

**Research Article**

**Phytotoxicity of coal fly ash on plant growth and heavy metal uptake by plant in an acid soil**

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**Abstract:** Considering the content of various elements in coal fly ash (CFA), it has the potential to be used as an additional source of nutrients in the soil. However, the use of CFA for agriculture is still debated because CFA contains several metal elements. The purpose of this study was to study the CFA phytotoxicity to seed germination and metal uptake by plants on acid soil. The first experiment was the study of the effect of extracts of various CFA and soil mixtures (5% - 45% CFA) on the germination of mustard seeds. The second study was a CFA phytotoxicity test for plant growth and metal uptake by the mustard plant. Results of the first experiment showed that the application of CFA had no significant effect on mustard seed germination, but had a significant effect on radicle length. CFA application increased the uptake of Pb and Cu by plant along with the increase in the proportion of CFA in the CFA + soil mixture. The contents of Cu and Pb in the mustard plant due to CFA application up to 30% were still below the threshold levels of Cu (36 ppm) and Pb (2 ppm).

**Keywords:** coal fly ash, phytotoxicity, germination, radicle length

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**Introduction**

Coal fly ash (CFA) is mostly produced from coal combustion in Steam Power Plants and in coal-fired industries (Gupta et al., 2002; Singh and Pandey, 2013). Generally, the colour is grey and vary to black. The colour of this CFA is influenced by the burning time of the furnace (Kurniawan et al., 2010). The CFA contains mainly of silicate dioxide, aluminium, iron, and calcium, as well as a small amount of magnesium, potassium, sodium, titanium, and sulfur (Nugraha and Antoni, 2007). The amount of CFA produced in Indonesia, according to Aziz et al. (2006) in 1996 was as much as 0.25 million tons and in 2000 was about 1.41 million tons. The estimated amount of CFA produced in Indonesia in 2006 was 1.7 million tons and in 2009 was 2.78 million tons. No additional new available data about the CFA produced in

Indonesia, but along with the rapid construction of coal-fired power plants, it is estimated that the production of CFA increases. In general, the CFA particles are round with sizes ranging from 0.5-100 µm (Kurniawan et al., 2010). CFA also contains elements such as phosphorus, bases, and microelements that are essential for plants (Chaudhuri et al., 2003; Singh and Pandey, 2013). Inthasan et al. (2002) reported that CFA contains 17% Si, 11% Fe, 9.8% Al, 6.4% Ca, 1.4% K, 1.2% Mg, 0.4% Na, 582 ppm Mn, 53 ppm Ni, 34 ppm Co, 67 ppm Cr, and 20 ppm Mo, so that it can be potentially used as an additional source of nutrients in agricultural land and used as an ameliorant material in improving problem soils, such as acid soils. However, the use of CFA for agriculture is still debated. According to Government Regulation No. 85 of 1999 Jo Government Regulation No. 18 of 1999 CFA is classified as

hazardous and toxic waste because it contains heavy metals. Several research results showed the potential of CFA phytotoxicity (Yunusa et al., 2012; Ukwattage et al., 2013; Shaheen et al., 2014). The purpose of this study was to elucidate the effect of CFA on seed germination, crop yields, and uptake heavy metals by plants on acid soil.

