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Toxicity of Copper (Cu) and Chromium (Cr) on the Seed Germination of Mung Bean (*Vigna radiata* L.)

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ABSTRACT

Rapid industrial growth may have implications for an increase in cases of pollution of soil ecosystems through untreated waste disposal. Copper and chromium are metals found in some types of industrial wastewater, such as electroplating and silversmithing, that are essential at low concentrations but toxic at high concentrations. *Vigna radiata* was chosen as the test organism because it has been reported that the yield and production of the crop have decreased worldwide due to heavy metal pollution. This study aims to evaluate individual and mixed Cu and Cr toxicity in the seed germination of *V. radiata*. The individual and mixed Cu and Cr toxicity test was started with a range-finding and definitive test for 96 h. The toxicity level of the individual metal was expressed as IC50-96 h by probit analysis. For the mixed test, the organism was exposed to concentration ratios of Cu and Cr: 10%:90%; 35%:65%; 50%:50%; 65%:35%; and 90%:10% of individual IC50-96 h, each conducted for 96 h. The results showed that the individual IC50-96 h of Cu was 127.4 mg/L, while for Cr, it was 615.23 mg/L, indicating that Cu is more toxic than Cr. The mixed test showed that the IC50-96 h of Cu was 247.5 mg/L, 579.85 mg/L for Cr. The highest inhibition value at the mixed test was at a Cu and Cr concentration ratio of 90%:10%. Based on the mixed test, the interaction of Cu and Cr resulted in an additive effect (CI=1).

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1. Introduction

The development of industry seems to be something inevitable for humans. Along with the development of technology from time to time, various industries built for the benefit of human life have a significant impact on the environment because all industrial activities inevitably produce waste. This usually leads to the transformation of lakes, rivers, and water bodies into dumps where eventually, the ecosystem will be polluted, and its biological balance will be chaotic (Forstner & Wittmann, 1981). Pollution is the entry of a contaminant into the environment that causes instability, disorder, damage, or discomfort to an ecosystem or a living organism. Pollutants can be chemical or energy, such as sound, heat, or light (Ghosh, 2014).

Pollution by heavy metals occurs a lot in developing countries. This is due to the uncontrolled pollution level caused by the industry's growth and the increased use of fuel oil. Heavy metals are non-biodegradable and persistent. Heavy metal contamination, even in low environmental concentrations, can harm exposed organisms (Jain, 2004 in Priadi *et al*., 2014).

Large amounts of copper (Cu) and chromium (Cr) are mined/produced every year worldwide owing to their uses in various industrial and agricultural activities (Li *et al.*, 2018). Copper is commonly used in electrical wiring components, building materials, and industrial machinery. Cu is one of the essential metals for plants. This metal is helpful for the growth of plant tissues, especially leaves, as a site of photosynthesis. Generally, Cu levels in plant tissues range from 5-25 mg/L. But at high concentrations, Cu metal can be toxic to plants. Humans exposed orally to high concentrations of Cu may experience nausea and abdominal pain (acute toxicity) (Liestianty *et al.*, 2014; Taylor *et al.*, 2020).

While chromium (Cr) is one of the heavy metals that is corrosion resistant, gray in color, and commonly found in the form of chromate ore, Cr is used in the industrial field, including in the production of alloys (alloys), electroplating, and pigments (Ali *et al.*, 2004; Gomes *et al.*, 2017). Cr is also essential for humans and animals, helping insulin regulate blood sugar. However, continuously chronically exposed to Cr in humans can result in toxicity and multiple pathophysiological defects, including allergic reactions, anemia, burning, and sores, especially in the stomach and small intestine, damage to sperm and the male reproductive system, and affect various biological systems. Cr in the soil can suddenly change shape from Cr (III), which is not toxic, to Cr (VI), which is highly toxic, carcinogenic, and mutagenic. (Hossini *et al.*, 2022; Singh & Tripathi, 2007; Triatmojo, 2001). Large amounts of Cu and Cr in the soil can affect the growing plants (Triatmojo, 2001). The toxic effects of metal combinations in organisms can be stronger than those of individual metals (Nugroho *et al.*, 2020).

