Phytoremediation of heavy metal copper (Cu\(^{2+}\)) by sunflower (Helianthus annuus l.)

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Abstract. A study in microcosmic condition has been carried out to determine the effectiveness of Helianthus annuus as a hyperaccumulator plant for heavy metal, Copper (Cu\(^{2+}\)), that exposed in the soil. Artificial pollutants containing Copper (Cu\(^{2+}\)) 0, 60, 120, 180 ppm are exposed to uncontaminated soil. The 12-weeks old H. annuus seedling were grown in Cu\(^{2+}\) contaminated soil, with variations of absorption time 3, 6, and 9 weeks. Analysis of Cu\(^{2+}\) concentration on soil and H. annuus (root, stem, leaf) was analysed by Atomic Absorbtion Spectrometry (AAS). H. annuus are capable for Cu\(^{2+}\) removal, and the highest removal of Cu\(^{2+}\) is 85.56%, the highest metal accumulation/bioconcentration factor (BCF) is 0.99 occurred at roots with 9 weeks of exposure time and the highest translocation factor (TF) is 0.71. This highest removal is five times better than absorption by stems and leaves. The results concluded, the use of H. annuus for phytoextraction of heavy metals Cu\(^{2+}\) in contaminated soil can be an alternative to the absorption of heavy metal Cu\(^{2+}\) with low concentration metals which is generally very difficult to do in physical-chemical removal.

Keywords: bioconcentration factor, helianthus annuus, hyperaccumulator, phytoremediation, translocation factor

1. Introduction
Phytoremediation is a method to recover contaminated soil by heavy metals. Phytoremediation through phytoextraction process is a biotechnology that utilizing plants to clean the environment [1]. This technology is suitable to clean up the environment contaminated by toxic metals in-situ [2]. Unlike organic compounds, metal compounds could not be degraded. Therefore, this phytoremediation method aims to remove or eliminate pollutant components.

Phytoextraction is one of the phytoremediation’s strategies. The terms of phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept while phytoextraction is a specific cleanup technology [1]. Phytoextraction involves the absorption of contaminants by roots followed by translocation and accumulation in the aerial parts [3]. This method using hyperaccumulation plants that have a natural ability to extract metals from the soil, translocating metals from roots to shoots, and accumulate and tolerate high metal concentrations within the plant tissues [4].

The application of this technology required a type of heavy metals hyperaccumulator plant. Hyperaccumulator plants are hypertolerant, able to absorb heavy metals from high absorption soil solution, and have the ability of translocation from root to stem and to leaf. Hyperaccumulator does not refer to plants that have familial relations, but both have the ability to live in metal-contaminated soil and are able to accumulate high amounts of metals in their organs far beyond the accumulation rate at other plants without experiencing phytotoxic effects.
The process of phytoextraction occurs with the mechanism as shown in Figure 1. In process 1 showing the absorption of the metal by the root (root uptake), process 2 showing translocation of heavy metals from the roots to other plant parts, and process 3 showing localization of metals in plant parts. Research on a microcosmic scale aims to exclude Cu\(^{2+}\) heavy metals exposed in soil by Sunflower (Helianthus annuus L.) as hyperaccumulators.

![Figure 1. Mechanism of Phytoextraction [3].](image)

2. Research Method

2.1. Planting H. annuus

H. annuus is grown on sterile (heavy metal-free) soil with polybag container. The soil media used for each plant is 5 kg. H. annuus was grown to 12-weeks old, to be further used as a hyperaccumulator plant in this study.

2.2. Contamination of Cu\(^{2+}\) on Soil

Solution of Cu\(^{2+}\) concentration 60, 120, 180 ppm was exposed to sterile soil. The research design can be seen in Table 1.

| Cu\(^{2+}\) concentration (ppm) | 3-weeks | 6-weeks | 9-weeks |
|-------------------------------|---------|---------|---------|
| 0                             | A₀ R₁   | A₀ R₂   | A₀ R₃   |
| 5                             | A₁ R₁   | A₁ R₂   | A₁ R₃   |
| 10                            | A₂ R₁   | A₂ R₂   | A₂ R₃   |
| 100                           | A₃ R₁   | A₃ R₂   | A₃ R₃   |

2.3. Atomic Absorption Spectrophotometer (AAS)

This method is used to analyse Cu\(^{2+}\) absorbed by roots, stems, leaves, and soil, at week 3, 6, and 9.