**Experiment 1: CFA phytotoxicity test on mustard seed germination**

The materials used in this study were coal fly ash (CFA), soil, and seeds of mustard plant as a metal accumulator plant. Raharjo et al. (2012) reported that the mustard plant effectively absorbed Cu and Hg. In Indonesia, mustard plant is a horticultural plant that is consumed and has high economic value after cabbage and broccoli (Zulkarnain, 2009). CFA was obtained from the PLTU PT. Cahaya Fajar Kaltim, located in Embalut Village, Tenggarong Seberang District, Kutai Kartanegara Regency, Tenggarong Regency, East Kalimantan. The CFA had been piled in a landfill for one month. The soil was obtained from Tenggarong Regency, East Kalimantan. The characteristics of CFA are as follows: pH = 9.8, organic-C = 0.82%, total-N = 0.05%, total-P = 1378.56 ppm, available-P = 5.73 ppm, total-K = 719.35 ppm, Zn = 60.80 ppm, Cu = 13.50 ppm, Cd = 2.60 ppm, and Pb = 5.70 ppm (Fahrussyah et al., 2018). The characteristics of Ultisol from East Kalimantan are as follows: pH = 4.1, organic-C = 1.23%, organic matter = 2.12%, total-N = 0.14%, total-P = 137.22 ppm, available-P = 3.76 ppm, total-K = 232.87 ppm, Ca<sup>2+</sup> = 3.17 me/100 g, Mg<sup>2+</sup> = 1.08 me/100 g, K<sup>+</sup> = 0.12 me/100 g, Na<sup>+</sup> = 0.13 me/100 g, H<sup>+</sup> = 1.08 me/100 g, Al<sup>3+</sup> = 4.45 me/100 g, CEC = 21.18 me/100 g, and base saturation = 44.87% (Fahrussyah et al., 2018). The treatments tested were combinations of CFA and soil with the following proportions, (1) 0% CFA + 100% soil; (2) 5% CFA + 95% soil; (3) 10% CFA + 90% soil; (4) 15% CFA + 85% soil; (5) 20% CFA + 80% soil; (6) 25% CFA + 75% soil; (7) 30% CFA + 70% soil; (8) 35% CFA + 65% soil; (9) 40% CFA + 60% soil; and (10) 45% CFA + 55% soil. A mixture of CFA and soil as much as 50 g was put in a container, and 100 mL of distilled water was then added to the mixture. The soil-CFA-water mixture was shaken for 6 hours at 100 rpm, then filtered. Fifteen mustard seeds were germinated in a petri dish (diameter: 100 mm, height: 15 mm) coated with Whatman No. filter paper. 41) followed by addition of 5 mL of CFA + soil extract according to treatment. The treatment dosage, extraction method, and application method of extracting a mixture of CFA and soil followed the method used by Mtisi and Gwenzi (2018). Petri dish was then covered to reduce the evaporation of the solution and was incubated in a 25°C dark room

for 7 days. Ten treatment combinations of CFA and soil were arranged in a completely randomized design with three replications. Parameters observed on the 7th day of germination were the number of germinated seeds and the radicle length of the seedlings. Radicle length was observed in three seedlings randomly selected from each petri dish so that nine observations were obtained for each treatment. The data obtained were used to calculate the toxicity index, namely (1) seed germination (SG), and (2) radicle elongation (RE). SG is the percentage of seed that germinates, while the RE is the length of the seed root that germinates (Bagur-Gonzalez et al., 2011; Mtisi and Gwenzi, 2018) using the following formula:

$$SG = \frac{G_E(i) - G_c}{G_c}$$

$$RE = \frac{R_E(i) - R_c}{R_c}$$

where:

- GE (i) = The average number of seeds germinating in extract (i)
- GC = The average number of seeds germinated in control (without extracts)
- RE (i) = The average length of the radicle in the extract (i) (mm)
- RC = The average length of the radicle in control (mm)

Index values vary from -1 (maximum phytotoxicity) to > 1 (stimulation of seed germination or root growth). Positive SG and RE values indicate seed germination stimulation or radicle elongation, while negative values indicate phytotoxicity. Phytotoxicity levels are grouped into four based on SG or RE values, namely: (1) Low (0 > SG or RE > -0.25), (2) moderate (-0.25 > SG or RE > -0.5), (3) high (-0.5 > SG or RE > -0.75), and (4) very high (-0.75 > SG or RE > -1) (Bagur-Gonzalez et al., 2011). The effect of the application of CFA doses on germination and radicle length were statistically analyzed by one-way analysis of variance (one-way ANOVA, followed by the least significant difference (LSD) test used if there were significant differences between treatments at p = 0.05.