V. radiata, commonly called mung bean, is a legume plant (Fabaceae) that is drought-resistant, so it has excellent potential to be developed (Atman, 2007). In addition, the selection of mung bean seeds as a model plant because these seeds meet the criteria as a test organism. They are available in abundant quantities in nature, are easy to get, have fast growth, and are affordable. In addition, *V. radiata* has also been reported that the yield and production of the crop have decreased worldwide due to heavy metal pollution (Mao *et al.*, 2018).

Previous studies generally evaluated the toxicity of various metals individually in seed plant germination. Pokorska-Niewiada *et al.* (2018) reported that exposure to individual Cu (40-480 mg/L) and Cr (150-500 mg/L) inhibited the seed germination of vetch (*Vicia sativa* L.), rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), winter rape (*Brassica napus* L.), pea (*Pisum sativum* L.), and cress (*Cardamine pratensis* L.). However, the study of the toxicity of Cu and Cr, especially in the mixed concentration of the two metals, on *V. radiata* still needs to be explored. This study evaluates individual and mixed Cu and Cr toxicity on the seed growth of *V. radiata*.

2. Methods

2.1. Chemical materials and glassware

This study used chemical materials with analytical grade (Merck). Stock solutions of Cu and Cr with concentrations of 100 mg/L and 1000 mg/L, respectively, were prepared using $CuSO_4 \bullet 5H_2O$ and $CrCl_3 \bullet 6H_2O$ for toxicity exposure experiments.

All glassware was washed twice with 50% HNO_3 solution (65%, 225 g/L), distilled water, and bidistilled water and then sterilized in an autoclave at 121°C for 15 min.

2.2. Mung bean seed preparation

Mung bean seeds of about 150 g were placed in a 250-mL Beker glass and added distilled water until half volume of the glass was. Floating seeds were not selected for toxicity tests.

2.3. Acute toxicity test

The acute toxicity test consisted of range-finding and definitive tests to obtain the individual IC50–96 h of Cu and Cr on *V. radiata*. For the range-finding test, 20 seeds in a 12-cm Petri dish were exposed to Cu and Cr individually for 96 h, respectively, with the concentration of each metal, i.e., 0, 0.1, 1, 10, and 100 mg/L (n=3). The stock solution was used to establish the concentrations. The metals were administered by adding 25 mL of the metal solution. The seed germination, i.e., forming a primary root with a length of 5 mm, was recorded every 24 h. Furthermore, the concentration range that produced the inhibition rate of 50% was further used as the definitive test range.

For the definitive test, 20 mung bean seeds were placed in a 12-cm Petri dish and exposed to Cu or Cd for 96 h. The stock solution was used to establish concentrations of Cu, i.e., 0, 15, 23, 34, 51, 76, and 90 mg/L; for Cr, the concentrations were 0, 150, 230, 340, 510, 760, and 900 mg/L (n=3). Exposure of the seed to the individual metal was conducted by adding 25 mL of the metal solution. The seed germination of *V. radiata* was recorded every 24 h. Linear regression analysis is performed to evaluate the relationships between metal concentration and inhibition of seed germination, followed by Pearson correlation analysis for testing the strength of linear relationships. The probit analysis was based on definitive test results to obtain the IC50–96 h for individual metals.

2.4. Mixed test

The results of individual IC50-96 h Cu and Cr were used to determine the mixed concentrations of Cu and Cd. In this mixed test, 20 mung bean seeds in a 12-cm Petri dish were exposed to concentration ratios of 0%:0%, 10%:90%, 35%:65%, 50%:50%, 65%:35%, and 90%:10% of the individual values of IC50-96 h Cu:IC50-96 h Cr, respectively (n=3). The stock solution was used to establish the concentrations. The metals were administered by adding 25 mL of the metal solution. The seed germination was recorded every 24 h. The probit analysis was based on mixed test results to obtain each metal's IC50–96 h in a mixed exposure experiment.

