

# Effectiveness of Green Coconut (*Cocos nucifera* L.) Water against Heavy Metal Levels in the Blood of *Rattus norvegicus*

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Abstract: The environmental pollution can endanger human health if it comes from waste that has high toxicity such as heavy metal waste. Heavy metals that are often found in our environment are metal lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), and chromium (Cr), but Pb, Hg, and Cd are the most common causes of human health problems. Green coconut (*Cocos nucifera* L.) water is widely consumed and believed to be an antidote. Green coconut water itself is one of the local wisdoms of herbal plants. Even though substances contained in coconut water are unknown to the ordinary people, coconut water is believed to be able to neutralize toxins in the body. The purpose of this research is to examine the effectiveness of green coconut (*C. nucifera* L.) water in reducing level of heavy metals (Pb, Hg, and Cd) in the blood of *Rattus norvegicus* L. The Pb, Hg, and Cd groups were treated each 2 mg/mL of 3 mL/week, while the combination group was given Pb-Hg-Cd each of 1 mg/mL as much as 3 mL/week. Treatment of heavy metals was carried out 3 times in a week for 4 weeks, while coconut water treatment to the *Cocos* group was given every day. The results showed that there were significant differences between the average Cd levels (p.value 0.009) and the average Hg levels (p.value 0.007). Cd level in the Cd group was found to be 0.223 ppm, whereas in the Cd-*Cocos* group, it was 0.096 ppm. The average Hg level in the Hg group was 276.15 ppm while in the Hg-*Cocos* group was 81.81 ppm. In the Pb group, there was a difference in the mean of treatment results, even though the difference was not significant in the Kruskal-Wallis test. This proves that *C. nucifera* can effectively reduce heavy metal levels, especially in Cd and Hg, in the blood of *Rattus norvegicus*.

## 1. INTRODUCTION

Environmental pollution is a classic problem that continues to haunt until now. One of the pollutions that still becomes a concern is due to heavy metals. Water in Surabaya, in Kalimas sediment, to be exact, contained Pb (103,219-138,621 ppm) and Hg (11,984-13,525 ppm), according to a survey conducted by Ariestyra in 2008. Similar research conducted on sea cucumber in the coastal area of Kenjeran found metal level of Hg ranged from 0.099 to 0.112 ppm and Pb 9.86 to 15.27 ppm on sea cucumber (Lestari, 2015). In Surabaya, Kupang traditional foods contain heavy metals as well; Pb metal is 1,281 ppm and Cd is 1,254 ppm (Indasah, 2011). The measurement results of heavy metal concentrations in air ambient in 10 major cities in Indonesia found that Surabaya was ranked first in Pb

pollution, followed by Tangerang and Jakarta. The average Pb level in ambient air in Surabaya is 10.43-2664.2 ng/m<sup>3</sup> (Mukthar, 2013). In 2017, a research conducted in the area of Bandung found that one of the rice fields there contained heavy metal Hg of 92.2 mg / L. This value is above the Hg quality standard, which is 25.9 mg/L (Komarawidjaja, 2017).

Metal pollution can endanger human health. Health problems can be caused by heavy metals, depending on the level of poisoning experienced by the individual. For example, Pb Poisoning can attack the central nerve, inhibit enzyme reactions, shorten the life of red blood cells, increase iron (Fe) content in blood plasma, damage the Cerebrum, and inhibit fetal growth (Ratmini, 2009).

Lead entering through breathing process will also circulate to all tissues and organs of the body. More than 90% of lead absorbed by the blood is

bound to red blood cells (Rahmawati *et al.*, 2016). Consumption of lead 0.2-2 mg/day can cause food poisoning. While an increase of lead level by 10µg/dl in blood can result in an IQ reduction of 6-9 points (Anggraini, 2014). Meanwhile, mercury level in the tissues of 0.1-1 ppm can cause disruption of body function.

*Cocos nucifera* L. is an important member of the family Arecaceae (palm family), usually called coconut (Aragao, 2002). The plant is originally from Southeast Asia (Malaysia, Indonesia, and the Philippines) and the islands between the Indian and Pacific Oceans (Purseglove, 1972). All parts of the coconut can be used. *C. nucifera* L. gives human many benefits, especially from its water. Compared to other types of coconut, water of green coconut (*C. nucifera* L.) contains more bioactive enzymes such as tannins. Tannins are anti-toxic substances, so that green coconut water is often considered capable of decomposing and removing toxins in the body (Candra, 2016).

