

Toxicity Test of Copper (Cu) and Chromium (Cr) on the Growth of Mung Bean (*Vigna radiata* L.) Sprouts

^{1*}Sutan Nur Chamida Tri Astuti & ²Andhika Puspito Nugroho

^{1,2}Ecology and Conservation Laboratory, Faculty of Biology, Gadjah Mada University

¹sutannurchamida@mail.ugm.ac.id*; ²andhika_pn@ugm.ac.id

*corresponding author

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ABSTRACT

Industries that are growing rapidly contribute a big role in environmental pollution, one of which is the production of heavy metal waste. One example of heavy metals that are toxic when present in large quantities is Copper (Cu) and Chromium (Cr). The purpose of this experiment is to test the toxicity of the two types of heavy metals Cu and Cr against the seed growth of the *Vigna radiata* L. plant, find out the type of metal that gives the highest percentage of inhibition to the growth of *V. radiata* L., and find out the relationship between the two types of heavy metals. The method to determine the level of toxicity and the relationship between the two metals can be known by toxicity tests which include preliminary tests, actual tests and mixed tests on the seeds of *V. radiata* L. while data analysis is carried out by linear regression and the data is presented in the form of tables and graphs. Preliminary test to determine the ideal range of toxicity concentrations to be continued with the actual test with the addition of Cu and Cr metal toxins to the seeds of *V. radiata* L. The results of the study on the actual test showed that the IC₅₀ of Cu metal was 127.4 ppm while the IC₅₀ of Cr metal was 615.23 ppm with Cu metal having a more significant negative impact than Cr. In the mixed test, it showed that Cu metal IC₅₀ was 247.5 ppm while Cr metal IC₅₀ was 579.85 ppm with the highest inhibition value at Cu:Cr concentration of 90:10. The toxicant relationship of Cu and Cr based on the additive index is the additive (value S = 0).

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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 the existence of various industries built for the benefit of human life ultimately has a great 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 itself is actually 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 substances or energy such as sound, heat or light (Ghosh, 2014).

One of the pollutants or factory waste that needs to be watched out for is heavy metals. Pollution by heavy metals occurs a lot in developing countries. This is due to the uncontrolled level of pollution caused by the growth of the industry and the increased use of fuel oil. Heavy metals are non-



biodegradable and persistent. Exposure to heavy metal contamination present even in low concentrations in the environment can be harmful to exposed organisms (Jain, 2004 in Priadi *et al.*, 2014).

The test organism used in this experiment was *V. radiata* L. *V. radiata* L. or commonly called green beans is a legume plant (Fabaceae) that is drought-resistant, so it has great 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, that is, they are available in abundant quantities in nature, are easy to get, have fast growth and are affordable.

Cu metal (Cuprum) commonly called copper is used, which is a type of metal that is easily corroded. Pure copper is soft and reddish-orange in color. This metal is commonly used in electrical wiring components, building materials and industrial machinery. Cu heavy metal is a type of essential metal for plants. Cu metal is useful for the growth of plant tissues, especially leaves as a place of photosynthesis. In general, Cu metal levels in plant tissues range from 5-25 ppm. But at high concentrations Cu metal can be toxic to plants and carcinogenic if it accumulates in the human body (Liestianty *et al.*, 2014).

While Cr (Chromium) metal is a type of heavy metal that is corrosion resistant, gray in color and commonly found in the form of chromate ore, Cr is useful in life in the industrial field, including in the production of alloys (alloys), electroplating and pigments. Cr metal is needed essentially for humans and animals, as in helping insulin regulate blood sugar. However, when exposed to large amounts of Cr metal and continuously, it can cause dermatitis, asthma, cancer, and death (Singh & Tripathi, 2007).

The presence of large amounts of Cu and Cr heavy metals in the soil can affect the plants growing in the area. Such metals can accumulate in plants. In the case of Cr metal contained in the soil, it can suddenly change shape from Cr (III) which is not toxic to Cr (VI) which is highly toxic, carcinogenic and mutagenic. Chrome, both Cr (VI) and Cr (III) can fit into plants, animals and humans. This metal can be absorbed by plants, and these plants when given to livestock can also damage their organs because the metal can be deposited in the liver, kidneys, meat and other body tissues (Triatmojo, 2001). So it is necessary to conduct further research on the level of toxicity and tolerance limits of plants to these two metals.

