

The lead level in rice plant leaves planted in Sukawening Village, Jatinangor District, West Bandung Regency, West Java

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ABSTRACT: Lead (Pb) is the most common heavy metal contaminant in plants. It occurs as organic and inorganic salts in plants. Lead is harmful when its amounts in food are higher than the optimum. Rice plants with high levels of lead may trigger the generation of reactive oxygen species and eventually cell death. Our study aimed to assess lead levels in rice plant leaves planted in Sukawening Village, Jatinangor District, West Bandung Regency, West Java. The rice plant leaves were collected each month until four months. The samples were dried and wet-destructed using concentrated sulfuric acid and nitric acid. The qualitative analysis of lead in the samples was carried out using visible spectrophotometry with the addition of xylenol orange. The lead level was measured using a standard addition photometric titration method. Our results indicated that lead was present in the samples since the plant was 1 month old, as proven by the lead-xylenol orange peak at 580 nm. Lead content in the rice plant leaves was as follows, in the 1st-month plants = 0.4118 mg/kg, 2nd-month = 0.5232 mg/kg, 3rd-month = 0.6206 mg/kg, and 4th-month = 0.5264 mg/kg. We concluded that the lead level in the rice plants is in the range of that required by the Verdict of the Director General of the National Agency of Drug and Food Control No. 03725/B/SK/89 about the Maximum Limit of Heavy Metal Contamination in Food, which is 2.0 mg/kg calculated as the dried samples.

Keywords: heavy metal; hypertension; lead (Pb); *Oryza sativa*; toxicity.

Introduction

Statistics Indonesia announced that in 2021 the paddy harvested area reached 10.41 million hectares and the rice production for food consumption was 31.36 million tons. However, the paddy fields located near industrial areas and highways are easily contaminated by heavy metals. Previous studies carried out in China, Pakistan, Thailand, Bangladesh, and Indonesia, have reported the heavy metals content in rice [1-6]. Lead is harmful when its amounts are higher than the optimum. Plants with high levels of lead content may trigger the generation of reactive oxygen species (ROS) [7,8]. Lead pollution may rise to health problems, particularly when it enters the body and harms the cells by decreasing their functions or inducing oxidative stress and apoptosis [9]. Lead can be distributed to all organs but mostly to the bones in the body. Its long-term exposure contributes to elevated blood pressure (BP) [10].

Previous studies in animal models have revealed that long-term subjection to lead could increase BP by

developing oxidative stress. Chronic lead contamination in the body also reduces nitric oxide (NO) availability by altering its pathway and affecting the renin-angiotensin system [11]. Considering this, the harmful effect of lead exposure raised our concern, particularly in areas where rice plants are grown nearby highways and textile industries. Thus, our study aimed to assess lead levels in rice plant leaves using standard addition photometric titration with disodium-EDTA titrant.

Methods

Rice Plant Leaves Materials and Growth Conditions

The selected location of the paddy field was at Sukawening Village (Figure 1), Jatinangor District, West Bandung Regency, West Java. The rice plant leaves were collected each month since the plants were of one month to four months of growth (Figure 2). The plants were taxonomy identified at the