The Javanese believe that water of green coconut (*C. nucifera* L.) contains an antidote, without knowing what substances are in the water. Green coconut water itself is one of traditional medicines based on local wisdom and is believed to be able to neutralize toxins in the body.

The results of giving young coconut water to painting workers exposed to Pb for 2 weeks have shown significant reduction of the average Pb level in the blood, from 35,009 µg/dl to 9,0089 µg/dl (Candra, 2016). Research conducted on workers exposed to mercury (Hg) in the gold mining area in Selogiri has showed an increase in the activity of glutathione peroxidase (GPx) enzymes after the treatments of coconut water as an antioxidant (Zulaikha *et al.*, 2015).

The effectiveness of coconut water in reducing the level of Cadmium (Cd) in the blood has not been widely studied. This study will measure the effectiveness of *C. nucifera* L. in reducing the level of heavy metals (Cd, Pb and Hg) in the blood of *Rattus norvegicus* or white rats.

## 2. METHOD

The research was conducted at the Integrated Laboratory of UIN Sunan Ampel Surabaya from October to December 2017. The experimental animal used for this study was *Rattus norvegicus* L., or white rats, that were controlled for all treatments. Experimental research method was conducted as Complete Randomly Design (CRD) with the

treatment group, namely 'the control group', 'metal treatment group of Pb, Hg, and Cd', 'the treatment group of green coconut water', and 'the combination of the 3 treatments of heavy metals with green coconut water'. Each group has 3 repetitions.

Heavy metal treatments were as follows: Lead (Pb) group was given Pb 2 mg/mL as much as 3 mL/week; Mercury (Hg) group was given 2 mg/mL of Hg as much as 3 mL/week; Cadmium (Cd) group was given Cd 2 µg/mL as much as 3 mL/week as much as 3 ml/week; Combination group was given Pb+Hg+Cd each of 1 µg/mL, 3 mL/week. Heavy metal treatment was given 3 times in 1 week for 4 weeks. Coconut water was given to the *Cocos* group every day.

The data in this study were primary data, obtained from direct measurements of total Hg levels in blood (THg), total Pb in blood (TPb), and total levels of Cd in blood (TCd) of *Rattus norvegicus* L. taken intracardially. Heavy metal levels testing in rat blood used the Atomic Absorbance Spectrophotometry (AAS) method.

The measurement results of metal content in the blood were in the form of data ratios. Comparison between levels of heavy metals in experimental animals with the provision of mineral water and coconut water and the metal content after the treatment of giving coconut water was then tested using statistical analysis. The results of the data were statistically analyzed using Kruskal-Wallis and Kolmogorov-Smirnov tests, while further testing was done with Mann-Whitney if there was significance in the results.

## 3. RESULT AND DISCUSSION

### 3.1 Cadmium Analysis

On table 1 showed measurement results of cadmium heavy metals in various treatments, including control group, control group with coconut water (Control-Cocos), Cd-treatment group, Cd-treatment group with coconut water (Cd-Cocos), group combination of Pb + Hg + Cd (Mix) treatment, and combination treatment group Pb + Hg + Cd with coconut water (Mix-Cocos).

Table 1: Result of average Cd concentration

Treatment	Average Cd Concentration (ppm)	P-Value
Control	0.003 <sup>a</sup>	0.009
Control- <i>Cocos</i>	0.003 <sup>a</sup>	
Cd	0.223	
Cd- <i>Cocos</i>	0.096	
Mix	0.069	
Mix- <i>Cocos</i>	0.014	

<sup>a</sup>unsignificantly different

Based on the calculation of Kruskal-Wallis test, a significance value of 0.009 (smaller than  $\alpha$ ) was obtained. So, it can be concluded that there is a significant difference in the average Cd level between each group. Further analysis with Mann-Whitney showed a significant difference (P-Value 0.04) between the Cd group and the Cd-*Cocos* group, and a significant difference between the groups of Mix and the mix-*Cocos* group. It can be seen in the table that the average Cd level in the treatment group was 0.223 ppm, while in the Cd-*Cocos* group, the average Cd lowered to 0.096 ppm. It can be said that the administration of coconut water treatment can reduce cadmium level in the blood of rats.

Cadmium accumulated in the body can cause nephrotoxicity (kidney toxicity), symptoms of proteinuria, glycosuria, and aminoaciduria accompanied by a decrease in the renal glomerular filtration rate. Cadmium causes osteomalacia symptoms because of the balance between calcium and phosphate in the kidneys (Muhajir, 2009).

