Analysis of the copper removal kinetics of the Philippine giant bamboo (*Dendrocalamus asper*) in hydroponics

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**Abstract**

Copper is the third most utilized metal and is a versatile resource with multiple beneficial uses, but it may also become toxic to aquatic life in excess amount. Thus, there is a need to develop methods to reduce the copper contamination in the environment, particularly in bodies of water. Phytoremediation using *Dendrocalamus asper* may offer an environment-benign and potentially effective method for copper removal though its effectiveness may take several years to materialize for this technology to become cost-effective. By growing *D. asper* in synthesized contaminated water and analyzing the change in the copper content of the substrate via atomic absorption spectrophotometry, the removal was found to be optimal at 20 ppm Cu and pH 5. The rate of removal was found to have an order of 2.71 and a kinetic constant of 0.0013 ppm/Cu$^{1.71}$/day. With this, it may be possible to estimate the treatment length of phytoremediation given an initial level of copper contamination and a target concentration.

**1. Introduction**

Because of its malleability, electrical conductivity, and ability to form various alloys, copper has found widespread use in electronics, construction, as well as automotive industries; however, as the demand for copper increases, its polluting effects on the environment become more apparent [1]. Once the main copper source of the British Commonwealth, the untreated soils of the Britannia Beach Mine became a daily source of 600 kg of copper leachate to nearby bodies of water. Consequently, their copper concentrations rose to 20 ppm, which lead to the inhabitability of multiple rivers [2]. Increased copper concentrations in soils pose a threat to local biodiversity and land usability, and the inability to properly treat copper-contaminated soil also puts nearby bodies of water at risk [3]. Groundwater may become unsafe for drinking, and surface waters may become uninhabitable [4].

In Bulacan, Philippines, industries involved in electronics, metal-working and jewelry are forced to discharge copper-containing wastes to the Meycauayan-Marilao-Obando River System (MMORS). In one of the test points near a dump site, copper concentrations of up to 3.14 ppm were detected [5, 6, 7]. This could have been caused by surface runoff during the rainy season. Sediments tested exceeded the threshold element level and were possibly toxic to the milkfish being reared in the area through aquaculture [6, 7]. As such, various techniques and technologies are being further developed in order to restore the copper concentrations back to habitable levels [8]. One effective method to address this concern is through a technology known as bioremediation, which involves the use of living entities to clean environmental contaminants.

Phytoremediation is one form of bioremediation that uses plants in removing pollutants such as heavy metals from a water/soil matrix. It was the recommended solution to treating the MMORS [6]. Although the mechanism for copper removal differs among plant species, the treatment is proven to be cheaper, less invasive and requires less energy/labor intensive compared to conventional in-situ and ex-situ methods such as pump and treat, thermal desorption, encapsulation, soil washing, electrokinetic removal, and chemical immobilization [8]. However, phytoremediation is often limited by two factors: the ability of plant species to resist the toxic effects of high copper concentrations, and the long treatment period involved [9]. Even in the presence of a viable plant species, the inability to accurately estimate the length and effectiveness of...
of treatment is detrimental to field applications of this cost-efficient and non-energy intensive technology.

Bamboos were used in order to address the issue of low biomass and slow growth experienced in order to address copper hyperaccumulator [10]. They have been shown to be highly resistant to heavy metals. Of the 21 endemic bamboo species in the Philippines, Dendrocalamus asper (Schult.) Backer is highly recommended for riverbank rehabilitation due to its fast growth and large culm formations [11]. D. asper has also been used in countries such as Kenya and has been shown to be highly capable of tolerating and removing chromium from contaminated Tannery sites [12]. Other giant bamboo species such as the “Moso Bamboo”, Phyllostachys pubescens have also been observed to resist elevated copper concentrations (up to 300 ppm) [10].

An earlier study performed on D. asper determined the effects of varied initial copper concentration, pH and water hardness on copper uptake [13]. Some of the data used in that study will be presented here in order to justify the pH that would be used in these experiments, but the focus of this study is the kinetics behind copper uptake using D. asper.