**Experiment 2: A test of the effect of CFA application on heavy metal uptake, and plant biomass yield**

The materials used in experiment 2 (a pot experiment in a glasshouse) were similar to those used in experiment 1. The treatments tested were

the same as the treatments in experiment 1, i.e. ten combinations of CFA and soil. For each treatment, 2 kg of each CFA + soil mixture was put into a 3 kg plastic pot with a hole at the bottom for drainage. The pot hole was layered with a Whatman filter paper to prevent soil loss. The ten treatments were arranged in a randomized block design with three replications. Water was supplied to the field capacity and allowed for one week for CFA to react with the soil. Five mustard seeds which were previously soaked for 24 hours in distilled water were then planted in pots, which were left with the two best seeds at one week after growing. Basal fertilizers of 100kg N/ha, 50 kg P<sub>2</sub>O<sub>5</sub>, and 50kg K<sub>2</sub>O/ha were supplied to each pot. During the experiment, well water was periodically added to each pot to maintain the water content of the pot about 80% of field capacity. Plants were harvested after eight weeks by separating the plant shoots and roots. The plant shoots were washed with distilled water. The roots were separated from the soil by soaking in water. Root samples were washed in a 0.1 mm sieve equipped with a container under the sieve, to capture fine roots (Gwenzi et al., 2011). Observations made at harvest included fresh and dry weights of shoots and roots, and the content of metal elements (Zn, Cu, Cd, and Pb) in the plant shoots and roots, and soils which were carried out using Atomic Absorption Spectrometer. Uptake of elements by plants = dry weight of plants x% elements in plants. The ability of mustard plant in absorbing and translocating metal elements was measured through Translocation Factor (TF). TF shows the ability of plants to translocate metal elements and roots to the plant canopy. Metal elements that are

accumulated by plants and partially stored in plant roots are shown with a TF value <1, while a TF value > 1 indicates a logical translocation from the root to the plant canopy. TF = [metal concentration in plant shoots] / [metal concentration in plant roots] (Mellem et al., 2009). The effect of CFA application on heavy metal (Zn, Cu, Cd, and Pb) uptake, and crop yields were statistically tested by one-way analysis of variance (one-way ANOVA, followed by the least significant difference (LSD) test at p = 0.05.

## Results and Discussion

### *Seed germination, radicle length, and toxicity index*

The observation of seed germination presented in Figure 1 shows that the CFA treatment of 5% - 20% had no significant effect on mustard seed germination, but had a significant effect on the CFA treatment of 25% - 45%. The effect of 30%, 35% and 40% CFA treatments on seed germination was not significantly different. The 45% CFA application significantly reduced the percentage of seed germination. When compared with the control (CFA0%), application of 25% - 40% CFA to the soil only reduced the percentage of mustard seed germination by 2%, but the application of 45% CFA to the soil decreased seed germination by 5%. The effect of CFA application on the SG toxicity index was also not significant even though the application of CFA5% - CFA 20% gave a positive value meaning it did not cause toxicity while the negative value was indicated on the application of CFA 25% - 45%.

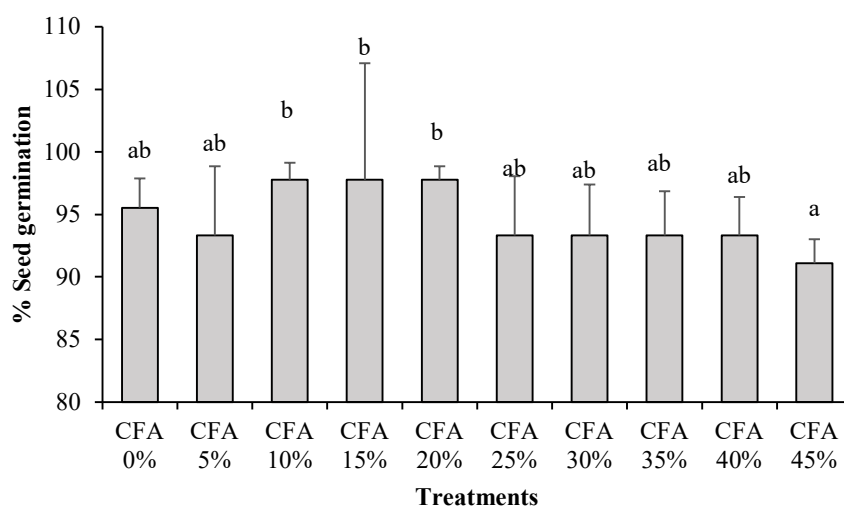


Figure 1. Germination of mustard seed on various combinations of CFA and soil, Bars represent standard deviations.