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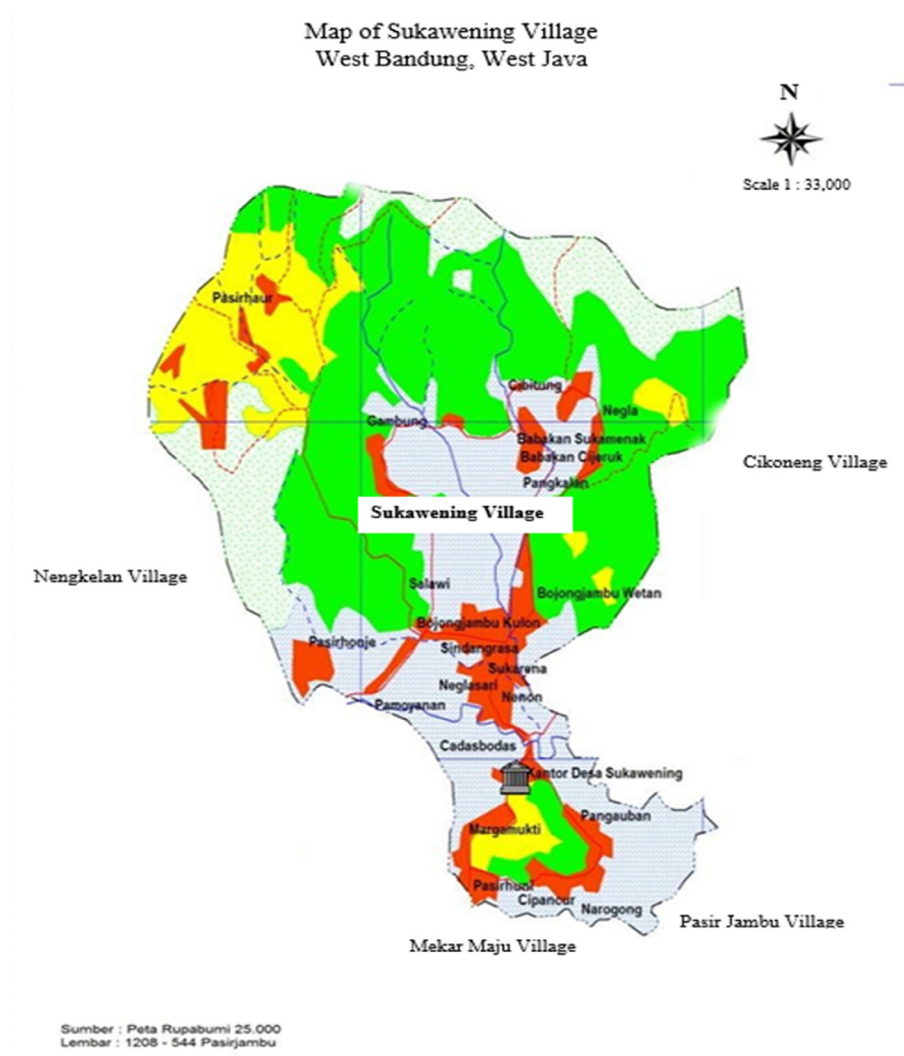


Figure 1. Map of Sukawening Village, Jatinangor District, West Bandung Regency, West Java. The light grey color on the map indicates the paddy fields area, the red indicates the villagers' houses and offices, the green indicates vegetable fields, and the yellow represents grass fields and public areas.

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Sample Preparation

The leaves were sun-dried and ground to powder (Figure 3a). 50 g of the powder was wet-destructed using concentrated sulfuric acid and nitric acid (1:3) (Figure 3b).

Determination of Lead in the Samples using Standard Addition Photometric Titration with Disodium-EDTA Titrant

Determination of lead content in the rice plant leaves was carried out by adding a small amount of lead nitrate, containing 3.312 g of $\text{Pb}(\text{NO}_3)_2$ in 1000 ml of demineralized water, and titrated using a freshly prepared

0.01 M Na_2EDTA standard solution, containing 1.86 g of Na_2EDTA dissolved in 500 ml of demineralized water. One to two drops of xylenol orange solution were used as the indicator [12]. In this study, the complex was detected by a visible spectrophotometer (Specord 200) at 580 nm. For each addition of 0.1 ml titrant, the absorbance of the mixture was measured at 580 nm.

Result and Discussion

Sukawening Village is located in Jatinangor District, West Bandung Regency. This village was selected because it is located nearby our campus, and surrounded by rice fields, textile industries, and busy streets. The rice plant sample was taxonomy identified by a certified botanist