With the use of empirical kinetic models, the rate of copper removal using D. asper may be estimated based on the desired final pollutant concentration. Though few and far between, there have been studies aimed at predicting the kinetics of pollutant removal through phytoremediation. These involve the use of nth order kinetic models [14], Michaelis-Menten models [15, 16] as well as various sorption isotherms and equations [17, 18]. As the Michaelis-Menten model is more commonly applied to organic pollutants, and adsorption kinetics on macrophytes with large surface area, the nth order model seems most applicable for establishing the kinetics of copper uptake using D. asper [15, 16, 17, 18, 19]. Although very powerful, a general kinetic model does not necessarily describe the mechanism involved in phytoremediation. It provides an understanding of the overall process, but does not often yield the elementary reactions involved in the process.

The objective of this study is to analyze the rate of copper removal from synthesized contaminated water using Philippine giant bamboo, Dendrocalamus asper. This involves an evaluation of the effect of varying initial copper concentration and pH on the amount of copper, followed by a kinetic analysis of the effect of copper concentration at any time, t, on the copper removal rate.

2. Materials and methods

2.1. Hydroponic setup

Hydroponics was used in order to limit the effect of organisms found in soils. The hydroponic solutions used in this study were made by adding 5 mL of liquid fertilizer (Simple Nutrient Addition Program - SNAP) to 3 L of distilled water. The addition of liquid fertilizer is needed for the growth of plants in hydroponic systems in the absence of liquid circulation [20]. CuSO₄·5H₂O and NaOH crystals were then added in order to simulate increased copper concentrations at varying pH. Each of these hydroponic solutions housed one six-month-old D. asper propague. Although there was no written record on the exact date when each of the propagules germinated, it was assured that they were of the same age based on the height, number of leaves as well as nodes formed (morphological parameters used by the growers). The SNAP solution was bought from the Soil Science Department of the University of the Philippines Los Baños (UPLB) while the propagules were sourced from the Department of Environmental and Natural Resources - Ecosystems Research and Development Bureau (DENR - ERDB) in Los Baños, Laguna, Philippines.

Each hydroponic jar of 23.5 cm diameter contained 3-L of hydroponic solution. This liquid level started at around 7 cm, but throughout the testing period, the liquid volume of these hydroponic jars decreased by around 75 mL/day.

2.2. Effect of initial copper concentration & pH on copper uptake

In order to determine the initial copper concentration and pH to use in the kinetic studies, plants were grown for in 3.14, 11.57 & 20 ppm Cu at pH 5, 6 & 7. The copper levels were chosen as previously discussed. Plant uptake of heavy metals is improved at slightly acidic conditions [21, 22]. Additionally, copper pollution due to acid mine drainage tend to be acidic [2]. After 16 days, liquid aliquots were taken from each hydroponic jar, and the plant biomass was dried under the sun for three days. Each plant was then sorted into its leaves, branches, roots and shoots then dried at 65 °C for three days before weighing. The sum of the weights of the individual parts was used as the total dried plant biomass.

The design of experiments was based on Response Surface Methodology with 4 corner points (co-extremes), 6 center points, 8 axial points. Instead of using a full factorial method with 3 replications which would require 27 trials. This design decreased that number to only 18 plants. Two-way analysis of variance (ANOVA) was then used to determine the significance of initial copper concentration & pH on copper removal with a 95% confidence interval. Calculations were performed using Design-Expert® Software Version 10. A corresponding equation relating the various factors was also produced.