CFA applications reduced the length of radicles from 20% (CFA5%) to 49% (CFA 40%). Overall, the application of CFA significantly reduced the length of the radicle compared to control (Figure 2). The decrease in radicle length in applications of 5-30% CFA was not significantly different, with a mean of 29% reduction. The reduction in radicle length in the CFA treatment of 35%, 40%, and 45% was also not significantly different from the average reduction of 48%. Based on the RE toxicity index proposed by Bagur-Gonzalez et al. (2011), the application toxicity level of 5% CFA up to 30% CFA was included in the low category,

while the application of 35% CFA to 45% was included in the moderate category.

The results indicate that the use of CFA to improve soil fertility should not exceed 30% of the proportion applied to the soil. Fahrnsyah et al. (2018) showed that the application of 80 t CFA / ha on Ultisol (about 5%) combined with 20 t TKKS / ha increased the yield of maize seeds by 15 times. Based on the toxicity index criteria proposed by (Bagur-Gonzalez et al., 2011), the application of CFA up to 45% only caused low to moderate toxicity in mustard plants because the SG value was between -0.05 to -0.05 (Figure 3).

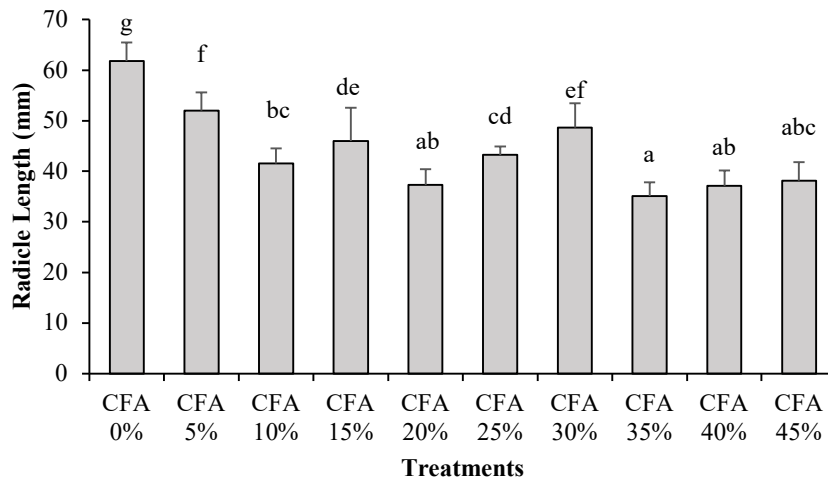


Figure 2. Radicle elongation of germinated mustard seeds on various combinations of CFA and soil. Bars represent standard deviations.

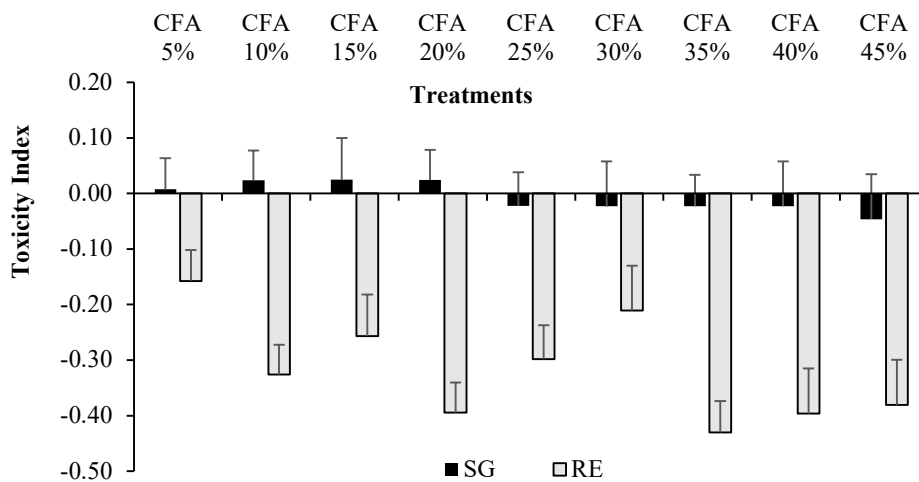


Figure 3. Toxicity index of CFA on seed germination (SG), and radicle elongation (RE). Bars represent standard deviations.