To evaluate the effects of the mixed metals, i.e., additive, synergism, or antagonism, the combination index (CI) was calculated using the following classic isobologram combination index: $\frac{Am}{Ai} + \frac{Bm}{Bi}$, where Am is the IC50–96 h value of Cu in mixed test, Bm is IC50–96 h value of Cr in mixed, Ai is IC50–96 h value of individual Cu, and Bi is IC50–96 h value of individual Cr.

The values of CI are defined as synergism (CI<1), additive (CI=1), or antagonism (CI>1). If the values were plotted graphically, then the CI point position on the above additive line represents an antagonistic effect, the under-additive line being synergistic (Markovsky *et al.*, 2014).

2.5. Seedling growth

Seedling growth is a bioindicator of metal exposure on a plant's survival ability (Houshmandfar and Moraghebi, 2018). Seed growth is evaluated by counting the number of leaves and measuring seedling height at hour 96 for each treatment.

3. Results and Discussion

In the range-finding test, inhibition of Cu on the seed germination of *V. radiata* L. was the highest at a concentration of 100 mg/L, above 50% inhibition, reaching 85% (Figure 1). Therefore, the concentration range used for the actual test ranged from 10 to 100 mg/L. Based on the results, concentrations of Cu for the definitive test were 15, 23, 34, 51, 76, and 90 mg/L. For Cr, there was no inhibition effect of 50% up to 100 mg/L (Figure 2). It indicated that Cr produced less toxic effect than at similar concentrations of Cu. Therefore, for the definitive test, the Cr concentrations were increased to 900 mg/L, i.e., 150, 230, 340, 510, 760, and 900 mg/L.

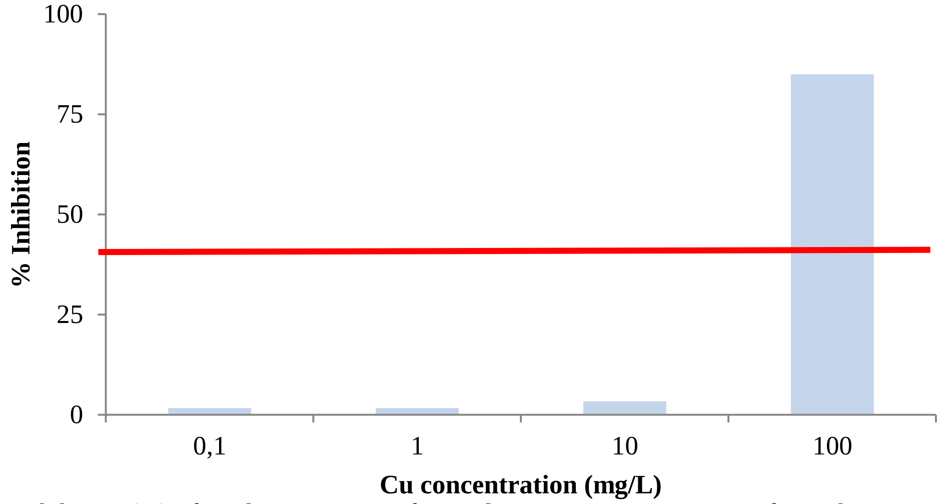

Figure 1. Inhibition (%) of seed germination of *V. radiata* upon exposure to Cu for 96 h in a range-finding test.

