FB	Cd	0,14276	0,47587
	Pb	15,2868	50,9559
PR	Cd	0,18458	0,61525
	Pb	9,13428	30,4476

Sample Preparation

Destruction is a process to break down or decompose organic bonds that bind metal ions in the samples so that metals are in a form free that can be measurable (Asmorowati DS et al, 2020). The deconstruction method is categorized into two, wet deconstruction and dry deconstruction. In this study, sample preparation used the wet deconstruction method because the equipment used was more simplified and the time required was also relatively shorter than dry deconstruction which requires a relatively longer time and uses more complex equipment, such as a muffle furnace (Abata et al., 2019). Based on previous research, the wet deconstruction method also produces a higher percent recovery of analytes than dry deconstruction in the analysis of Pb metal in soil, namely 98-99% (Asmorowati DS et al, 2020).

To dissolve the sample's organic bonds, a 4:1 mixture of acid solvents HNO_3 (65%) and H_2O_2 (30%) was utilized. The combination of HNO_3 and H_2O_2 with a ratio of 4: 1 is the result of optimization from previous research that gets the best results with a % recovery value of 104.2% (Cd metal) and 104.75% (Pb metal) for the combination of HNO3 and H2O2 and a % recovery of 99% for the ratio of 4: 1 (Gende & Schmeling, 2022; Rezeki et al., 2019). HNO_3 is a strong oxidizer that serves to dissolve or release metal elements from bonds of organic compounds so that metals are in a measurable free form that can be measured, while H_2O_2 also acts as an oxidizing agent in helping the course of the destruction process. The mixture of strong acids as a destructive agent will increase the strength of the acid so that the destruction process will run faster and maximize in destroying organic bonds in the sample so that metals are released and dissolved (Wulandari EA & Sukesi, 2013).

The deconstruction process was carried out for ± 50 minutes using a temperature of 120ºC. The temperature selection is because HNO_3 has a boiling point of 121ºC while H_2O_2 has a boiling point of 150ºC (Anothairungrat et al., 2019). Heating should be done below the solvent's boiling point so that the solvent does not evaporate before the destruction process is complete. When the

heating process is in progress, gas bubbles and brownish-red smoke will arise due to the reaction of the sample when oxidized using HNO_3, namely CO_2 gas and NO gas. Brown smoke indicates that the organic bonds in the sample have been successfully destroyed (Wulandari EA & Sukesi, 2013). The deconstructed solution after completion will be clear, yellowish in color, thick, and no sediment.

Qualitative Analysis

Qualitative tests for Cd and Pb metals use complex reactions, namely ARS and Ditizon for Cd and Pb metals (Rahman Kustiawan & Pratiwi, 2016; Ullah & Haque, 2010). Complexing reagents are used because the analytes to be analyzed are small, and hence by using these complexing reagents the visualization of the color formed will be more clearly in showing the positive results of the sample to be analyzed. Positive samples containing Cd metal, if it reacts with ARS, will produce a yellow color, while positive samples containing Pb metal, if it reacts with ditizon, will produce a pink color (Faradilla et al, 2020; Wongthanyakram & Masawat, 2019).

FB and PR samples are positive for Cd metals shown in **Figure 4** by changing the color from light yellow to dark yellow when adding ARS. The color change from less intense was produced before dropping the ARS reagent and after adding the ARS reagent because the results of the sample destruction used for qualitative tests are already light yellow. The results of this study are from previous studies that analyzed heavy metal Cd in kale, mujaer fish, catfish, and cob samples using the wet deconstruction method with ARS reagent to get positive results in the qualitative test, which gives a yellow color (Rismiarti et al, 2017).

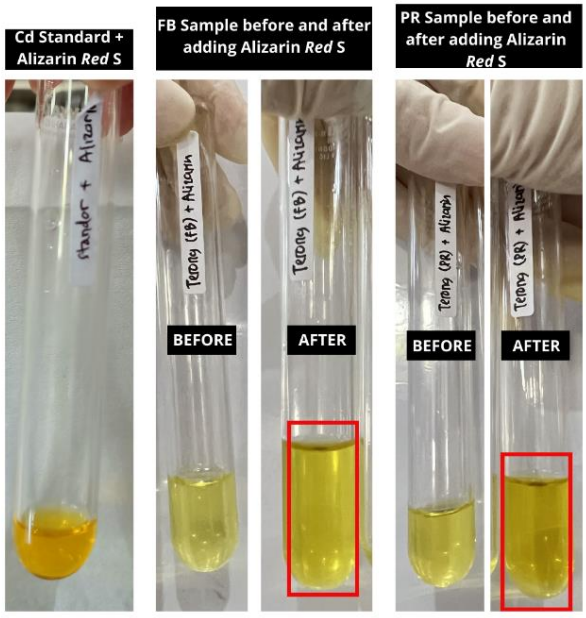

Figure 4. The qualitative test results of Cd metal in FB and PR samples using ARS reagent formed a dark yellow color (personal documentation).

Samples FB and PR are also confirmed to contain Pb metal marked by the formation of a pink color when adding Ditizon in **Figure 4.** The color produced in the positive control, namely the addition of Pb metal standard and Ditizon reagent produces a very clear red or dark pink color compared to the sample. It is due to the FB and PR samples containing very little Pb metal so the qualitative analysis of the color obtained is very light. The results of this study are from previous research analyzing Pb metal in cassava by wet deconstruction method using ditizon reagent and found positive results that give a pink color (Wongthanyakram & Masawat, 2019).