The purpose of this experiment is to test the toxicity of the two types of heavy metals Cu and Cr against the seed growth of the *Vigna radiata* L. plant, find out the type of metal that gives the highest percentage of inhibition to the growth of *V. radiata* L., and find out the relationship between the two types of heavy metals.

2. Methods

2.1. Tools

The tools used in this study are: 1) measuring pipettes of 1 mL and 10 mL which function to move the solution with a maximum volume of 1 mL and 10 mL accurately; 2) filler pipette that serves to suck the solution installed at the base of the measuring pipette; 3) a drip pipette that serves to move the solution from one container to another drop by drop; 4) 50 mL, 100 mL, and 250 mL beakers that serve as a container used to stir and mix a stock solution; 5) a 10 mL measuring cup that functions as a measuring instrument with a maximum volume of 10 mL to make a new solution from the stock laruan; 6) erlenmeyer 50 mL which serves to hold a solution with a certain concentration but not to measure volume; 7) petri dish a glass container that serves to grow green bean seeds; 8) cotton wool that serves as a medium for the germination of green bean seeds absorbing metal solutions; 9) a funnel that serves to insert the solution from one container to another; 10) a ruler that serves to measure the length of green bean sprouts; 11) a label that serves to mark a petri dish or tray; 12) a tray that serves to put a petri dish; 13) glass stock bottles that serve to store Cu and Cr metal stock solutions; 14) a glass jar of small size that serves to soak green bean seeds; 15) a data table that serves to write down the data obtained during the study; and 16) *tissue* that serves to dry tools that are still wet, a practical cleaning solution to carry.

2.2. Materials

The materials used in this study are: 1) a 100 ppm Cu stock solution which functions as a stock solution which is then diluted at the time of preliminary test and actual test; 2) Cu metal crystals that serve as stock solutions during mixed tests; 3) a 1000 ppm Cr stock solution that serves as a stock solution which is then diluted at the time of preliminary test, actual test, and mixed test; 4) mung bean

seeds that function as ingredients that are tested for growth by being exposed to metal solutions of Cu and or Cr (treatment) and akuades (control); 5) akuades that serve to dilute metal stock solutions or as a solution for the growth of green bean seeds (control); and 6) 70% alcohol that serves to clean the petri dish before use at the time of testing.

2.3. Greenbean seed preparation

Green bean seeds are laid out in a glass jar of small size and soaked with water for 10 minutes. After that, good seeds are selected for each test (preliminary test, actual test, and mixed test), seeds that are not intact, only the seed coat remains, or floating ones are not selected for testing. Furthermore, 20 good seeds are placed in each petri dish during the preliminary test, actual test, or mixed test.

2.4. Preliminary test

Preliminary tests with varying concentrations of each were 0.1 ppm; 1 ppm; 10 ppm; 100 ppm (4 treatments) for Cu metal and 0.1 ppm; 1 ppm; 10 ppm; and 100 ppm (4 treatments) for Cr metal as well as control. The dilution of Cu solution from a stock solution of 100 ppm uses the formula while the $M1 \times V1 = M2 \times V2$ dilution of the Cr solution of a stock solution of 1000 ppm uses the formula. $M1 \times V1 = M2 \times V2$ Each of these treatments was repeated 3 times. Green bean seeds of 20 grains each are laid into 27 petri dishes filled with cotton. A solution of Cu and Cr of 30 mL each is watered on green bean seeds already laid on a petri dish. Preliminary tests were carried out for 96 hours and observed the number of germinated seeds every 24 hours. The number of seeds germinated at the 96th hour was used for the determination of the actual test.

2.5. Test as true as possible

Based on the number of germinated seeds, in the concentration range that inhibits the germination of mung bean seeds 50% preliminary test up to 96 hours, a new concentration is created for the actual test. The new concentration for the actual test in this study is that the concentration variation is 15 ppm each; 23 ppm; 34 ppm; 51 ppm; 76 ppm; 90 ppm (6 treatments) for Cu metal and 150 ppm; 230 ppm; 340 ppm; 510 ppm; 760 ppm; 900 ppm (6 treatments) for Cr metal as well as control. Each of these treatments was repeated 3 times. Green bean seeds of 20 grains each are laid into 39 petri dishes filled with cotton. Then a solution of Cu and Cr of 30 mL each is watered on the seeds of green beans that have been placed on a petri dish.