### 3.2 Mercury Analysis

Table 2: Result of Average Hg Concentration

Treatment	Hg Average Concentration (ppm)	P-Value
Control	0 <sup>a</sup>	0.007
Control- <i>Cocos</i>	0 <sup>a</sup>	
Hg	276.15	
Hg- <i>Cocos</i>	81.81	
Mix	65.39	
Mix- <i>Cocos</i>	13.11	

<sup>a</sup>unsignificantly different

Table 2 has showed measurement results of mercury metals in various treatments, including control group, control group with coconut water (Control-*Cocos*), Hg-treatment group, Hg-treatment group with adding of coconut water (Hg-*Cocos*),

group combination of Pb + Hg + Cd (Mix) treatment, and combination treatment group Pb + Hg + Cd with coconut water (Mix-*Cocos*).

Kolmogorov-Smirnov test showed that the data were not normally distributed (P-value is smaller than 0.05), so that non-parametric analysis was done by Kruskal-Wallis test. The test results showed that there was a significant difference in the average Hg level between each treatment group. The significant different groups were the Hg group with the Hg-*Cocos* group, the mixed group, and the mix-*Cocos* group.

The content of mercury in the body is very dangerous. Mercury poisoning can interfere with blood components, namely an increase in the levels of amino acids levulinie (ALA) in the blood and urine, increased level of protoporphyrin in red blood cells, reduced number of red blood cells, the cause of hemopoietic, and increased level of hematocytes in the blood (Aryani, 2013).

### 3.3 Lead Analysis

The Table 3 has showed measurement results of lead in various treatments, including control group, control group with adding of coconut water (Control-*Cocos*), Pb-treatment group, Pb-treatment group with adding of coconut water (Pb-*Cocos*), group combination of Pb + Hg + Cd (Mix) treatment, and combination treatment group of Pb + Hg + Cd with coconut water (Mix-*Cocos*).

Table 3: Result of Average Pb Concentration

Treatment	Pb Average Concentration (ppm)	P-Value
Control	0 <sup>a</sup>	0.081
Control- <i>Cocos</i>	0 <sup>a</sup>	
Pb	0.12 <sup>a</sup>	
Pb- <i>Cocos</i>	0.04 <sup>a</sup>	
Mix	0.11 <sup>a</sup>	
Mix- <i>Cocos</i>	0.03 <sup>a</sup>	

<sup>a</sup>unsignificantly different

Based on the Kolmogorov-Smirnov test, the results showed that the average data distribution of Pb levels was not normal, so the analysis was continued with the Kruskal-Wallis test. Significance value of 0.081 showed that there was no significant difference in the average Pb level between groups. Although the statistical analysis showed no significant differences, there was a decrease in the level of lead concentration in the blood of rats treated with coconut water. In

coconut water, there are many amino acids such as glutamate acid, arginine, leucine, lysine, proline, aspartate acid, alanine, histidine, phenylalanine, serine, cysteine and tyrosine. This allows the reduction of lead levels in the blood (Anggraini, 2014).

The research of Jesuorsemenwen *et al.* in 2016 determined the ameliorative effect of coconut water on haematobiochemical changes due to lead poisoning in wistar albino rats. For six weeks, sixty rats were assigned to four groups. Lead 0.10g/l and 75 ml coconut (*C. nucifera* L.) water were given orally for six weeks with the mean values of red blood cells, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution width and platelets, white blood cells, lymphocytes, total cholesterol, triglyceride, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, low density lipoprotein-cholesterol/ high density lipoprotein-cholesterol, and total cholesterol/high density lipoprotein-cholesterol indicate that coconut water could ameliorate effects of lead toxicity.

The Javanese believe green coconut water (*C. nucifera* L.) is an antidote to poison without knowing what it is inside coconut water. This belief is passed down through generations, so that when symptoms of poisoning occur, parents often recommend consuming green coconut water. Green coconut water itself is one of traditional medicines based on the local wisdom which are believed to be able to neutralize toxins in the body. Throughout history, humans have used medicinal plants therapeutically. Minerals, plants, and animals traditionally have been the main sources of drugs. The constituents of *C. nucifera* have some biological effects, such as antihelminthic, anti-inflammatory, antinociceptive, antioxidant, antifungal, antimicrobial, and antitumor activities (Lima *et al.*, 2015).

Compared to other types of coconut, green coconut water contains more bioactive enzyme called tannins. Tannins are anti-toxic substances so that green coconut water can decompose and remove toxins in the body. Coconut water, aside from being a fresh drink, also contains various minerals, vitamins, and sugars as well as essential amino acids, so that it can be categorized as a highly nutritious soft drink and can cure various diseases.