2.3. Calculation of copper uptake

The copper uptake was measured by filtering the extracted aliquots then vaporizing them in an Atomic Absorption Spectrophotometer (Shimadzu AA-6300). The corresponding calibration curve was con-
structed using 0.05, 1, 5, 10 & 20 ppm Cu solutions made from copper sulfate pentahydrate with the proportional amount of liquid fertilizer. With the final liquid concentration and dry plant mass known, the copper removed was calculated through a mass balance. In Eq. (1), the amount of copper removed, \( q \), was taken to be the difference between the original \( (C_{0}V_{0}) \) and the final \( (CV) \) mass of copper in the system on a per dry plant mass basis. A summary of the steps performed in shown in Figure 1 below.

\[
q = \frac{C_{0}V_{0} - CV}{m}
\]  

(1)

2.4. Effect of time on copper uptake

Using the data gathered from the first experiment, 9 bamboo propagules were grown in pH 5 solutions initially containing 3.14, 11.57 & 20 ppm copper (3 bamboo per concentration). This new batch of 9 bamboos were grown in 3-L hydroponic solutions using the same hydroponic jars. For each concentration, an additional pH 5 3-L hydroponic solution was stored in a hydroponic jar to serve as a control. Multiple samples were taken from each hydroponic jar as opposed to the previous experiment wherein only one sample was taken at the end of 16 days.

As the concentration of the liquid changed over time, liquid aliquots were taken from each hydroponic jar after 9 days of experimentation. This was repeated at days 16, 25 & 34. At the end of 34 days, the plants were then dried under the sun for three days, separated by hand into its individual parts then dried in an oven at 65 °C for three days prior to being weighed on an analytical scale. The liquid samples were filtered and vaporized in the AAS to test their concentration against the calibration standards (see Figure 1). Analysis of the three control setups showed no significant change over 34 days.

3. Results and discussion

3.1. Determination of initial copper concentration and pH

All the treated solutions showed a decrease in copper concentration. The hydroponic solutions initially at 3.14 ppm copper decreased to 1.66–2.75 ppm; those that were initially at 11.57 ppm decreased to 3.08–7.34 ppm, while those that were initially at 20 ppm decreased to 5.03–19.15 ppm. After accounting for the change in liquid volume, as well as the mass of each plant, the calculated copper removal was summarized in Table 1 below.

Using Design-Expert, the data was forced into a quadratic equation containing three types of terms the independent factors, their interaction term, as well as the square of each independent term. After hierarchical removal of insignificant terms (p-value > 0.05), it was determined that both initial copper concentration, pH and their interaction were significant to copper removal using D. asper (see Table 2).

After determining which terms were significant to copper removal, they were fit into a linear equation using the Design Expert software, thus generating Eq. (2). Taking into consideration the fact that difference exists even among individual plants of the same species, an adjusted \( R^{2} \) of 0.71 shows that the mathematical model adequately represents the effects of initial copper concentration and pH on copper removal after 16 days of treatment. More importantly, Eq. (2) shows that increased initial copper concentration and pH have inherently positive effects on copper removal; however, when both are present in higher levels, copper removal is hindered.

\[
q = -0.115 + 0.046C_{0} + 0.017pH - 0.0045C_{0}pH
\]  

(2)

Figures 2 & 3 present the graphical representation of the copper removal of the plants at varying initial copper concentration and pH. Comparing this to Eq. (2), it can be observed that there is a general increase in the copper removed. Although, the effect of pH is not as evident until the highest copper concentration was reached. At an initial copper concentration equal to 20 ppm, the interaction term in Eq. (2) may be combined with the pH term leading to a coefficient of -0.073, meaning at high copper concentrations, increasing the pH drastically decreases the copper uptake. From Figure 3, it can also be observed that a lower pH of 5 generally provides an increased copper uptake than a neutral pH of 7. In the range of 3.14–3.78 ppm Cu, the copper uptake at pH 7 is very slightly greater than that at pH 5. However, for most of the tested range, the copper uptake at pH 5 is better than that at pH 7. As such, a pH of 5 will be used in order to analyze the kinetics of copper uptake by D. asper.

In order for the plant to absorb copper, the copper ions must first bind with the active sites of the plant roots known as chelators or ligands [23]. These ligands may be proteins such as COPT1 which bind with copper to allow entry through the cellular membrane of the roots, or enzymes such as ATPase that utilize an ATP pump to achieve the same effect. Copper chaperones then transport the copper ions from the root cytosol to the necessary sites of protein/enzyme synthesis [24].