The dilution of Cu solution from a stock solution of 100 ppm uses the formula $M1 \times V1 = M2 \times V2$ while the dilution of the Cr solution of a stock solution of 1000 ppm uses the formula $M1 \times V1 = M2 \times V2$. Preliminary tests were carried out for 96 hours and observed the number of germinated seeds, the length of sprouts, and the number of leaves every 24 hours. The number of seeds germinated at the 96th hour was calculated IC50 and then the concentration obtained was used for the determination of the mixture test.

2.6. Mixed test

Based on the results of IC50 Cu and IC50 Cr in the actual test, a stock solution with the same concentration was made for each metal. Then a ratio of the volume of Cu:Cr in the mixed test was made with a total volume of 30 mL. The ratio of Cu:Cr volume in mixed tests in this study was 10:90; 35:65; 50:50; 65:35; and 90:10 (5 treatments) and controls. Each of these treatments was repeated 3 times. Green bean seeds of 20 grains each are laid into 18 petri dishes containing cotton. Then the ratio of the finished Cu:Cr solution mixture is watered on the seeds of green beans that have been placed on a petri dish.

Cu stock solution in mixed test is prepared from metal crystals using the formula because the concentration of Cu solution in mixed test exceeds Cu stock solution (more than 100 ppm) while $X = \frac{BM \text{ senyawa}}{BM \text{ logam}} \times [larutan]$ dilution of Cr solution from stock solution 1000 ppm uses the formula $M1 \times V1 = M2 \times V2$. Cu:Cr ratio of 100% of 30 mL of watered solution. For example, in the ratio Cu: Cr 10: 90 then 10% of 30 mL is Cu and 90 % of 30 mL is Cr so that the mixture Cu: Cr 10: 90 is 3 mL Cu plus 27 mL Cr. Preliminary tests were carried out for 96 hours and observed the number of germinated seeds, the length of sprouts, and the number of leaves every 24 hours. The number of seeds germinated at the 96th hour is calculated IC50 and then calculated using the additive index formula, which is $S = \frac{Am}{Ai} + \frac{Bm}{Bi}$ to determine whether the mixture of the two metals is antagonistic, additive, or synergistic.

2.7. Data Analysis

Tabulation of data is performed in excel which is then analyzed using linear regression. Based on the y equation obtained on linear regression, the IC50 is sought for the preliminary test and the actual test while in the mixed test, the IC50 is sought to be calculated by the additive index formula, namely

$S = \frac{Am}{Ai} + \frac{Bm}{Bi}$. The final data obtained are presented in the form of tables and figures which are then analyzed descriptively qualitatively.

3. Results and Discussion

The research that has been carried out includes preliminary tests, actual tests and mixed tests. In the preliminary test, researchers usually use concentration variations with distances following a long geometric series, as in this study, namely 0 ppm; 0.1 ppm; 1 ppm; 10 ppm; and 100ppm. Based on the preliminary tests that have been carried out, the following results are obtained (Figure 1 and 2):

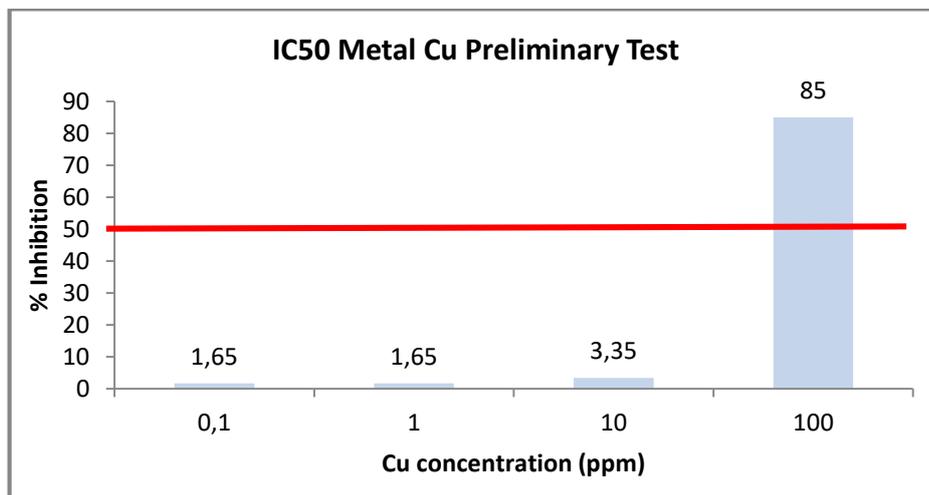


Fig. 1. Inhibition (%) Cu on preliminary test

Based on the picture above, it is known that the inhibition of seed germination of *V. radiata* L. is the highest at a concentration of 100 ppm, reaching 85%. So the concentration range used for the actual test is 10 ppm for the lower threshold and 100 ppm for the upper threshold. The concentration range used for the actual test of Cu metal is 15 ppm; 23 ppm; 34 ppm; 51 ppm; 76 ppm; and 90 ppm.

