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Absorption of Cadmium and its Effect on the Growth of Halfsib Family of Three Cocoa Clones Seedling

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Abstract

The issue of cadmium in cocoa beans has become one of the primary considerations in setting quality criteria in chocolate-consuming countries. Using rootstock genotypes that are tolerant to cadmium uptake can provide data on the absorption of cadmium into shoots for recovery. This study examined cocoa plants' growth and physiological response to cadmium (Cd) accumulation. The research was carried out using polybags in the Greenhouse of the Indonesian Coffee and Cocoa Research Center using a half-sib family of cocoa aged 4 months after sowing. The study was designed using a completely randomized split-plot design with 3 replications. The main plots were the half-sib families of Sulawesi 1, Sulawesi 2, and Scavina 6. The subplots were added with cadmium solution at 0 (control), 2, 4, and 8 ppm. The results showed that adding cadmium up to 8 ppm did not affect the growth and biomass of cocoa seedlings; however, it had a negative effect on plant physiological processes of the net assimilation rate and relative growth rate. Cadmium content in the tissue increased with increasing cadmium concentration in the growing medium. The accumulation of cadmium in roots was higher than that of shoots. Absorption of cadmium in the root tissue of Sulawesi 1 was higher than that of Scavina 6 and Sulawesi 2; genetic factors did not affect cadmium uptake in the shoots. There was a positive correlation between root dry weight and cadmium concentration in the tissue; the higher the dry weight of the root tissue, the higher the cadmium uptake in plants.

Keywords: cadmium, half-sib family, *Theobroma cacao* L.

INTRODUCTION

Cacao is a plantation crop that offers economic benefits. Cacao cultivation currently pays attention not only to quantity but also to production quality. One of the largest cocoa-producing countries is Indonesia. However, there are problems related to the quality of cocoa produced in Indonesia, including the issue of cadmium.

Many developing countries these days have regulations related to the quality of the cocoa beans to consume; one of the quality criteria is the content of the heavy metal such as cadmium (Cd) found in cocoa beans. The global market has forbidden the sale of commodities with specific heavy metal concentrations (Zarcinas *et al.,* 2004) due to health reasons.

The maximum threshold for heavy metal content in food in Indonesia for As, Cd, Hg, and Pb has been stipulated through the standard SNI Number 7378 of 2009, including on products like candies or confectionary, chocolate, and chocolate powder. The maximum threshold for Cd in chocolate and cocoa products, according to SNI Number 7378 of 2009, is 0.5 ppm. For the European Union market, importers set the maximum threshold of Cd at < 0.5 ppm. Cocoa beans with a Cd content up to 0.8 ppm are still allowed, yet assessment must be done on the products from the beans, such as the paste, nibs, and butter.

Cd is a type of heavy metal commonly found in plants due to natural soil and rock conditions and cultivation practices, especially the use of pesticides and phosphate fertilization. Accumulation of Cd in cocoa plants should not exceed 1 mg kg^{-1} because it will bring a negative impact on health (Zarcinas *et al*., 2004). Absorbed Cd will interfere with specific metabolic processes in plants. Stress caused by Cd stimulates plants to induce several resistance mechanisms for survival.

Pollution caused by Cd can be minimized using technical culture, for example, planting materials as a cadmium hyperaccumulator or applying straw and brown algae extract. Research on the Cd uptake of cocoa plants has been done by many, including Oliva *et al*. (2020) in Peru, Ramtahal *et al*. (2016) in Trinidad and Tobago, and Correa *et al*. (2021) on cocoa nurseries. Fernandez-Paz *et al*. (2021) also carried out a study on open-pollinated and controlled-pollinated cocoa seedlings. However, studies on cacao rootstock on Cd response are still limited. This research was conducted to examine the physiological response of cacao to the accumulation of cadmium metal (Cd).

MATERIALS AND METHOD

This study took place from June to December 2020 at the Greenhouse of the Indonesian Coffee and Cocoa Research Center. The study was designed using a completely randomized split-plot design with 3 replications. The parent plots were the half-sib families of propeligitim seedlings of Sulawesi 1, Sulawesi 2, and Scavina 6. The subplots were added with cadmium solution at 0 (control), 2, 4, and 8 ppm. We applied different concentrations of Cd solutions to plants to determine cadmium accumulation in plant tissues.

We used 4-month-old seedlings placed in 15 x 15 cm polybags. The planting medium was a mixture of soil and manure with a ratio of 2:1. Seedlings selected were in a suitable category, with at least 5 mature leaves and a minimum height of 30 cm. The plants were then placed on a collection plate according to the treatment. The application was carried out once the seeds were selected by giving 200 mL of Cd solution to the plants according to the treatment on the 4-month-old seedlings.