2.4. Removal Percentage

Determination of metal percentage that removed can be calculated by the following equation:

\[
\% \text{ removal} = \left[ \frac{C₀ - Cₑ}{C₀} \right] \times 100 \%
\]  

\(C₀ = \) the concentration of the initial metal solution (mg/L),  
\(Cₑ = \) final concentration in the soil (mg/L)
2.5. Bioconcentration Factor (BCF)
The value of BCF is the effectiveness of the plants ability to accumulate metals in their tissues. BCF can be calculated by the following equation [5]:

\[
BCF = \frac{\text{Cu}^{2+} \text{ concentration on plant} \left( \frac{\text{mg}}{\text{L}} \right)}{\text{Cu}^{2+} \text{ concentration on soil} \left( \frac{\text{mg}}{\text{L}} \right)}
\]  

(2)

2.6. Translocation Factor (TF)
TF is the value of Cu2+ translocation from soil to a particular part of the plant. TF can be calculated by the following equation [6]:

\[
\text{TF} = \frac{\text{Cu}^{2+} \text{ concentration on the part of the plant} \left( \frac{\text{mg}}{\text{L}} \right)}{\text{Cu}^{2+} \text{ concentration on soil/root} \left( \frac{\text{mg}}{\text{L}} \right)}
\]

(3)

3. Results and Discussion

3.1. Cu\(^{2+}\) Removal from Soil
In Table 2 show that Cu\(^{2+}\) concentration in soil is decreasing every week. This is happening due to the absorption process by \textit{H. annuus}, resulting in the translocation Cu\(^{2+}\) into the plant’s organ.

\textbf{Table 2. Cu}\(^{2+}\) concentration in soil. 

| Initial Concentration Cu\(^{2+}\) in soil (ppm) | Concentration of Cu\(^{2+}\) (ppm) | 3 weeks | 6 weeks | 9 weeks |
|-----------------------------------------------|-----------------------------------|---------|---------|---------|
| 0                                            | 0                                 | 0       | 0       | 0       |
| 60                                           | 21.61                             | 21.57   | 21.46   |
| 120                                          | 21.28                             | 21.12   | 21.01   |
| 180                                          | 27.37                             | 26.62   | 26.00   |

Based on Table 2 percentage of Cu\(^{2+}\) absorption could be measured in soil (Table 3). The highest elimination percentage (reaching 85.56%) happen at the concentration of 180 ppm with the exposure time Cu\(^{2+}\) for 9 weeks. It means that the heavy metal availability in the soil affects the capability of \textit{H. annuus} to absorb the copper (Cu\(^{2+}\)).

\textbf{Table 3.} % Removal of Cu\(^{2+}\) from soil. 

| Initial Concentration Cu\(^{2+}\) in soil (ppm) | Absorption of Cu\(^{2+}\) | 3-weeks  | 6-weeks  |
|-----------------------------------------------|---------------------------|----------|----------|
| 0                                            |                           | 0.00%    | 0.00%    |
| 60                                           |                           | 63.98%   | 64.05%   |
| 120                                          |                           | 82.27%   | 82.40%   |
| 180                                          |                           | 84.79%   | 85.21%   |

Based on Figure 2 the longer the exposure time, the higher percentage of removal (R\(^2\) = 0.9).
3.2. Cu$^{2+}$ Absorption by H. annuus

The measurement of Cu$^{2+}$ absorption was done with H. annuus, to make sure that the absorption is really happening with the plant (Table 4). Copper (Cu$^{2+}$) that goes in to the plant’s tissue, cannot be degraded, however it will be accumulated in the plant tissues [7]. In this research, absorption that happened at concentration of contaminated soil 60 ppm, not yet reached in stem or in leaf.