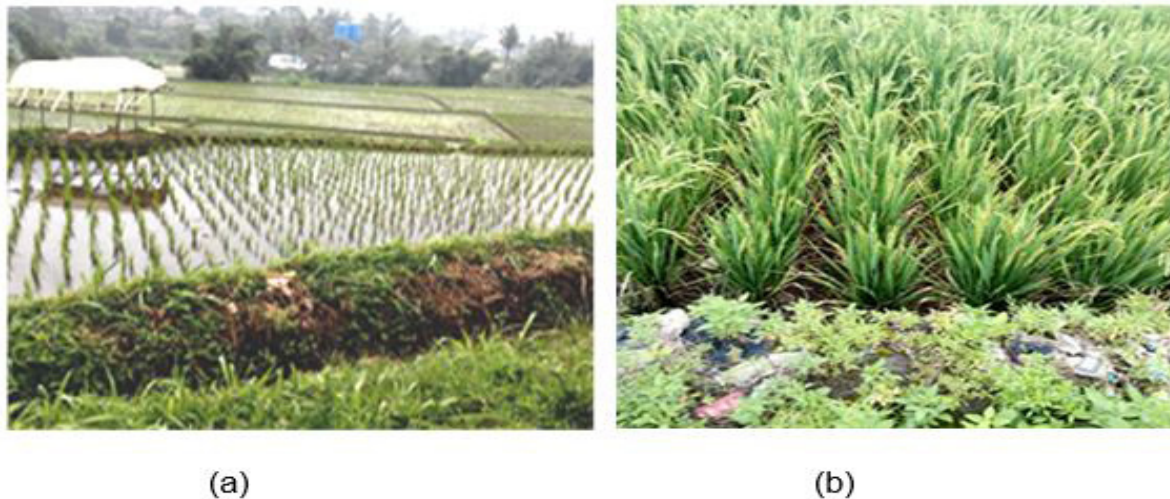


Figure 2. Rice plants in Sukawening Village at (a) 1st month and (b) 4th month.

at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, and was confirmed as *Oryza sativa* L. synonym *Oryza communissima* Lour. (Family Poaceae).

The rice plant leaves were collected every month since the plants were of 1 month to 4 months of growth. The height of the plants was measured every month. The result is presented in Table 1.

Our study revealed that lead was positively identified in the samples since the plant was 1 month old as proven by the lead-xylene orange peak at 580 nm (Figure 4). Xylene orange is an excellent complexometric pH-dependent color reagent for the determination of many metal ions.

Xylene orange reacts with the metal in the sample and produces a purple lead-xylene orange complex [12]. The level of lead in the rice plant leaves (50 g of rice plant leaves powder) is depicted in Figure 5 and Table 1.

A previous study reported that excess contamination of lead in plants could trigger chlorosis and stunted growth [13]. But, despite their exposure to lead, the rice plants in Sukawening Village still grew normally as indicated by their normal range in height and appearance. However, in our study, the lead content in the rice plant leaves is not correlated with the height or the age of the plant.

The stages of rice plant growth indicated that during stage 1 vegetative (24-42 days), the height of the plants

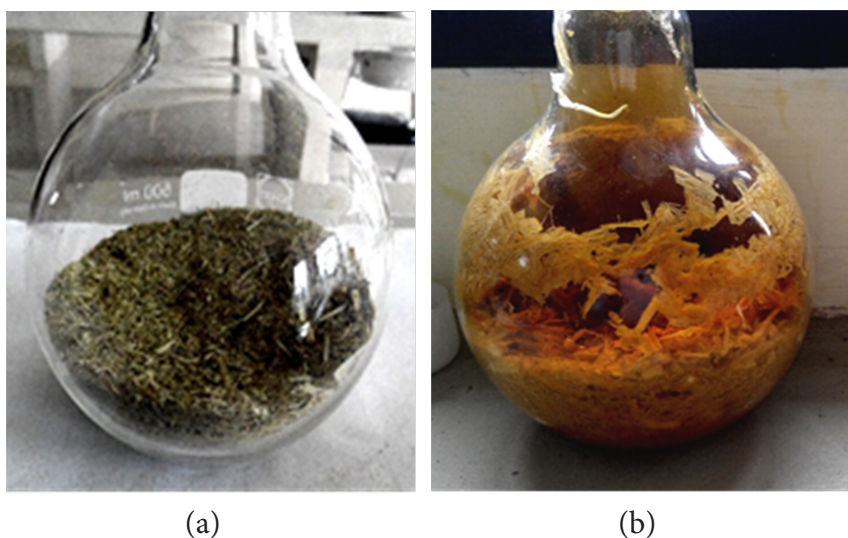


Figure 3. Rice plant leaves (a) after grinding and (b) after wet destruction using a mixture of concentrated sulfuric acid and nitric acid (1:3).