Exposure of *V. radiata* to individual Cu or Cr (in the definitive test) and mixed Cu and Cr resulted in the inhibition of seed germination (Table 1). For individual Cu and Cr, the inhibition tended to increase following the increased concentrations. However, concentrations of Cu up to 51 mg/L did not significantly affect seed germination (inhibition of seed germination below 20%). According to Iqbal *et al.* (2018), it might be due to its copper resistance. For Cr, a significant effect also occurred on the high concentration of Cr, i.e., 760 and 900 mg/L. Pokorska-Niewiada *et al.* (2018) reported similar patterns; inhibition of seed germination on vetch (*V. sativa*), rye (*S. cereale*), wheat (*T. aestivum*), barley (*H. vulgare*), oat (*A. sativa*), winter rape (*B. napus*), pea (*P. sativum*), and cress (*C. pratensis*) was also increased upon exposure to the increased Cu or Cr. Garba *et al.* (2021) also reported the seed germination inhibition on *Zea mays* L. upon exposure to Cu or Cr. Significant inhibition was only observed in the high concentrations of the metals. In this study, the lowest and highest Cu concentrations for mixed experiments showed significant seed germination inhibition (min. 20%), indicating that Cu is more toxic than Cr in *V. radiata* (Table 1). A concentration ratio of 50%:50% indicates that this interaction may have an antagonistic effect.

According to Ruhling *et al*. (1987), Singh *et al.* (2013), and Srivastava *et al.* (2021), in terms of seed germination, the ability of a plant to withstand/tolerate metal toxicity depends upon its capacity to sustain germination in a metal-contaminated environment. Plants show a significant variation in their sensitivity/tolerance to metal in the environment, and metal toxicity depends upon the plant species and the source. According to Kranner and Colville (2011), seed germination depends on the taxon, population, and metal concentrations. Baderna *et al.* (2015) and Houshmandfar and Moraghebi (2018) stated that the decrease in seed germination of plants can be attributed to the accelerated breakdown of stored food materials in seeds by applying a heavy metal mixture.

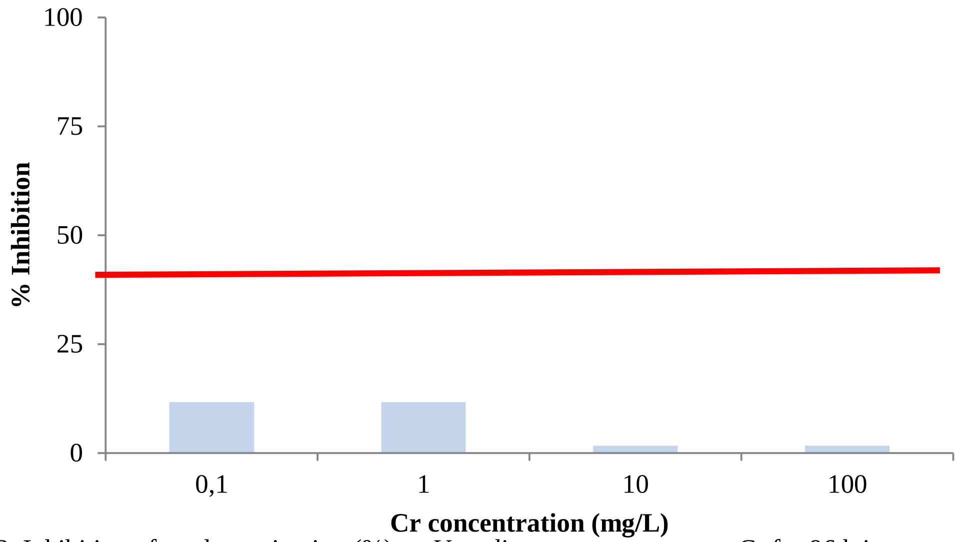

Figure 2. Inhibition of seed germination (%) on *V. radiata* upon exposure to Cr for 96 h in a range-finding test.

Regression and correlation analysis indicated strong relationships between concentrations of individual Cu or Cr and the inhibition of seed germination (Figures 3 and 4); the values of R^2 for Cu and Cr were close to 1.00, i.e., 0.96 and 0.97, respectively.