Table 4. Cu$^{2+}$ Concentration on H. annuus.

| Initial Concentration of Cu$^{2+}$ in H. annuus | Part of Flower | Concentration of Cu$^{2+}$ in H. annuus |
|-----------------------------------------------|----------------|--------------------------------------|
|                                               | 3-weeks       | 6-weeks     | 9-weeks     |
| 60 ppm                                        | roots          | 34.79       | 35.68       | 42.36       |
|                                               | shoots         | 0.00        | 0.00        | 0.00        |
|                                               | leaves         | 0.00        | 0.00        | 0.00        |
| 120 ppm                                       | roots          | 57.59       | 58.40       | 70.78       |
|                                               | shoots         | 22.36       | 22.50       | 22.85       |
|                                               | leaves         | 18.51       | 19.11       | 19.28       |
| 180 ppm                                       | roots          | 79.65       | 89.28       | 128.84      |
|                                               | shoots         | 24.74       | 25.17       | 25.37       |
|                                               | leaves         | 23.47       | 23.63       | 24.09       |

Referring to Table 5, the highest Cu$^{2+}$ content is at the root followed by shoots and leaves of H. annuus. This is in accordance with previous studies where it was found that the metal content was accumulate more in the root than the shoots and leaves because the roots were the first to utilize nutrient uptake and the metal content [8]. Roots of the plants act as a barrier against heavy metal translocation, and it causes the concentration in the roots are higher.

After knowing the concentration of Cu$^{2+}$ absorption with H. annuus then the calculation could be done with BCF to know the effectiveness of H. annuus to accumulate metals in the tissue (root, stem, leaf) (Table 5). The highest BCF is 8.1 obtain in the initial concentration of 50 ppm with absorption time of 9-weeks.
Table 5. *H. annuus* Bioconcentration Factor (BCF).

| Initial Cu$^{2+}$ Concentration in Soil | Bioconcentration Factor (BCF) |
|---------------------------------------|-------------------------------|
|                                       | 3-weeks | 6-weeks | 9-weeks |
| 60 ppm                                | 0.58    | 0.59    | 0.71    |
| 120 ppm                               | 0.82    | 0.83    | 0.94    |
| 180 ppm                               | 0.71    | 0.77    | 0.99    |

Compared to the previous research [9], the largest bioconcentration factor found in heavy metal absorption by *H. annuus* was 1.78 in the absorption of heavy metals Zn. The bioconcentration factor obtained is quite high due to the addition of compost and vermicompost that can increase the absorption of heavy metals in the study.

3.3. Translocation Factor (TF)

The translocation of Cu$^{2+}$ from soil to specific parts of *H. annuus* can be seen in Table 6. From the table it can be seen that the largest translocation factor is 0.71 at 6-weeks absorption. According with previous studies [9], translocation factors obtained greater than or equal to ($\geq$) 1 indicate that the plant includes hyperaccumulator plants and is able to perform phytoextraction.

Table 6. *H. annuus* Translocation Factors (TF).

| Initial Cu$^{2+}$ Concentration in Soil | Translocation Factors (Roots $\rightarrow$ Shoots and Leaves) |
|----------------------------------------|----------------------------------------------------------|
|                                        | 3-weeks | 6-weeks | 9-weeks |
| 60 ppm                                 | 0.00    | 0.00    | 0.00    |
| 120 ppm                                | 0.71    | 0.71    | 0.60    |
| 180 ppm                                | 0.61    | 0.55    | 0.38    |

However, that result not happened in this study, due to the heavy metals concentration of Cu$^{2+}$ tend to be low. The translocation factor is less than 1.0, does not mean that *H. annuus* is unable to absorb heavy metal Cu$^{2+}$ but at that concentration, translocation will run more slowly or take more than 9-weeks.

4. Conclusion

Phytoremediation by *H. annuus* as a hyperaccumulator has been shown to be effective in removing heavy metals from the soil. The removal efficiency of Cu$^{2+}$ by *H. annuus* reached 85.56% in the soil with Cu$^{2+}$ exposure concentrations 180 ppm for 9 weeks. Measurement of TF and BCF can be concluded that the highest accumulation of Cu$^{2+}$ occurs in the root, which is 5 times better compared to shoots and leaves.

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