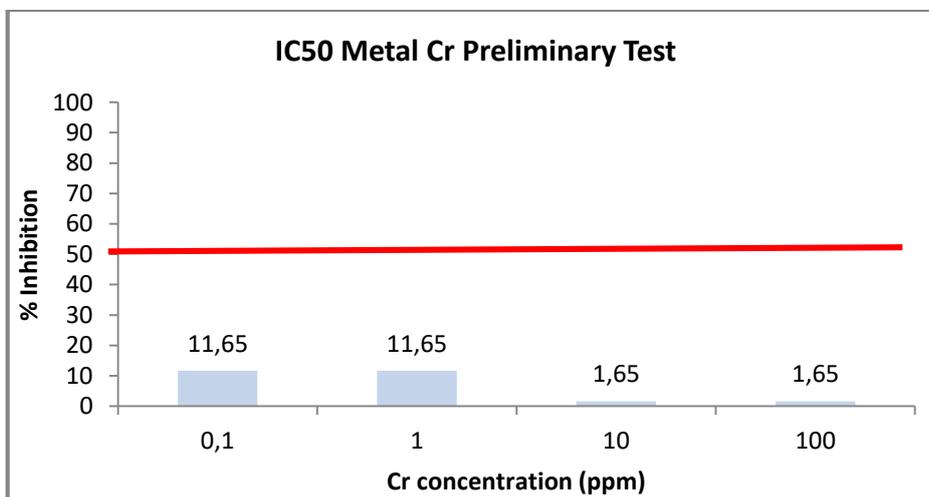


Fig. 2. Inhibition (%) Cr on preliminary test

The figure above shows that the inhibition value of *V. radiata* L. seed germination is highest at a concentration of 0.1 ppm and 1 ppm with a peer inhibition percentage of 11.65%. Of all the concentrations, none of them had an inhibition value exceeding 50% (IC50) so that the concentration used in the actual test was increased to exceed 100 ppm. Therefore the concentration range used for the actual test is 100 ppm profit lower threshold and 1000 ppm for the upper threshold. The concentration range used for the actual test of Cr metal is 150 ppm; 230 ppm; 340 ppm; 510 ppm; 760 ppm; and 900 ppm.

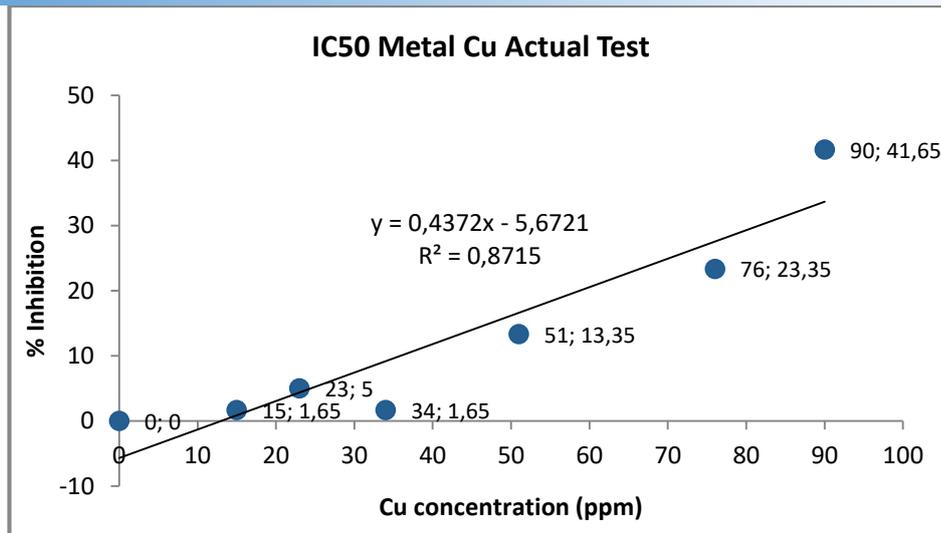


Fig. 3. Concentration-response percentage of inhibition of Cu on the actual test

In the Fig 3. above, it can be seen that the percentage value of inhibition has not reached 50% in the germination of *V. radiata* L seeds, at a concentration of 90 ppm new inhibitions of 41.65%. Therefore, a linear regression analysis was carried out and it was found that the equation $Y = 0.4372X - 5.6721$ where Y inserted was 50%. The IC50 result obtained for Cu metal is a concentration of 127.4 ppm so this concentration is used in mixed tests.