Growth observations were made by measuring the height, the number of leaves, stem diameter, leaf area, and dry weight of plants at 0 and 28 weeks after treatment (WAT). The total dry weight was observed destructively by taking the whole plant stover. The samples were dried in an oven at 60-80^{O}C (until reaching constant weight) and weighed using an analytical balance.

Relative Growth Rate refers to the rate of increase in plant dry weight (W) per unit dry weight at a particular time (T), which is expressed mathematically as follows (Ivonne, 2013):

$$RGR = \frac{\ln W2 - Ln\, W1}{T2 - T1}$$

In which RGR = Relative growth rate; W1 = Harvested dry weight 1; W2 = Harvested dry weight 2; T1 = Harvesting time 1 (0 WAT); T2 = Harvesting time 2 (28 WAT)

Net Assimilation Rate is calculated using the following formula:

$$NAR = \frac{\ln W2 - Ln\,W1}{T2 - T1} x \frac{\ln L2 - Ln\,L1}{L2 - L1}$$

In which LAB = Net assimilation rate, W1 = Dry weight at 0 WAT, W2 = Dry weight at 28 WAT, T2 = age at week 1, T1 = age at week 2, and L = leaf area at 0 WAT and 28 WAT.

Cd content was analyzed on root and shoot tissues represented by leaves at 28 WAT. Samples were extracted using the open destruction method by inserting 0.5-1.0 g of the sample, which had been mashed into the digestion flask. Then 5-10 mL of HNO_3 p.a and 1-2 mL of $HCLO_4$ p.a were added to the flask. Then it was heated to 130°C for 1 hour, and the temperature was increased to 150°C for 2 hours and 30 minutes (until the yellow steam ran out; if the yellow steam was still present, heating was continued). After the yellow steam had all gone, the temperature was increased to 170°C for 1 hour, and then the temperature was increased to 200°C for 1 hour (until white steam was formed). The process was completed by the formation of a white precipitate, or about 1 mL of clear solution remained. The extract was cooled and diluted with free ionized water to 25 mL, then shaken until homogeneous before being left overnight. The clear extract was used to measure the weight of metals using flame-atomic absorption spectroscopy. Titrisol® standard solution Cd 1000 ppm was diluted, and 25 mL of each test sample was taken and then given 2 mL of 8M HCl and 0.1 mL of 20% KI. The sample and standard solution were left for 2 minutes; then, the absorbance was measured.

Observational data included variables analyzed using Analysis of Variance (ANOVA) with α at 5%. If the results of ANOVA show that F calculated > F table, there is a significant difference between treatments so we can proceed with Duncan's Multiple Range Test (Gomez & Gomez, 1995). The closeness of the relationship between the observed variables was determined by correlation and regression analysis.

RESULTS AND DISCUSSION

Cd has been a concern for food safety, including in the cocoa cultivation sector. Cd from the soil transported to the shoot can provide an overview of Cd uptake levels up to the seeds. The results showed that the Cd treatment had no significant effect on plant growth, but a significant effect was seen on the genotypic factor on the number of leaves. Sulawesi 1 had more leaves than Sulawesi 2 and Scavina 6 (Table 1). This shows that the administration of Cd up to 8 ppm did not have a detrimental effect or

Table 1. Plant height, stem girth, leaf number and total leaf area of three clones as affected by cadmium concentration treatment at 28 weeks after treatment

Clones	Plant height (cm)	Stem girth (cm)	Leaf number	Total eaf area (cm²)
Scavina 6	24.56 a	0.96 a	16.46 b	1773.26 a
Sulawesi 1	20.15 a	1.02 a	19.54 a	1625.22 a
Sulawesi 2	21.64 a	1.05 a	16.79 b	1631.81 a
Significance	ns	ns	*	ns
Cadmium concentration				
0	21.46 p	0.40 p	18.44 p	1832.39 p
2	27.00 p	0.41 p	19.22 p	1773.70 p
4	18.89 p	0.43 p	15.78 p	1492.05 p
8	21.12 p	0.39 p	16.94 p	1608.93 p
Significance	ns	ns	ns	ns
Interaction	(-)			

Notes: Figures in the same column and treatment group followed by the same letter are not significantly different according to Duncan Multiple Range Test 5%; ns = not significant, * = sinificant.

was not yet toxic to the morphology of the cacao plant. This is supported by Correa *et al.* (2021), showing that in a condition where the soil pH is above 5, the increase in stem height and diameter is not affected by the concentration of Cd in the planting medium up to 10 ppm. Correa *et al.* (2021) added that at low pH (pH < 5), a Cd concentration of 5-10 ppm in cocoa could reduce plant growth, while a concentration of 20 ppm can cause plant death. Cadmium poisoning in plants occurs when the Cd levels in plant tissues reach 30 ppm (Solís-Domínguez *et al.*, 2007; Chen *et al.*, 2011). Excess Cd levels will also disturb plant metabolism and interfere with the nutritional content of plants (Sandalio *et al.*, 2001; Sanchez-Pardo *et al.*, 2013).