The first step then for copper removal is for the copper ions to form bonds with the ligands present within the plants. According to the collision theory, the more copper present in solution, the more likely it is for these randomly moving ions to come in contact with the ligands with the right orientation and energy [25]. Thus, the higher the initial copper concentration, the more copper is removed from the system.

One study showed that increased copper concentrations in the soil lead to increased copper absorbed by the bamboo. Moreover, the copper absorption peaked when the soil concentration was 600 ppm, where the treatment almost killed the plant [10]. Another showed that bulk of the copper absorbed by Narihira bamboo were stored as cuprous sulfides in various sulfur-containing inorganic compounds found in the plant roots [26].

It was observed that pH mainly affects copper removal through its interaction with copper concentration. At higher copper concentrations, a decreased pH was needed in order to ensure the favoring of

### Table 1. Copper uptake of D. asper at varied initial copper concentration and pH.

| Plant # | Initial Copper Concentration (ppm) | pH | Q (mg Cu/g dried biomass) |
|---------|-----------------------------------|----|--------------------------|
| 1       | 3.14                              | 5  | 0.0267                   |
| 2       | 3.14                              | 6  | 0.0356                   |
| 3       | 3.14                              | 7  | 0.0423                   |
| 4       | 3.14                              | 7  | 0.0334                   |
| 5       | 11.57                             | 5  | 0.5504                   |
| 6       | 11.57                             | 5  | 0.1889                   |
| 7       | 11.57                             | 7  | 0.1653                   |
| 8       | 11.57                             | 7  | 0.1799                   |
| 9       | 11.57                             | 6  | 0.2051                   |
| 10      | 11.57                             | 6  | 0.3082                   |
| 11      | 11.57                             | 6  | 0.2437                   |
| 12      | 11.57                             | 6  | 0.1949                   |
| 13      | 11.57                             | 6  | 0.2409                   |
| 14      | 11.57                             | 6  | 0.2049                   |
| 15      | 20                                | 5  | 0.7596                   |
| 16      | 20                                | 6  | 0.3410                   |
| 17      | 20                                | 6  | 0.2765                   |
| 18      | 20                                | 7  | 0.3197                   |

### Table 2. Factors significant to copper removal using D. asper.

| Source       | p-value | Prob > F |
|--------------|---------|----------|
| Initial Cu   | 0.0003  |          |
| pH           | 0.0010  |          |
| Interaction  | 0.0025  |          |
bioavailable copper. In acidic conditions, copper is more likely to exist as free ions which are predicted to more easily bind with biotic ligands [27].

3.2. The kinetic model for copper removal

Similar results have been found by other researchers. Lettuce, tomato and onion grown in slightly acidic soils (pH 5.4–6.7) absorbed significantly more copper than those in neutral soils (pH 7.0–7.6) [28]. Experiments in sunflowers also show increased heavy metal absorption at lower pH. However, below pH 5, the copper absorption was significantly reduced; it was theorized that hydrogen protons could compete with copper ions for binding sites [28]. From an overall mass balance of a batch system, the rate of decrease in the mass of copper in the system is directly proportional to the volume of the solution as well as its concentration raised to the power of $n$. This is then the general model for an $n$th order kinetic equation (see Eq. (3)).

$$\frac{d(CV)}{dt} = -kC^nV$$

By rearranging Eq. (1) and representing the volume of the liquid, $V$, as a function of time, $t$, it is possible to reduce Eq. (3) into an equation that contains only copper removal, $q$, as well as the kinetic constants, $k$ & $n$ (see Eq. (4)). Further integration and simplification then lead to Eq. (5).

$$\frac{d(CV_m - mq)}{dt} = k \left( \frac{C_mV_m - mq}{V_m - 0.747t} \right)^n (V_m - 0.747t)$$

$$q = \frac{C_mV_m - \left( \frac{V_m - 0.747t}{k} \right)^{1-n} \left( \frac{V_m - 0.747t}{k} \right)^n + \left( \frac{C_mV_m}{k} \right)^{1-n}}{m}$$

Excel Solver was then used to iteratively calculate the kinetic constants $k$ & $n$ by minimizing the sum of squared errors between the predicted values and the experimentally determined values. An adjusted $r^2$ of 0.8058 was achieved with a 2.71th order equation and a $k$-value of 0.0013 ppm$^{-1.71}$ day$^{-1}$. As seen in Figure 4, although the model captures the trend of the remediation, it is unable to accurately determine the copper removal of the bamboo at a specific point in time.