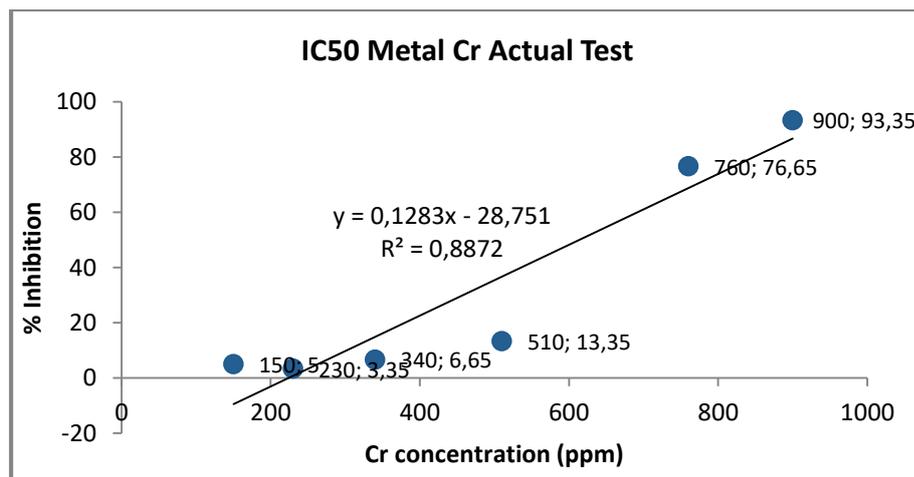


Fig. 4. Concentration-response percentage of Cr inhibition on the actual test

In the Fig. 4, it can be seen that the value of the percentage of inhibition of 50% germination of *V. radiata* L. seeds in the range of 510 ppm (inhibition 13.35 %) and 760 ppm (inhibition 76.65%). To see what the concentration of Cr in inhibiting 50% (IC50) was still carried out a linear regression analysis and found the equation $Y = 0.1283X - 28.751$ where Y is inserted is 50%. The IC50 result obtained for Cr metal is a concentration of 615.23 ppm so this concentration is used in mixed tests.

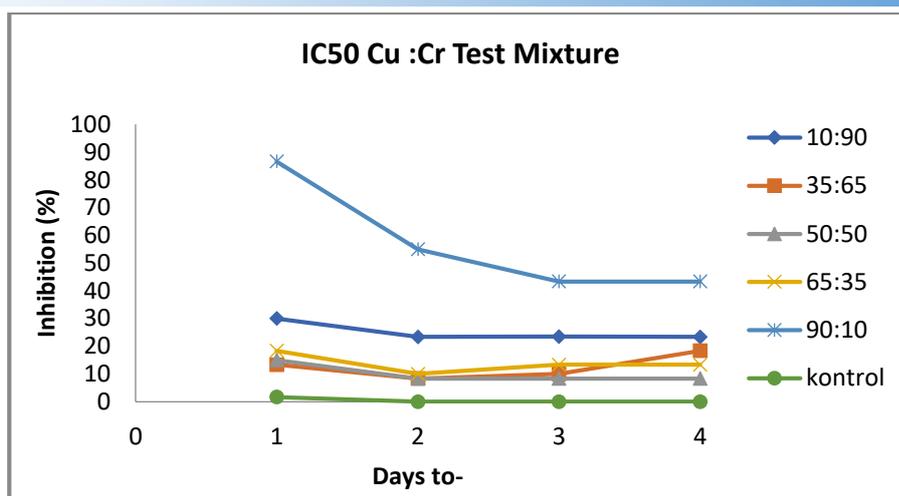


Fig. 5. Percentage of inhibition of Cu and Cr metal mixtures during 4 days of exposure

The Fig. 5, shows that the percentage of inhibition decreased further until the 4th day of exposure with both metal mixtures. The highest percentage of inhibition occurs in Cu:Cr metal mixtures in a ratio of 90:10 can be seen on day 1 to day 4. In both metal mixtures Cu:Cr 90:10 this from the first day (24 hours) already showed the highest inhibition so that the germination of *V. radiata* L. seeds decreased further on day 4. The graph also shows significantly declining results as indicated by the most sharply declining charts.

