Heavy metal concentrations in cocoa beans (*Theobroma cacao* L.) originating from East Luwu, South Sulawesi, Indonesia

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**Abstract.** Concentrations of some heavy metals (Pb, Cu, Cd, As and Hg) were assessed for cocoa beans (*Theobroma cacao* L.) originating from East Luwu, South Sulawesi, Indonesia after five-day fermentation. Consisting of PB 123, BR 25, and MCC 02 cocoa clones, the spectrophotometric analysis showed that concentrations of Pb, Cd, As and Hg in the cocoa beans over the three clones was below the detection limits of 0.100; 0.050, 0.010 and 0.005 mg/kg. For Cu, they were 19.343; 10.391, and 18.594 mg/kg respectively, but still below the maximum critical levels, established by the European Food Safety Authority (EFSA). Concentrations of those five heavy metals in the bean shells were found to be parallel to those in the cocoa beans, except for Pb.

1. **Introduction**

Cocoa (*Theobroma cacao* L.) is cultivated mainly in West Africa, Central and South America and Asia. Based on the ICCO 2017 data, eight largest cocoa producing countries today are Ivory Coast, Ghana, Indonesia, Nigeria, Brazil, Equador, and Malaysia. The total world cocoa production in 2015/2016, is estimated 3.972 thousand tonnes, in which Indonesia contributed for about 8.0 % of the total world production [1].

Studies on cocoa chemical composition and properties have proven that a moderate consumption of cocoa may be beneficial for human health, mainly due to cocoa contains high polyphenolic compounds, particular flavonoids as antioxidants [2], which are responsible for the overall health of the humans. Antioxidant capacity of cocoa has been reported higher than that of green tea and red wine [3].

The beneficial effects of cocoa range from its free radical scavenging capacity [4], reduction of the risk of cardiovascular diseases [4,5], prevention of autoimmune diseases and hepatopathy [6,4,7], anti inflammatory [8], reduction of anxiety and depressive symptoms [9], to anti cancer effect [10,4]. Moreover, extract prepared from cocoa beans or cocoa powder shows to exhibit an effect on
syrthocotozin induced diabetes mellitus [11] and antibacterial effect against Streptococcus mutants formulated in mouth washes [12].

Despite the health benefits of cocoa, recently, there has been a high food safety concern regarding the presence and levels of heavy metals, such as nickel (Ni), cadmium (Cd), chromium (Cr), and lead (Pb), both in cocoa beans and cocoa products i.e. cocoa butter, cocoa liquor, cocoa powder and chocolates. Rankin et al. [13] reported a relatively high level contaminants of lead in chocolates and other cocoa products, produced in Nigeria. The average lead level for 23 chocolates and cocoa products, measured by WCAS was 32.5 ng/g. The samples were taken in 2002. Dahiya et al. [14] have examined Cd, Ni and Pb levels of 69 chocolate brands sold locally in the suburban areas of Mumbai, India. They found that the Cd levels ranged from 0.001 to 2.730 g/kg. The Ni levels ranged from 0.041 to 8.290 g/kg, while the Pb levels ranged from 0.049 to 8.040 g/kg. If using the Polish National Standard for Pb, established by the Poland Regulatory Agency [15] as a reference, 42 chocolates out of 69 contained Pb, exceeding the authorized limits of 0.30 g/kg. In the case of Cd, 19 chocolates exceeded the authorized limits of 0.05 g/kg.

Cocoa beans, as a key raw material in the cocoa derived products, could also contain those heavy metals, obtained from the growing soils and/or during the production process. High levels of Cd, Pb, Cu, and As in the cocoa beans are of the interest as those trace elements are generally considered as toxic to human [16]. Chaves et al. [17] in investigating Cd contamination in cocoa plants and the growing soil in Southern Ecuador, found that the accumulation of Cd in the surface soil of the cocoa farms was most probably caused by the anthropogenic activities, such as irrigation of Cd rich water used. The contamination caused accumulation of Cd in the cocoa beans, in which 12 out of 19 sampling sites had a bean Cd level exceeding 0.6 mg/kg, the maximum critical level for Cd, established by European Union (EU). Yanus et al. [9] reported significant levels of Bi, Pb, and As in the cocoa bean shells, but not in the cocoa beans. Samples of the cocoa beans were received from the chocolate brand importers in Israel. This indicated that the metal contaminants occurred likely after the beans are harvested, sun-dried, and shipped.

Due to the toxicity of those heavy metals, there have been several agitations to reduce the concentrations to the safe levels by setting up stringent standards by international or state legislative bodies, and by chocolate manufacturers as well, in order to protect the health of consumers. This study assessed the heavy metal concentrations in the cocoa beans originating from East Luwu, South Sulawesi, Indonesia, and to ascertain their compliance with standards. Those heavy metals included lead (Pb), copper (Cu), cadmium (Cd), arsen (As), and mercury (Hg). As an additional information, East Luwu is one of the main producing areas of cocoa in Indonesia, located in South Sulawesi Province.