The lack of response of CFA application to seed germination is thought to be due to the inability of toxic compounds in coal CFA to reach embryogenic tissue in the seeds. In most plant seeds, the embryogenic tissue is physically protected by the seed coat having different permeability (Akinci 2010; Soares et al., 2016). This explanation is consistent with the results of a nickel ecotoxicity study that Ni accumulates in the seed coat but not in the endosperm (Léon et al., 2005). Contrary to germinating seeds, radicle elongation showed significant phytotoxicity of low to high levels (Figures 1 and 2). These results indicate that radicle elongation is a more sensitive endpoint for CFA phytotoxicity than germination as reported by Soares et al. (2016) that 100 and 500 mg/L nickel affected the germination of *Grevillea exul* var seeds. *rubiginosa*, but at lower nickel concentrations (50 mg/L) already inhibited root extension (Léon et al., 2005).

**Uptake of Pb and Cu by mustard plant**

Pb and Cu contents in the plant increased with increasing the proportion of CFA in the CFA + soil mixture (Figures 4 and 5). Compared with controls, CFA application increased the Pb content of the plant shoot from 73% in the CFA5% treatment to 964% in the CFA45% treatment, and the Pb content in the roots increased up to 1125% in the CFA45% treatment (Figure 4). For Cu, the application of CFA increased the Cu content in plant shoot starting from 73% in the CFA5% treatment to 611% in the CFA45% treatment, and the Cu in the roots increased up to 468% (Figure 5). The increase of Pb and Cu contents in the plant indicated the increase of Pb and Cu uptake by the plant (Figures 6 and 7). The highest increase in Pb

uptake (600%) occurred at CFA45% treatment. The increase of Pb content in the mustard plant due to the application of CFA 5-30% was still in a safe point for consumption because it was still below the threshold value of 2 ppm. However, the CFA application of more than 30% resulted in Pb content of the plant that exceeded the threshold so that the plant is not safe for consumption. Cu content in the mustard plant due to CFA application up to 45% did not exceed the actual threshold of 36 ppm. The maximum limit of heavy metal content in vegetables allowed according to Decree of the Directorate General of Drug and Food Control No. 03725 / B / SKVII / 89 is 2.0 ppm for Pb and 36 ppm for Cu. Several other researchers also reported that the addition of CFA caused an increase in the elements of Cu, Pb and Ni in the soil but within the normal range (Tripathi et al., 2009). Türkmen et al. (2015) reported that addition of CFA increased Cu content. Increased content of these Pb and Cu elements is related to the dose of CFA. The highest increase occurred in 24% CFA mixed soil. Wardhani et al. (2012) reported that on the 15<sup>th</sup> day, tomato plants treated with 75% of Lembang soil + 25% of coal ash exceeded control and other treatments. This change was because the Cu content found in coal ash was sufficient for micro-nutrient needs of tomato plants. At the concentration of 100% of coal ash occurred a slow down in growth or symptoms of toxification. This was probably caused by excess Cu content which affected tomato plants because of the small amount of Cu needed by tomato plants. The use of high coal ash would increase the synthesis of chlorophyll which will accelerate the degradation process, so that leaves can easily fall.

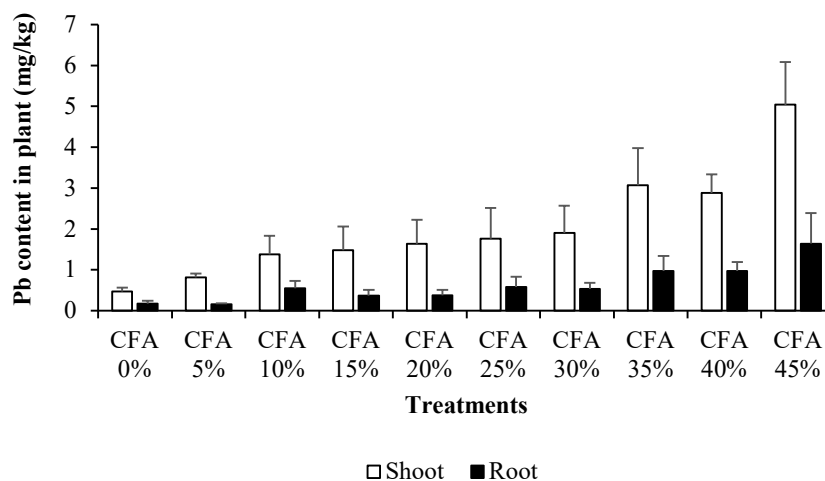


Figure 4. Pb content in the mustard plant as affected by application of CFA. Bars represent standard deviations.