Cd concentration did not affect the accumulated plant dry weight 28 weeks after treatment (Table 2). Root dry weight and root-shoot ratio were influenced by the genotype used. Sulawesi 1 had higher root dry weight and root-shoot ratio than Sulawesi 2 and Scavina 6. Cd administration had a physiological effect on the relative growth rate and net assimilation rate (Figure 1 and Figure 2). The net assimilation rate is a measure of the photosynthetic ability to produce plant dry matter, while the relative growth rate is the ability of plants to produce dry matter as assimilation results per initial dry weight unit per unit of time. Based on the regression analysis results, an increase in Cd concentration by 1 ppm could reduce the net assimilation rate by 0.4 gr m^{-2} $week^{-1}$ and the relative growth rate by 0.0019 gr gr^{-1} $week^{-1}$.

Excess Cd can be toxic to plants, and the threshold for Cd-tolerant hyperaccumulator plants is below 25 ppm (Wei *et al.*, 2008). On cocoa plants, de Araujo *et al.* (2017) have reported that administration of 50 ppm Cd reduced photosynthetic activity in seedlings aged 90 days after treatment. Dias *et al.* (2013) have reported that concentrations of 2.36–11.8 ppm could reduce the growth and photosynthetic efficiency of lettuce plants. The mechanism of Cd in inhibiting plant physiology was described by Haider *et al.* (2021) that Cd can inhibit photosynthesis by inhibiting the opening of stomata and damaging the photosynthetic apparatus, especially the light-harvesting complex and photosystem I and photosystem II.

Cd is a heavy metal that is easily translocated from the planting media to plant tissues (Haider *et al.*, 2021). Accumulation of Cd in the root of cocoa plants is higher than in the shoot (Table 3.). Genotypic factors and the concentration of applied Cd influence the concentration of Cd in the root. The Cd content in the root of the half-sib family Sulawesi 1 was higher than that of Sulawesi 2 and Scavina 6. The high Cd accumulation in the roots indicated that the plant could absorb Cd in the root tissue so that it could act as a Cd hyperaccumulator. However, the Cd content in shoots did not differ between genotypes. Giving Cd to the planting media

Table 2. Root, shoot, total drymass, and root/shoot ration of three clones at different concentration of cadmium

Clones	Root	Shoot	Total	Root/shoot ratio
Scavina 6	3.87 a	13.16 a	17.03 a	0.305 a
Sulawesi 1	5.51 b	14.17 a	19.67 a	0.399 b
Sulawesi 2	4.15 a	14.47 a	18.62 a	0.296 a
Significance	*	Ns	ns	*
Cadmium concentration				
0	4.53 p	14.79 p	19.32 p	0.311 p
2	4.16 p	14.73 p	18.89 p	0.296 p
4	4.48 p	12.68 p	17.16 p	0.361 p
8	4.86 p	13.53 p	18.39 p	0.365 p
Significance	ns	Ns	ns	ns

Notes: Figures in the same column and treatment group followed by the same letter are not significantly different according to Duncan MRT at 5%; ns = not significant, * = sinificant.