For systems with very large volumes of water, Eqs. (6) & (7) may be used to estimate the decrease of copper concentration with time. By inputting the initial copper concentration, $C_0$, as well as the length of remediation, $t$, it is possible to estimate the copper concentration of the volume of water after $t$ days.

When the initial copper concentration of the liquid is 3.14 ppm, one year of remediation is expected to decrease this value to 1.03 ppm. In the same span of time, a copper concentration of 20 ppm is predicted to decrease to 1.13 ppm. It may be observed that the rate of remediation relies heavily on the copper concentration of the water. This is because the order of the equation is high ($n = 2.71$). Consequently, the model phytoremediation is most effective when the copper concentrations are high, as long as D. asper is able to resist the phytotoxic effects.

$$\frac{dC}{dt} = -0.0013C^{2.71}$$

$$C = (0.0022r + C_o^{-1.71})^{-0.585}$$

At decreased copper concentrations, the copper concentration decreases very slowly. Decreasing the concentration of copper from 0.03 to 0.02 ppm (for class C waters) may take around 500 years. As such, the model suggests utilizing phytoremediation for rivers with very high concentrations of copper, then switching to another method in order to reach habitable levels. Realistically, the mechanism for copper removal may vary greatly between the tested concentrations (above 3.14 ppm) and the predicted 0.02 ppm range, so the model should be used with caution especially when veering from the tested range of 3.14–20 ppm.
Another weakness of the model is that as time increases, the effect of high initial concentrations quickly decreases. The model predicts that whether the initial concentration of the liquid is 20 or 100 ppm, D. asper would be able to decrease the copper concentration down to the 1.13 ppm range within a year of remediation. The model only accounted for the contaminant uptake of the plant and not the possible phytotoxic effects causing variations in the uptake rate. Copper absorption in bamboos has been shown to be most effective in elevated concentrations, however, accompanying adverse effects such as variation in thylakoid membrane, and inhibited enzymatic activities may likely lead to hindered remediation above threshold concentrations [9].

4. Conclusion and recommendations

4.1. Conclusion

The pH and heavy metal concentration of contaminated water greatly affects the rate at which copper is removed from the system. Increased copper concentrations yield higher copper removal rates especially at acidic pH. Maximum copper removal occurred at 20 ppm Cu and pH 5. Kinetic analysis showed that copper removal using D. asper followed a 2.71st order kinetic equation with a kinetic constant of 0.0013 ppm^{-1.71} day^{-1}. Using this, predictions for the copper removal of larger bodies of water can be achieved.

4.2. Recommendations

In order to build upon the knowledge gathered by this study several possible directions for further study are likely recommended. Because the optimum copper uptake occurred at the highest tested initial copper concentration and lowest tested pH, it may be beneficial to test more extreme conditions. However, there is a limit for both of these, after which, copper uptake would cease to improve. It may be more beneficial to analyze the copper removal between the minimum tested value of 3.14 ppm and the environmental standards set by the Philippine government of 0.02 ppm Cu.

Performing similar experiments with a continuous flow scheme or in soil may allow for longer testing periods and provide more realistic scenarios and improved kinetic models. In-vivo testing may also allow studies involving the phytotoxic effects of increased copper levels in the plants over time. The mechanism of copper uptake should also be further explored as this may lead to novel methods for application of D. asper in the clean-up of contaminated water and other toxic metals.

Declarations

Author contribution statement

Jerwin Lawrence C. Go: Performed the experiments; Wrote the paper.
Cynthia F. Madrazo, Aileen H. Orbecido: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Ma. Ellenita G. de Castro: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Custer C. Deocaris: Conceived and designed the experiments; Wrote the paper.
Lawrence P. Belo: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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