Based on the results of the IC50 data analysis, the mixture test of Cu and Cr obtained an additive index with a value of 0 which indicates that the mixture between Cu and Cr is classified as an additive, not an antagonist and synergistic. The value of each IC50 in the actual test and mixed test can be seen in the Table 1 below:

Table 1. Actual test IC50 value, mixed test IC50, and additive index

Metal	Ai/Bi (IC50 test actually)	Am/Bm (IC50 mixed test)	$S = \frac{Am}{Ai} + \frac{Bm}{Bi}$ (Additive index)	Conclusion
Cu (A)	127.4 ppm	247.5 ppm	0	Additives
Cr (B)	615.23 ppm	579.85 ppm		

Meanwhile, the number of leaves and the high average on the last day of observation (96 hours) in the actual test and mixed test can be seen in the Table 2 below:

Table 2. Number of leaves and mean height of Cu and Cr treatment on actual test

Treatment		Cu		Cr	
Cu	Cr	Number of leaves	Average High (cm)	Number of leaves	Average High (cm)
15	150	0	0	0	0
23	230	0	0	0	0
34	340	0	0	0	0
51	510	0	0	0	0
76	760	0	0	0	0
90	900	0	0	0	0
Control	Control	5	5,33	5	5,33

From the table above, it can be seen that the application of Cu and Cr metals in the test actually inhibits leaf formation and inhibits growth so that in the variation in the volume of the solution there

is no number of sprouted leaves or germinated seeds. The leaves only grew in the control treatment with an average number of leaves of 5 strands. While the seeds only germinated with the average plant height on the control treat was with an average of 5.33 cm.

Table 3. Number of leaves and mean height of treatment Cu:Cr on mixed test

Treatment (Cu:Cr)	Number of leaves	Average Height (mm)
10:90	0	0
35:65	0	0
50:50	0	0
65:35	0	0
90:10	0	0
Control	31,33	5,6

From the table above, it can be seen that the application of a mixture of Cu and Cr metals in the mixed test inhibits leaf formation and inhibits growth so that in the variation in the volume of the solution there is no number of sprouted leaves or germinated seeds. The leaves only grew in the control treatment with an average number of leaves of 31.33 strands. While seeds only germinate with the average plant height at the control treatment is with an average of 5.6 cm.

This study aims to test the toxicity of two types of heavy metals Cu and Cr to the growth of seeds of the *Vigna radiata* L. plant, determine the type of metal that provides the highest percentage of inhibition in the growth of *V. radiata* L., and find out the relationship between the two types of heavy metals. Results are expressed in IC50 96 hours. The study was conducted for 96 hours or 4 days for each preliminary test, actual test, and mixed test because there were no supporting data from the previous study so that if only 24 hours of observation were made, the results obtained were invalid or underrepresented.

V. radiata L. is a type of plant that belongs to the group of seeded plants (Spermatophyta). Based on the research conducted, in the preliminary test, results were obtained that the seeds of *V. radiata* L. experienced the highest germination inhibition (inhibition) of 85% when given Cu solution with a concentration of 100 ppm and 1.65% when given a Cr solution with a concentration of 100 ppm. So that a concentration of 100 ppm is not an effective concentration in inhibition of sprouts for Cr metal but is effective in inhibition of sprouts for heavy metal Cu, because in the administration of Cu with a concentration of 100 ppm can already inhibit more than 50%. Therefore, the concentrations used for the actual test of Cr metal are 150 ppm, 230 ppm, 340 ppm, 510 ppm, 760 ppm, and 900 ppm while Cu metal is 15 ppm, 23 ppm, 34 ppm, 51 ppm, 76 ppm, and 90 ppm. This is in accordance with the theory that Cu in high concentrations can be toxic to seeded plants (Ruhling *et al.*, 1987).