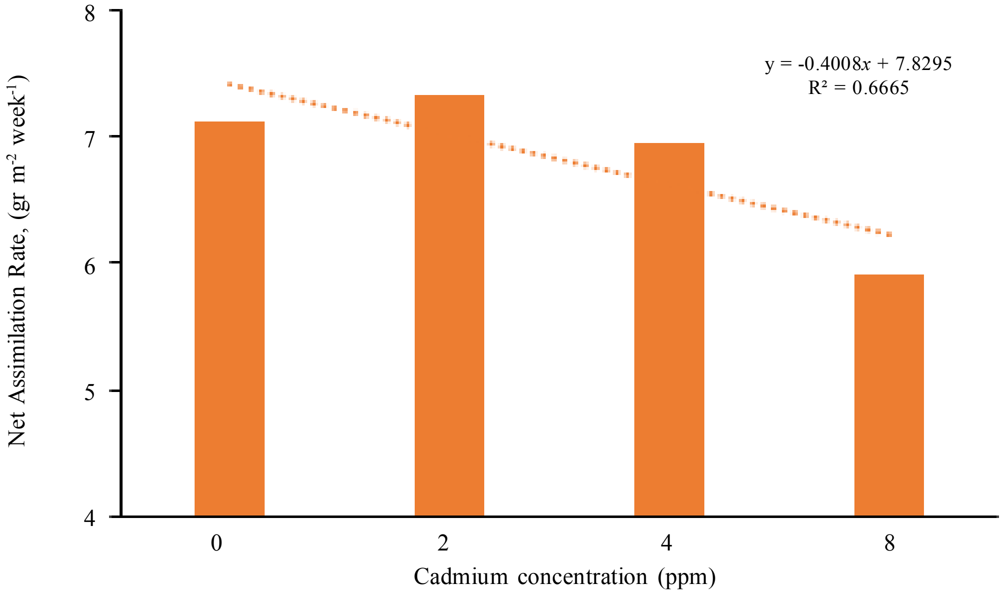

Figure 1. Net assimilation rate of cocoa seedlings treated with cadmium solution at 28 weeks after treatment

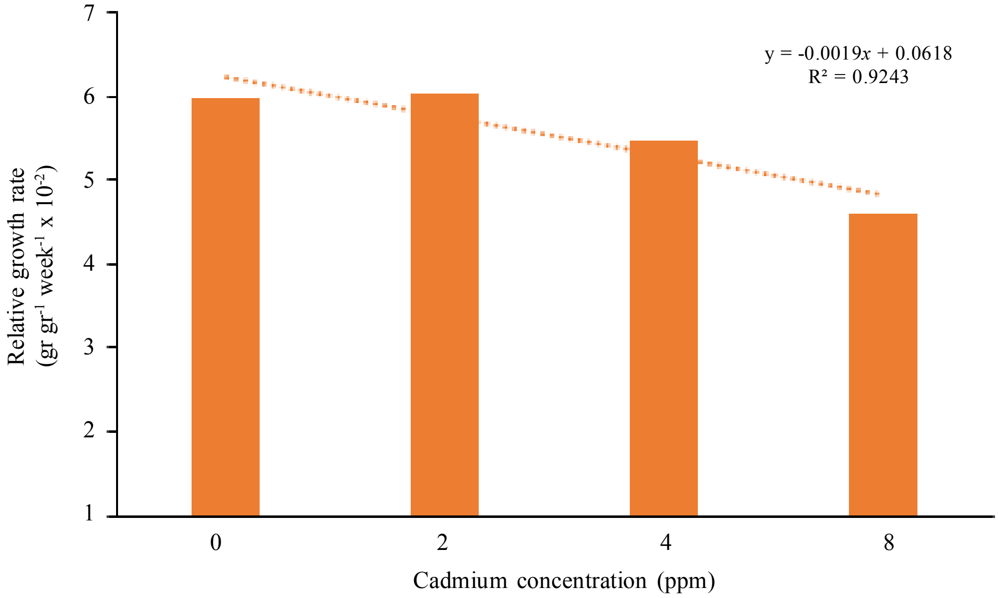

Figure 2. Relative growth rate of cocoa seedlingd as affected by treatment with cadmium solution at 28 weeks after treatment

increases the accumulation of Cd in the tissue. The regression analysis confirmed a strong linear relationship between the concentration of applied Cd and the accumulation of Cd in the root (R2 = 0.97) and leaves (R2 = 0.96). Giving 1 ppm Cd to the planting media can increase the absorption of 0.54 ppm in the root and 0.48 ppm in the leaves. Cd accumulation in plants is influenced by root interception of Cd. Greater root biomass indicates a wider distribution of roots causing greater potential for Cd uptake (Zakariyya, 2017). This is indicated by the positive relationship between root dry weight, root Cd content, and shoot Cd content (Figure 3).

Cd found in the root and shoot tissues indicates that the metal can be translocated (He *et al*., 2015). Haider *et al*. (2021) have explained that the Cd content in the roots is higher than in the shoots. A strong relationship between Cd content in soil and plants has been reported by Chaves *et al*. (2015), Ramtahal *et al*. (2016), and Barraza *et al*. (2017). Cd enters the plant through the roots and is translocated via transpiration streams and/or by transporters to the shoot in the form of ions to the xylem and phloem vascular bundles (Dong *et al*., 2019). Transporters, such as natural resistance-associated macrophage proteins (NRAMPs) and heavy metal transporting ATPases (HMAs), are involved in Cd transport (Vanderschueren *et al*., 2021).