The acute toxicity test showed that individual IC50-96 h of Cu on *V. radiata* was lower than that of Cd (Table 2), the toxicity of Cu on this species being higher than Cr. The study by Pokorska-Niewiada *et al.* (2018) stated that IC50-96 h of Cu on cress (*C. pratensis*), winter rape (*B. napus*), and vetch (*V. sativa*) was also lower than Cr. The opposite results were observed on rye (*S. cereale*), wheat (*T. aestivum*), barley (*H. vulgare*), and oat (*A. sativa*). However, in the mixture exposure experiment, the toxicity of both metals was lower than in the individual exposure experiment (Table 2). The combination index of Cu:Cr for *V. radiata* resulted in a value of 1, indicating that the two metals additive interacted.

Table 1. Inhibition of seed germination (%) on *V. radiata* upon exposure to individual Cu or Cr in the definitive test and mixed Cu and Cr

Metal	Concentration	Inhibition (%)
Cu	0 mg/L	0.0
	15 mg/L	1.7
	23 mg/L	1.7
	34 mg/L	5.0
	51 mg/L	13.4
	76 mg/L	33.4
	90 mg/L	71.7
Cr	0 mg/L	0.0
	150 mg/L	3.4
	230 mg/L	5.0
	340 mg/L	6,7
	510 mg/L	19,4
	760 mg/L	76,7
	900 mg/L	93,4
Cu:Cr	0% IC50-96 h:0% IC50- 96 h	0.0
	10% IC50-96 h:90% IC50- 96 h	23,4
	35% IC50-96 h:65% IC50- 96 h	18,4
	50% IC50-96 h:50% IC50- 96 h	8,4
	65% IC50-96 h:35% IC50- 96 h	13,4
	90% IC50-96 h:10% IC50- 96 h	43,4

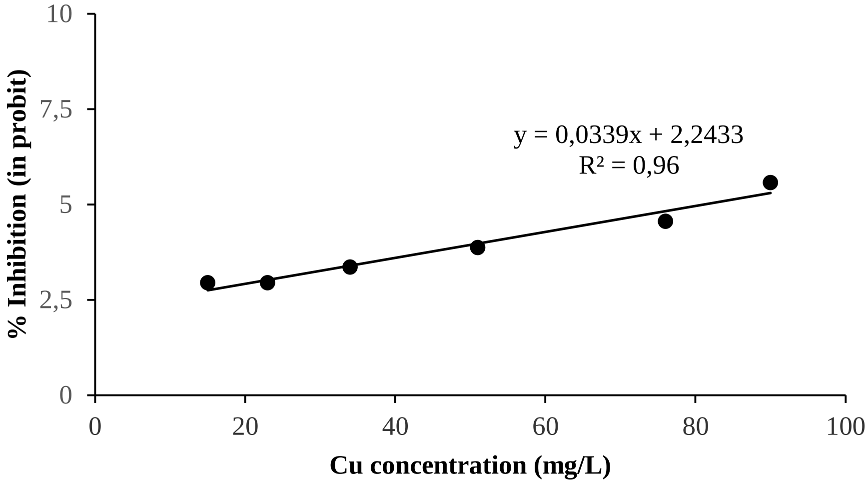

Figure 3. Regression and correlation analysis between Cu concentration and seed germination inhibition after 96 h of exposure.