2. Materials and Methods

2.1. Preparation of the cocoa beans

The cocoa beans for assessment were prepared as dried fermented cocoa beans. They consisted of three cocoa clones, namely PBC 123 (Sulawesi 1), BR 25 (Sulawesi 2), and MCC 02 (M 45).

Cocoa pods were procured from Sahar Cocoa Village (SCV) farm, a plasma cocoa farm of Mars Symbioscience Indonesia Ltd., located in Malioowowo Village, District of Angkona, East Luwu, South Sulawesi, Indonesia, while fermentation and sun-drying of cocoa beans took place in Mars Cocoa Research Station, about 12 km from the SCV location.

The mature cocoa pods were stored for six days, before the beans were removed from the pod husk. The cocoa beans were then separated from its placenta. Fermentation took place for five days, as recommended in the Sime-Cadbury method, but using fermentation boxes made of styrofoam, instead of wood materials. The capacity of each box is 7 kg of wet beans. After fermentation, the cocoa beans were sun-dried for 5 days, until reaching 6 to 7% moisture contents.
2.2. Preparation of the samples and analysis
Preparation and analysis of the cocoa bean samples were conducted at Laboratory of Chemical and Microbiological Testing, Center for Plantation Based Industry, in Makassar, South Sulawesi, Indonesia.

The cocoa nibs were separated from their bean shells and then ground with ceramic mortar. One gram of each sample was weighed into a teflon polytetrafluoroethylene (PTFE) vessel and digested with 5 mL HNO$_3$ and 3 mL H$_2$O$_2$. The digestion was carried out in a microwave for 50 minutes using the digestion protocol. Upon cooling to room temperature, the digested sample was transferred into 50 mL polypropylene tubes and made up to 25 mL with deionized water. The digested solution was filtered through a Whatman No. 42 filter paper, and diluted prior to analysis. The concentration of heavy metals was determined using Atomic Absorption Spectrophotometer (Shimadzu, AA-7000). Heavy metals of Pb, Cu, Cd, As, and Hg concentrations were determined at a wavelength of 248.3 nm, 324.8 nm, 228.8 nm, 193.7 nm, and 253.7 nm respectively, using acetylene as the fuel and air as the support gas [18].

The heavy metal concentration levels were then ascertained for their compliance with standards of European Food Safety Authority for cocoa beans.

3. Result and Discussion
The spectrophotometric analysis of Pb, Cu, Cd, As, and Hg concentrations in the cocoa beans are presented in Table 1. The cocoa beans were prepared as dried five-day fermented cocoa beans, consisting of PBC 123 (Sulawesi 1), BR 25 (Sulawesi 2), and MCC 02 (M 45) cocoa clones. Table 2 shows the same analysis results, but for the cocoa bean shells.

Table 1. Concentration levels of Pb, Cu, Cd, As, and Hg in the cocoa beans over the three clones.

| Heavy metals | Cocoa clones | Concentration level (mg/kg) | Maximum critical level (mg/kg) |
|--------------|--------------|-----------------------------|-------------------------------|
| Lead (Pb)    | PBC 123      | < 0.10                      | 1.0 mg/kg*                    |
|              | BR 25        | < 0.10                      |                               |
|              | MCC 02       | < 0.10                      |                               |
| Copper (Cu)  | PBC 123      | 19.343                      | 50 mg/kg*                     |
|              | BR 25        | 10.391                      |                               |
|              | MCC 02       | 18.594                      |                               |
| Cadmium (Cd) | PBC 123      | < 0.05                      | 0.10 mg/kg**                  |
|              | BR 25        | < 0.05                      |                               |
|              | MCC 02       | < 0.05                      |                               |
| Arsenic (As) | PBC 123      | < 0.01                      |                               |
|              | BR 25        | < 0.01                      |                               |
|              | MCC 02       | < 0.01                      |                               |
| Mercury (Hg)| PBC 123      | < 0.005                     |                               |
|              | BR 25        | < 0.005                     |                               |
|              | MCC 02       | < 0.005                     |                               |

* European Commission No.1881/2006
** European Union No. 488/2014

Concentrations of Pb in the cocoa beans over the three clones, shown in Table 1, were below detection limits of 0.10 mg/kg, thus far below the maximum critical level of 1.0 mg/kg, established by EFSA [19]. On the other hand, the concentrations of Pb in the cocoa bean shells shows quite high
levels; those are 9.724 mg/kg for the PBC 123 clone, 11.146 mg/kg for the BR 25 clone, and 5.810 for the MCC 02 clone. All the Pb intrusions were retained in the bean shells and did not pass into the nibs. Regarding this phenomenon, Rankin et al. [13] found that the cocoa bean shell can be regarded as an excellent protective shield against intrusions of lead into the nib from external sources before the cocoa beans are harvested. In fact, Meunier et al. [20] in their study have reported that the cocoa bean shells were particularly efficient in the removal of lead from very acidic solution ($q_{max} = 6.2$ mg/g of Pb). The presence of other metals and cations in the solution did not seem to affect the recovery of lead.