Table 3. Cadmium concentration in root and shoot of cocoa sedlings at 28 weeks after treatment

Clones	Root Cd concentration ($mg\ g^{-1}$ dry weight)	Shoot Cd concentration ($mg\ g^{-1}$ dry weight)
Scavina 6	1.91 ab	0.87 a
Sulawesi 1	2.85 a	1.40 a
Sulawesi 2	1.09 b	1.47 a
Significance	*	ns
Cadmium concentration treatment (ppm)		
0	0.00 p	0.00 p
2	1.50 q	0.90 pq
4	1.75 q	1.90 qr
6	4.56 r	3.85 s
Significance	*	*

Notes: Figures in the same column and tratment group followed by the same letter are not significantly different according to Duncan MRT at 5%; ns = not significant, * = sinificant. Data was transformed with $\sqrt{x + 0.5}$

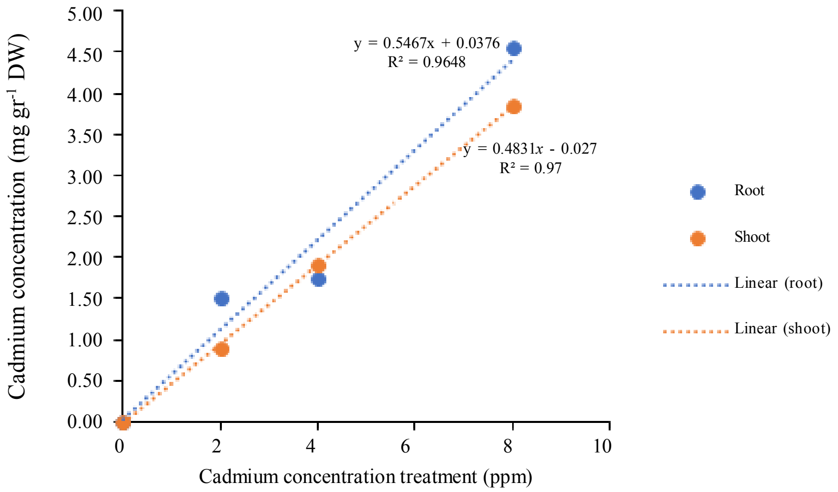

Figure 3. Relationship between cadmium concentration treatments and cadmium concentation in root and shoot tissues of cocoa seedlings at 28 weeks after treatment

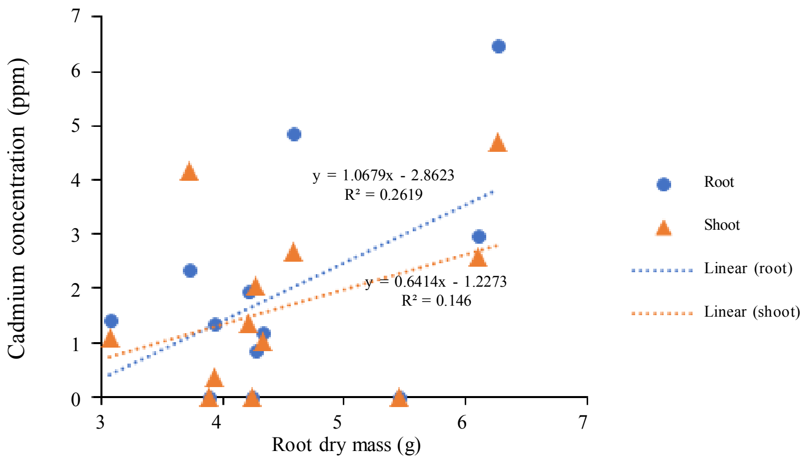

Figure 4. Relationship between root dry mass and cadmium concetration in root and shoot of cocoa seedlings at 28 weeks after treatment

CONCLUSIONS

Our findings confirmed that adding Cd up to 8 ppm had no effect on plant growth and biomass but had a negative effect on plant physiological processes in the form of net assimilation rate and relative growth rate. The Cd content in the tissue increased with the addition of Cd to the planting media. Cd accumulation in roots was higher than in shoots. The absorption of Cd in the root tissue of Sulawesi 1 was higher than that of Scavina 6 and Sulawesi 2, but the genotypic factor did not affect the uptake of Cd in the shoots. There was a strong relationship between root dry weight and Cd uptake in tissues; the higher the root dry weight, the higher the Cd uptake in plants.

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