Samples FB and PR are also confirmed to contain Pb metal marked by the formation of a pink color when adding Ditizon in **Figure 5.** The color produced in the positive control, namely the addition of Pb metal standard and Ditizon reagent produces a very clear red or dark pink color compared to the sample. It is due to the FB and PR samples containing very little Pb metal so the qualitative analysis of the color obtained is very light. The results of this study are from previous research analyzing Pb metal in cassava by wet deconstruction method using ditizon reagent and found positive results that give a pink color (Wongthanyakram & Masawat, 2019).

Figure 5. The qualitative test results of Pb metal in FB and PR samples using Ditizon reagent formed pink color at the bottom of the tube (personal documentation).

Analysis of Cd dan Pb Metal Levels with Standard Addition Technique

Pb and Cd metal levels are quantified using the SSA instrument and the standard addition method at wavelengths of 228.94 nm and 283.07 nm, respectively. The standard addition technique was used because Cd and Pb metal contaminants are so small that this technique is needed to enlarge the analyte response on

the instrument compared to the interference matrix response that is also in the sample. The obtained Cd metal concentrations (x-intercept) in FB and PR samples were 0.072 µg/mL and 0.099 µg/mL, respectively.

Meanwhile, the Pb metal concentrations obtained (x-intercept) in FB and PR samples were 4.450 µg/mL and 3.973 µg/mL, respectively. The LOD and LOQ values of each sample do not accept the results for the concentrations of the metals Cd and Pb. It is suspected that this is due to the limits of the instrument, which is less sensitive because it can only read the lowest concentration of the analyte in ppm (part per million) not in ppb (part per billion). In addition, it is also believed that the method of determining LOD and LOQ with linearity data is not suitable for the quantification technique used, namely the standard addition method.

According to previous research, the method of determining LOD and LOQ using linearity data is used for external standard and internal standard quantification techniques (Saadati et al, 2013). This research supports research that uses a standard addition quantification technique method, where in determining LOD and LOQ the method uses the signal-to-noise method by measuring ten blanks for 30 replicates. Furthermore, the LOD and LOQ values were calculated three times and ten times the standard deviation of the blank measurements (Craig et al., 2014; Landon et al., 2017).

Table 4. Pb and Cd metal levels in FB and PR Purple Eggplant Samples.

Metals/Markets	FB (mg/kg) wet weight	PR (mg/kg) wet weight
Pb	3,5066	3,957
Cd	0,056	0,0986

Based on the data in **Table 4**, shows that the Cd metal level in the FB sample is at the safe threshold value set by BPOM, which is 0.05 mg/kg, while the Cd metal level in the PR sample and the Pb metal level in the FB and PR samples exceeds the threshold value set by BPOM regulation No.5 of 2018, which is 0.2 mg/kg (Badan Pengawas Obat dan Makanan (BPOM), 2018). The results of previous studies that also obtained levels of Cd and Pb metals that were far above the safe threshold value, namely in mustard, kale, and spinach food products in Medan's agricultural land where the levels of heavy metal cadmium (Cd) were 2 mg/kg, 3 mg/kg, 3 mg/kg and heavy metal lead (Pb) were 2 mg/kg, 5 mg/kg and 6 mg/kg respectively (Yusuf et al., 2016).

According to WHO, the tolerance limit for heavy metal lead (Pb) entering the body in one consecutive week is 25 µg/kg (World Health Organization, 2009). If the Pb metal contamination entering the body exceeds the predetermined tolerance limit, it can cause health problems in the body (Balali-Mood et al, 2021; Mahurpawar M, 2015; Prasetya HR et al, 2021). However, in the process of measuring the levels of Cd and Pb metals in FB and PR samples, several disturbances or obstacles in the SSA instrument allegedly have on the measurement results obtained, namely the lack of sensitivity in the instrument used, because it cannot read the concentration of analytes in ppb (parts per billion) and the flame is not optimal and the hose for pipetting the sample detached during the measurement process.

The presence of heavy metals Cd and Pb in eggplant can be caused by several factors, such as agricultural activities, industrial activities, transportation activities, and air and water pollution (Alengebawy, 2021). Previous research conducted the application of phosphate fertilizer to the soil as a medium for growing eggplant, which resulted in higher concentrations of Cd and Pb metals in eggplant in soil added with phosphate fertilizer than those not given, which were 0.18 ± 0.02 mg/kg and 1.96 ± 0.23 mg/kg, respectively (Ahmed Youssef, 2018). According to a previous study that compared the growing location of eggplant, namely in industrial, traffic (roadside), and rural areas to the concentration of Cd and Pb metals, the results showed that the highest concentrations of Cd and Pb metals were found in traffic areas followed by industrial areas and then rural areas (Mor & Ceylan, 2008).

CONCLUSIONS

Purple eggplant samples purchased at FB and PR traditional wholesale markets in Pontianak City contained Cd and Pb metals. The level of Cd metal in the FB market is at the maximum threshold value, which is 0.05 mg/kg. Meanwhile, Cd levels in the PR market and Pb levels in FB and PR markets exceeded the maximum limit value of contamination set by BPOM Regulation No. 5 of 2018, which is 0.2 mg/kg. This study has limitations in the instruments and methods of determining LOD and LOQ used. Therefore, further research is necessary to reassess the analysis of heavy metals in samples.

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Competing Interests: The authors declare that there are no competing interests.

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