In the actual test on the exposure of Cu and Cr metals, leaves were not formed and the seeds did not germinate. *V. radiata* L. seeds given Cr solution experienced the highest inhibition at a concentration of 900 ppm with a percentage of inhibition in seed germination of 93.35%. Meanwhile, *V. radiata* L. seeds given Cu solution experienced the highest inhibition at a concentration of 90 ppm with a percentage of inhibition in seed germination of 41.65%. At such concentrations there is no appearance of leaves, seeds do not germinate, even the seed coat cannot open because of the high toxicity of the metal. This is in accordance with the theory that heavy metals have toxic properties that in high concentrations can affect the physiological processes of organisms, including plants (Rahmaniar and Kamil, 2015), where heavy metal solutions are absorbed into the seeds, then damage the parts in them, inhibit the activation of hydrolytic enzymes so that seed germination becomes slow, as well as the unavailability of food reserves so that seeds do not have nutrients to continue to grow to form leaves and increase in height.

At lower concentrations Cu metal is more toxic than Cr metal, this can be seen from the IC50 obtained. The seeds of *Vigna radiata* L. are stunted in growth by 50% (IC50) at a concentration of 127.4 ppm for Cu metal and 615.23 ppm for Cr. Metal Cr. This proves that with a lower concentration of Cu it can already inhibit the growth of *V. radiata* L seeds because Cu metal is an essential metal for plants. Essential metals will actually become more toxic if their levels exceed the threshold. This is in accordance with the theory that heavy metals can be classified into two types, namely: (i) Essential heavy metals are certain amounts of metals that are needed by organisms, but these metals can cause toxic effects if in excessive quantities. Examples are: Zn, Cu, Fe, Co, Mn, and others; (ii)

Non-essential heavy metals are metals whose presence in the body is still unknown, even toxic. Examples are: Hg, Cd, Pb, Cr, and others (Irhamni *et al.*, 2017). In addition, heavy metal Cu is a heavy metal that is more toxic than heavy metal Cr. In accordance with the classification of heavy metals according to the Ministry of State and Population and Environment (1990), namely metals that are classified as high toxic are Cu, Hg, Cd, Pb, and Zn; moderately toxic Cr, Ni, and Co; as well as low toxic are Mn and Fe.

V. radiata L. sprouts that are only exposed to Cu or Cr solution give a fairly high inhibitory reaction, while when exposed for four days with a mixed toxicant solution of Cu and Cr the inhibition actually varies from day to day. The highest inhibition occurred at a concentration of Cu:Cr in a ratio of 90:10 followed by inhibition of Cu:Cr in a ratio of 10:90 (Figure 5). It can be concluded that this concentration is a concentration that can greatly inhibit seed germination because the 90% ratio in both Cu and Cr both gives the highest inhibition results. The difference is that 90% of Cu metal is more toxic because the inhibition from the first day of the study has been the highest compared to other treatments.

It can be seen from the chart in Figure 5 where on the first day of inhibition it has been 90% (86.65%). In accordance with the classification of heavy metals according to the Ministry of State and Population and Environment (1990), metals that are classified as high toxic are Cu, Hg, Cd, Pb, and Zn; moderately toxic Cr, Ni, and Co; as well as low toxic are Mn and Fe. Cu metal is classified as more toxic than Cr metal so with a higher concentration of Cu, the percentage of inhibition is also greater. It can be assumed that both toxins at a balanced concentration in one mixture both become less effective for plants, while when one toxicant is more dominant than the other the effect is more noticeable.

Based on the additive index that has been carried out in this study, both mixtures of Cu and Cr are additives. The additive index is useful for classifying mixed interactions as additive, synergistic or antagonistic. The additive here means that the toxicity of the mixture is equal to the amount of toxicity of each component. The synergistic effect occurs when the experimental toxicity of the mixture is greater than the amount of individual toxicity. In contrast, the antagonistic effect occurs when the experimental toxicity of the mixture is less than that estimated by additivity. Understanding mixed interactions can prevent excessive toxicity and can be used to predict mixed toxicity in environmental regulation and management.

4. Conclusion

Based on the results and discussions that have been presented, it can be concluded that both types of metals have a negative effect on the germination of *V. radiata* L. and Cu metal has a more significant negative impact than Cr. Research results on the actual test show that IC₅₀ Cu metal is 127.4 ppm while IC₅₀ Cr metal is 615.23 ppm with Cu metal having a more significant negative impact than Cr. On the mixed test showed IC₅₀ metal Cu is 247.5 ppm while IC₅₀ metal Cr is 579.85 ppm with the highest inhibition value at Cu:Cr concentration of 90:10. The toxicant relationship of Cu and Cr based on the additive index is additive (value $S = 0$).

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