### Table 2. Concentration levels of Pb, Cu, Cd, As, and Hg in the cocoa bean shells over the three clones.

| Heavy metals | Cocoa clones | Concentration level (mg/kg) |
|--------------|--------------|-----------------------------|
| Lead (Pb)    | PBC 123      | 9.724                       |
|              | BR 25        | 11.146                      |
|              | MCC 02       | 5.810                       |
| Copper (Cu)  | PBC 123      | 12.861                      |
|              | BR 25        | 11.089                      |
|              | MCC 02       | 10.516                      |
| Cadmium (Cd) | PBC 123      | < 0.10                      |
|              | BR 25        | < 0.10                      |
|              | MCC 02       | < 0.10                      |
| Arsenic (As) | PBC 123      | < 0.02                      |
|              | BR 25        | < 0.02                      |
|              | MCC 02       | < 0.02                      |
| Mercury (Hg) | PBC 123      | < 0.005                     |
|              | BR 25        | < 0.005                     |
|              | MCC 02       | < 0.005                     |

Related to lead contamination in the cocoa beans, especially in some African countries, Rankin et al. [13] and Manton [21] hypothesized to derive from the atmospheric emission of leaded gasoline, (which is still used in the countries) during fermentation and drying of the cocoa beans.

As stated by Ellen [22], lead can be very harmful even at low concentrations if taken for a long period. After absorption, lead is initially distributed in soft tissue throughout the body via blood and then deposited in bone. It may cause damage to kidneys, cardiovascular, central nervous, and reproductive system [10].

Concentrations of Cu in the cocoa beans were 19.343 mg/kg for the PBC 123 clone, 10.391 mg/kg for the BR 25 clone, and 18.594 mg/kg for the MCC 02 clone, as shown in Table 1. All these concentrations were below the maximum critical level of 50 mg/kg, regulated by the EFSA (2006). Moreover, concentrations of Cu in the bean shells were found to be 12.861 mg/kg for the PBC 123 clone, 11.089 mg/kg for the BR 25 clone, and 10.516 mg/kg for the MCC 02 clone, as shown in Table 2. This indicated that some of the Cu intrusions were retained in the bean shells, and some others passed into the nibs. Compared to the removing lead from an acidic solution, the bean shells have less capacity to the removal of copper [20].

Some studies have proposed that high levels of copper in blood could increase the risk of artherosclerosis by promoting the oxidation of low-density lipoprotein (LDL) [23]. Increase in serum copper levels lead to increase in cardiovascular risk [23,24].

The concentration of Cd, As, and Hg in both the cocoa beans and the bean shells over the three clones, as shown Tables 1 and 2, were virtually below the detection limit of 0.050; 0.010 and 0.005.
mg/kg Cd, As and Hg, respectively. The maximum critical level of Cd itself is 0.10 mg/kg, as established by the EFSA [25], while for As and Hg, so far, have not been regulated for the cocoa beans.

Some evidence suggest that many cocoa farmers now apply pesticides, insecticides, and fertilizers to control pests and insects and to enhance maximum cocoa bean yields. These activities, if not controlled and managed well, could increase contamination of the heavy and trace metals like Cd, Fe, Pb, Cu, Mn, and Zn into the soil which eventually will end up in the edible parts of the cocoa through phytoextraction by the crops and translocation throughout the plant system [26,27].

High level of cadmium can lead to disturbance in calcium metabolism and formation of kidney stones, softening of the bones [28] and synthesis of pancreatic DNA [29]. Arsenic, in inorganic form is special of concern because of its cancer causing properties. Arsenic has been classified by the International Agency for Research into Cancer (IARC), as a human carcinogen. Mercury is often associated with a wide spectrum of adverse health effects, especially damage to the central nervous system and kidney [15].

Regarding the presence of Pb in the bean shells in our study, in which its levels were quite high, there was no available data whether the cocoa has uptaken Pb dominantly from atmospheric emission of leaded gasoline during the fermentation and sun-drying of the cocoa beans or from the soil. It was similar for the presence of Cu in both the cocoa beans and the bean shells, whether it comes from Cu contaminated soil due to the fertilizer and other agrochemical uses and/or from Cu rich sedimentary rocks. Alloway [30] reported his study that concentrations of Pb in the cocoa beans related to the presence of Fe, Zn and Cu in the soil. This can be attributed to the interactions of Fe, Zn, and Cu with Pb in competing for absorption by the plant. On the other hand, concentrations of Cu in the cocoa beans are mainly related to the concentrations of Pb and Mn in the soil. To examine the sources of these Pb and Cu contaminants in the cocoa beans, requires further studies.

Conclusion
Of the five heavy metals assessed, Pb, Cd, As and Hg were not detected in the cocoa beans over the PBC 123, BR 25 and MCC 02 cocoa clones. Cu was detected, but their concentrations were still below the maximum critical levels, established by the European Food Safety Authority (2006). Moreover, concentrations of those heavy metals in the bean shells seemed to be parallel with those in the cocoa beans (nibs), except for Pb.

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