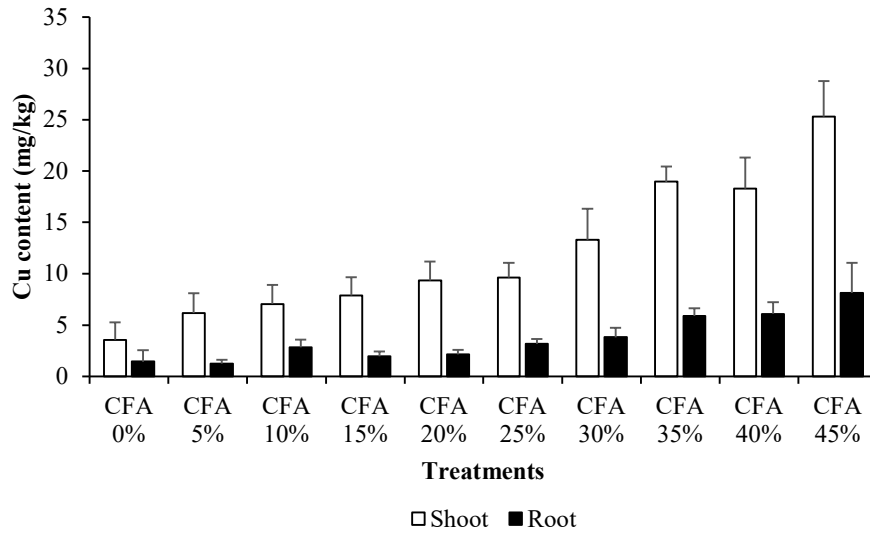


Figure 5. Cu content in the mustard plant as affected by the application of CFA. Bars represent standard deviations.

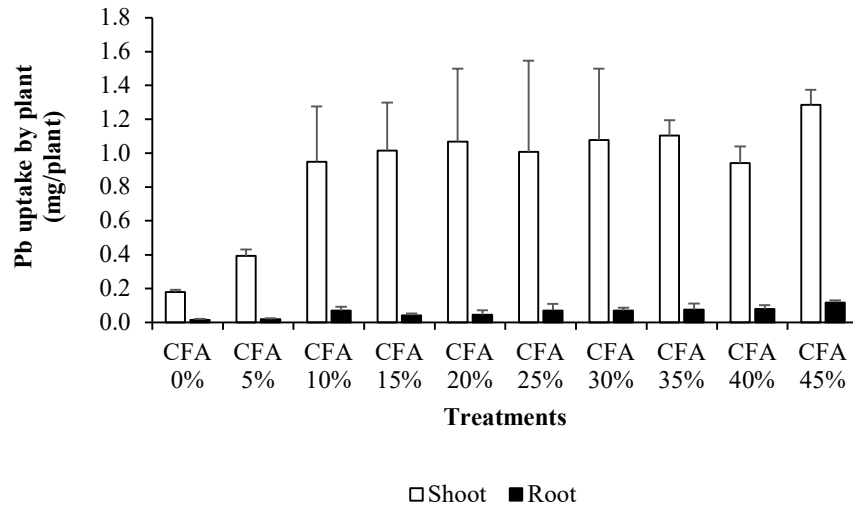


Figure 6. Pb uptake by the mustard plant as affected by the application of CFA. Bars represent standard deviations.

**Translocation Factors of Pb and Cu**

As the effect on Cu and Pb uptake in the plant, the application of CFA also had a significant influence on the translocation factor (TF) for Cu and Pb by the mustard plant which were all greater than 1 (Figure 8). The highest Pb translocation factor value (6.5) was found in the 1CFA5% treatment, while the lowest (2.54) was observed in the

CFA10% treatment. The highest Cu translocation factor value (4.5) was also found in the CFA5% treatment (2.9), and the lowest was in the CFA0% treatment (control). Based on TF values that are all greater than 1, it is known that mustard plant is a heavy metal accumulator plant that can be effectively used for phytoremediation of heavy metals contaminated in soils.