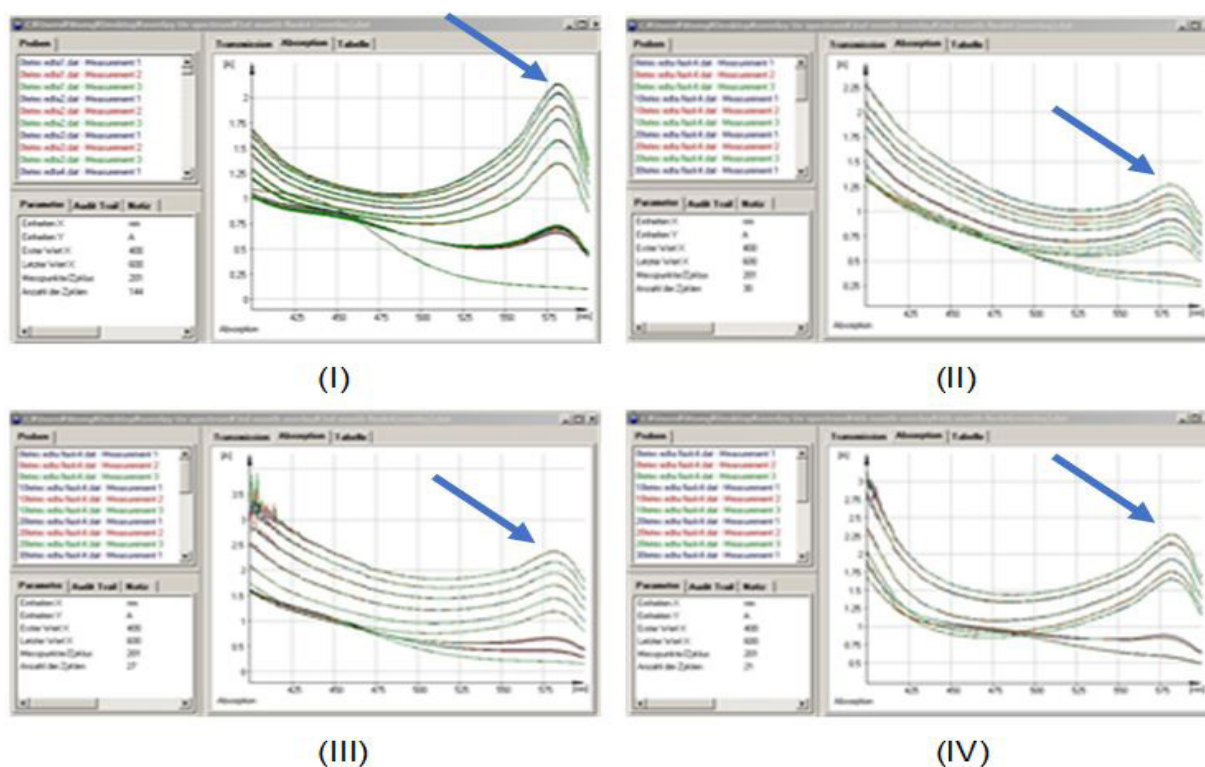


Figure 4. Identification of lead (Pb) in rice plant leaves by Pb-xylenol orange complex-visible-spectrophotometry: (I) 1st month; (II) 2nd month; (III) 3rd month; and (IV) 4th month. The peak of the Pb-xylenol orange complex is detected at 580 nm (shown by blue arrows).

reaches 30 cm, while at stage 3 grain filling and maturation the height reaches 90 cm, depending on the cultivar [14].

Toxic metal exposure, e.g., lead, via food intake has been a public health concern for decades. The lead content in rice plants has been reported previously in China, Pakistan, Thailand, Bangladesh, and Indonesia [1-6]. It was reported that lead was stored up in the order of root > stalk > leaf > grain of the rice plants [2]. The rice plants absorb lead from the atmospheric air via cellular respiration [15]. It was reported that when the lead is absorbed, it is mainly stored in the roots and only a small amount is delivered to the leaves [16].

The determination of lead by employing xylenol orange has been reported elsewhere [17-20]. Xylenol

orange is used to allow sensitive visible detection at 572-580 nm [20]. Xylenol orange has been described as an excellent indicator for the analysis of lead. This chemical is a pH-dependent color reagent [12,21-22]. However, washing the rice before cooking was proven to decrease the level of lead by 57% [23].

Referring to the Verdict of the Director General of the National Agency of Drug and Food Control No. 03725/B/SK/89 about the Maximum Limit of Heavy Metal Contamination in Food [24], the maximum limit of lead in rice is 2.0 mg/kg calculated as the dried samples, thus the lead content in our samples fulfills the requirement.