Micronutrients in coconut water, such as inorganic ions and vitamins, play vital roles in helping the antioxidant defense system of the human body (Evans & Halliwell, 2001). Some evidence points toward an antioxidant action of coconut water. From the research done by Loki & Rajamohan in 2003, administering coconut water (6 mL/100 g of body weight) to female rats intoxicated with carbon tetrachloride recovered the action of antioxidant enzymes (superoxide dismutase and catalase levels) and decreased lipid peroxidation. Coconut water is also rich in L-arginine (30 mg/dL), which significantly reduces the generation of free radicals (Boger *et al.*, 1995) and has antioxidant activity (Salil & Rajamohan, 2001). It is rich with ascorbic acid (15 mg/100 mL) as well, which decreases lipid peroxidation in rats (Das *et al.*, 2001). Furthermore, many parts of *C. nucifera* plants have proven to contain phenolic compounds and flavonoids that support antioxidant activity.

Coconut water contains antioxidants, such as vitamin C, vitamin B1, vitamin B6, amino acids like methionine, L-Arginine, selenium, cytokines, and minerals. Lead that enters the body will generally move freely in the form of Pb (II) ions and accumulates in body tissues. Accumulation occurs due to the high affinity between Pb (II) ions and sulfhydryl groups in amino acids. In coconut water, there are many amino acids such as glutamate acid, arginine, leucine, lysine, proline, aspartate acid, alanine, histidine, phenylalanine, serine, cysteine and tyrosine. This makes it possible to reduce blood lead levels.

Basically, almost all heavy metals, such as Hg and Cd, have high affinity for sulfhydryl (-SH) groups in amino acids. The decrease in these groups causes a decrease in the antioxidant system which is characterized by a decrease in the activity of the enzyme glutathione peroxidase (GPx). Decreased glutathione (GSH) and H<sub>2</sub>O<sub>2</sub> buildup will cause oxidative stress on cells. Giving natural antioxidants is an alternative to protect cell damage that occurs due to metal poisoning. One source of natural antioxidants is water of green coconut (*C. nucifera* L.). As mentioned earlier, in coconut water there is an amino acid in the form of methionine. Methionine acts as a precursor in the formation of cysteine, which is the main compound in synthesizing glutathione in the body. In addition, in coconut water there is L-arginine which plays a role in mercury detoxification.



L-arginine is a source of nitric oxide (NO) which can inhibit xanthine oxidase (XO) and increase SOD (superoxide dismutase). SOD is needed to reduce superoxide production in the body. *C. nucifera* L. has been recognized as an entity with multiple usages. Each of its components is biologically active in one way or other.

"Every disease has a cure" (hadith of Muslim history). Narrated by Muslims from the hadith Abu Zubair, Jabir bin Abdillah reported, the Prophet SAW said: "Each disease has a cure. If a cure is applied to the disease, then it is relieved by the permission of Allah SWT. "In Sahih Al-Bukhari and Muslim from 'Atha, from Abu Hurairah, that he said: "While in Musnad Imam Ahmad mentioned the hadith from Ziyad bin Ilaqah, from Usman bin Shaikh it was reported that he told, "One time I was with the Prophet SAW, then suddenly came a number of Bedouins. They asked, "Salam Messenger of Allah, should we not treat sickness?" He replied, "Treat sickness, for Allah has not created any disease except He has also created the cure, except for one disease." They asked, "What is the disease, O Messenger of Allah?" He replied, "Old age." In other words it is stated, "Every time Allah sends down disease, Allah definitely delivers the cure. There are only people who know it and there are people who do not know it."

The publication of the results of this study is expected to, at least, help to affirm the belief of the Javanese community in general that green coconut water is indeed proven to be able to ward off toxins, particularly toxins caused by the accumulation of heavy metals in the blood. Allah SWT has created a Coconut Plant with its water which can be used as a medicine.

#### 4. CONCLUSION

The results have showed that there were significant differences between the average Cd levels in (p-value 0.009) and the average Hg levels (P-value 0.007). Cd level in the Cd group was found to be 0.223 ppm, whereas in the Cd-*Cocos* group, it was 0.96 ppm. The average Hg level in the Hg group was 276.15 ppm while in the Hg-*Cocos* group was 81.81 ppm. In the Pb group, there was a difference in the mean of treatment results, even though it was not significant in the Kruskal-Wallis test. This proves that *C. nucifera* L. can effectively reduce the level of heavy

metal, especially Cd and Hg, in the blood of *Rattus norvegicus*.

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