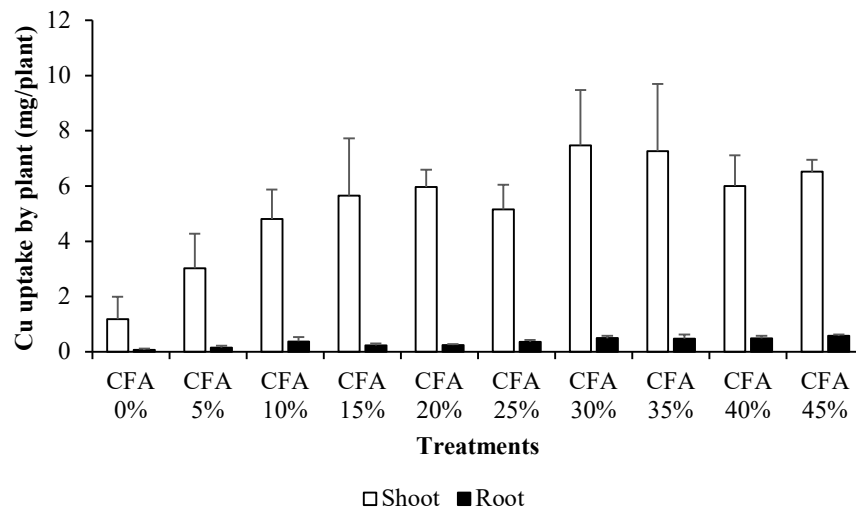


Figure 7. Cu uptake by the mustard plant as affected by the application of CFA. Bars represent standard deviations.

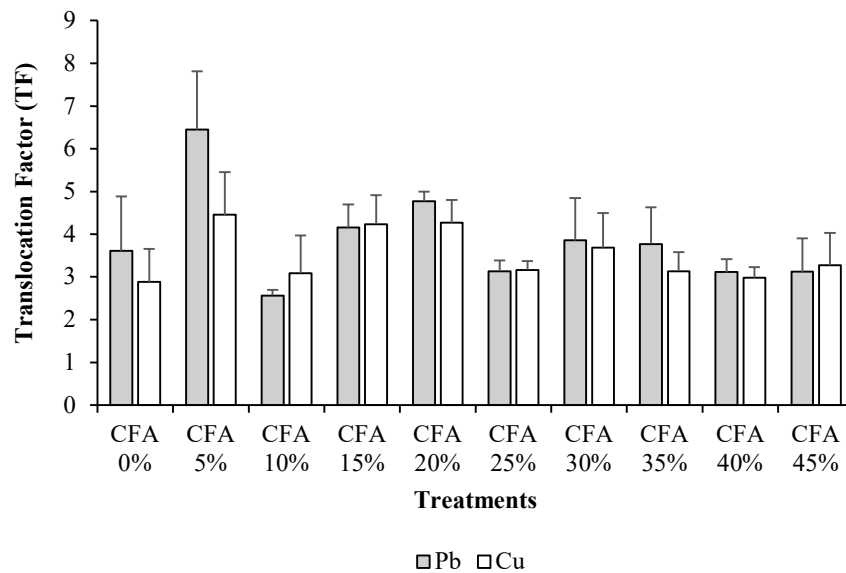


Figure 8. Translocation Factors of dan Pb and Cu of the mustard plant as affected by the application of CFA. Bars represent standard deviations.

### Conclusion

Application of CFA had no significant effect on the germination of mustard seeds but had a significant effect on the radicle elongation of mustard seedlings so that phytotoxicity of elongation was identified. Phytotoxicity test results showed that CFA caused low to moderate

toxicity to mustard plant. The Cu and Pb contents due to CFA application up to 30% were still below the threshold of Pb (2 ppm) and Cu (36 ppm). Application of CFA of more than 30% increased the Pb and Cu contents exceeding the threshold of Pb and Cu contents in vegetables in accordance with the Government of Indonesia regulations. The values of Translocation Factor (TF) for Pb and

Cu were all greater than one, indicating that mustard plant can be considered as a heavy metal accumulator plant that can be used for phytoremediation of heavy metals contaminated soils.

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