Table 1. The height of rice plants and the lead content during the 1st to the 4th month of growth

Rice plant leaves collected at	Height (cm)	Lead content (mg/kg)
1st month	28.8	0.4118
2nd month	39.3	0.5232
3rd month	55.0	0.6206
4th month	60.3	0.5264

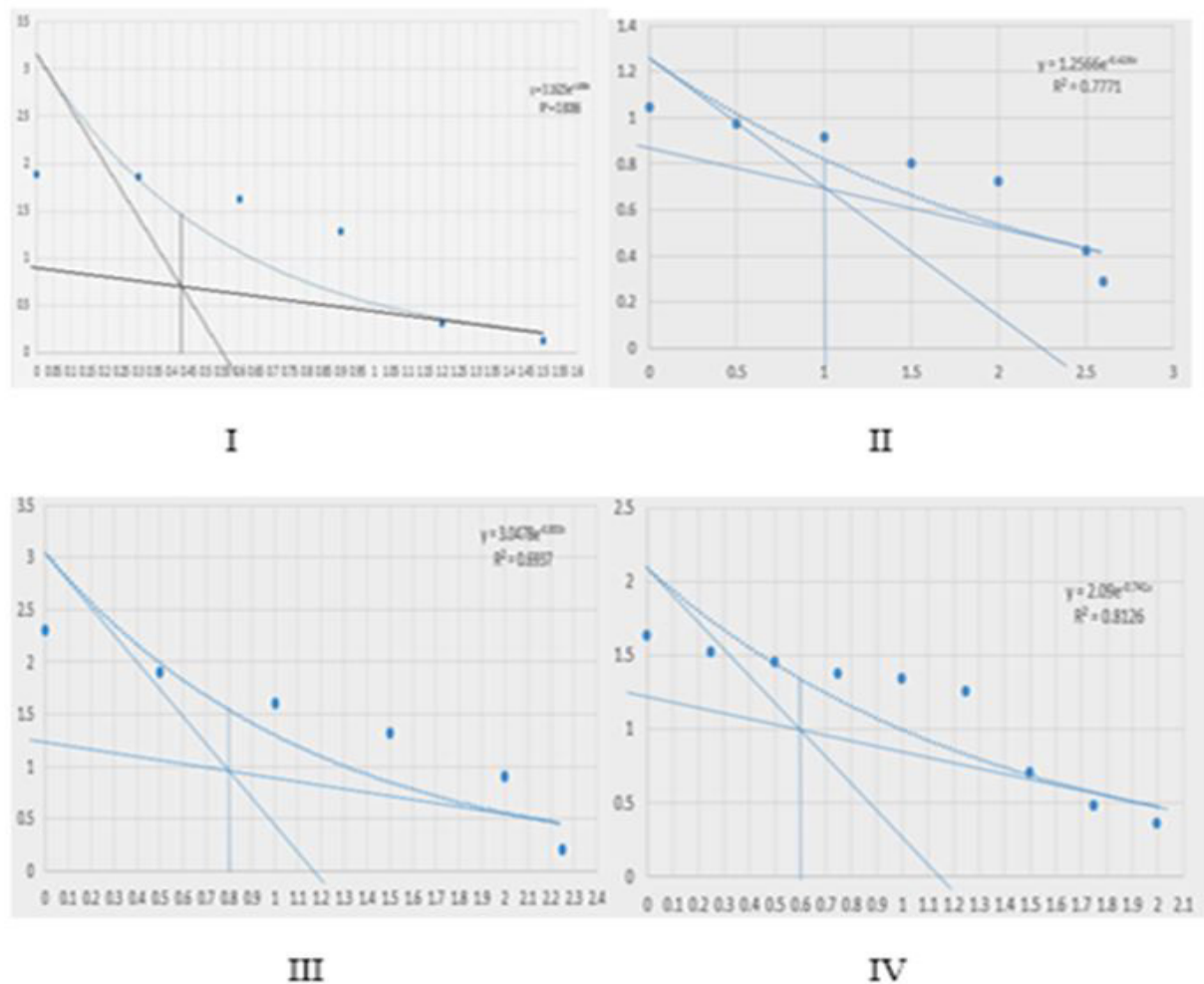


Figure 5. Disodium-EDTA standard addition photometric titration curves of lead in rice plant leaves resulted in (I) 1st month (0.4118 mg/kg); (II) 2nd month (0.5232 mg/kg); (III) 3rd month (0.6206 mg/kg); and (IV) 4th month (0.5264 mg/kg).

The x-axis represents the volume of the Na_2EDTA solution

The y-axis represents the absorbance of the mixture

Conclusion

Our findings revealed the presence of lead in a small amount in the rice plant leaves samples from Sukawening Village, Jatinangor District, West Bandung Regency, West Java. The data was based only on selected spots and may not cover a large area. The lead level is in the range of that required by the Verdict of the Director General of the National Agency of Drug and Food Control No. 03725/B/SK/89 about the Maximum Limit of Heavy Metal Contamination in Food.

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