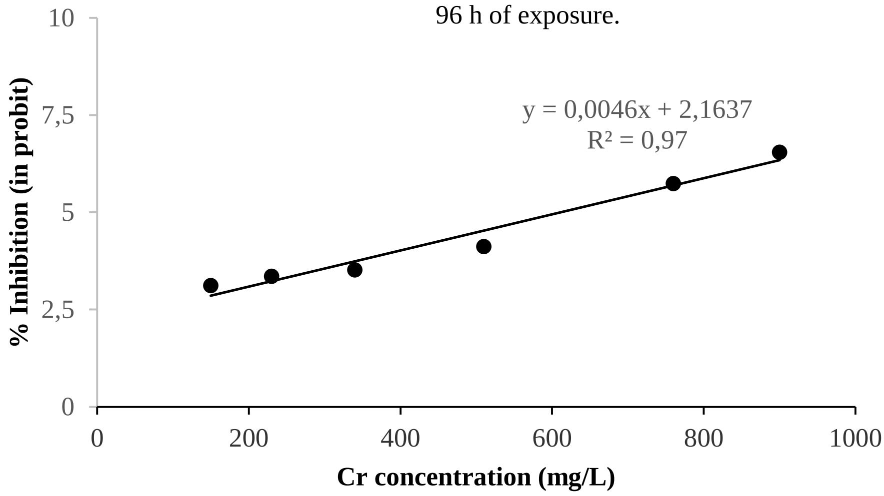

Figure 4. Regression and correlation analysis between Cr concentration and seed germination inhibition after 96 h of exposure.

Table 2. IC50-96 h of individual Cu or Cr, and Cu and Cr in a mixed exposure experiment against *V. radiata*.

Metal	Exposure to *V. radiata*	IC50-96 h (mg/L)
Cu	Individual	81.3
	Mixture with Cr	50.8
Cr	Individual	616.6
	Mixture with Cu	252.0

Exposure to Cu and Cr individually and in the mixture affected seed germination. The seeds will develop into a shoot in the control after forming the primary root (Table 3). The final step of seed germination is when the first embryonic leaf or the cotyledon appears. Gradually, tiny leaves sprout from the shoot ends. Conversely, upon exposure to the metals, the seeds only formed a primary root. Even the coat of some seeds cannot open. Metals can inhibit root growth; further, they directly decrease nutrients, water absorption, and transportation to aerial plant parts, inhibiting shoot growth (Singh et al., 2013). Houshmandfar and Moraghebi (2018) stated that germination and seedling establishment are vulnerable stages in the plant life cycle; the reduced seedling growth upon exposure to the metals might be a result of the reduction in meristematic cells present in this region and some enzymes contained in the cotyledons and endosperm. Proteomics studies have revealed that Cu toxicity inhibits seed germination by down-regulating the activity of alpha-amylase or enolase. It has been reported to affect overall metabolism, water uptake, and failure to mobilize reserve food (Gautam *et al.*, 2016; Rahmaniar and Kamil, 2015; Sethy and Ghosh, 2013; Verma *et al.*, 2011).

Table 3. Number of leaves and height average of *V. radiata* upon exposure to individual Cu or Cr in the definitive test and mixed Cu and Cr

Metal	**Concentration**	**Number of Leaves**	**Height Average (cm)**
Cu	0 mg/L	5	5.3
	15 mg/L	0	0
	23 mg/L	0	0
	34 mg/L	0	0
	51 mg/L	0	0
	76 mg/L	0	0
	90 mg/L	0	0
Cr	0 mg/L	5	5.3
	150 mg/L	0	0
	230 mg/L	0	0
	340 mg/L	0	0
	510 mg/L	0	0
	760 mg/L	0	0
	900 mg/L	0	0
Cu:Cr	0% IC50-96 h:0% IC50- 96 h	5	5.6
	10% IC50-96 h:90% IC50- 96 h	0	0
	35% IC50-96 h:65% IC50- 96 h	0	0
	50% IC50-96 h:50% IC50- 96 h	0	0
	65% IC50-96 h:35% IC50- 96 h	0	0
	90% IC50-96 h:10% IC50- 96 h	0	0

4. Conclusion

Exposure of *V. radiata* to Cu and Cr individually and in a mixture produces a toxic effect on seed germination and seedling growth. The effects of the metals on *V. radiata* were dependent on metal concentration. The toxicity of individual Cu on *V. radiata* was relatively higher than Cr. Conversely, the mixture exposure test resulted in lowered values of IC50-96 h compared to individual IC50-96 h. The combination index of Cu:Cr for *V. radiata* resulted in a value of 1, indicating that the two metals additive interacted. This finding can provide a better understanding of metal toxicity individually and

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in a mixture and be used as a reference in policy-making, mainly to prevent and reduce metal pollution in soil and aquatic ecosystems.

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