Copper uptake potential of Philippine giant bamboo (*Dendrocalamus asper*) under varied initial copper concentration, water hardness and pH

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Abstract. Copper is a commonly used metal in construction, engineering, agriculture and water treatment. Consequently, increased copper concentrations resulting in adverse environmental effects is inevitable. Phytoremediation using *Dendrocalamus asper* or Philippine giant bamboo (PGB) is a viable option for treatment of copper-contaminated media, but their copper uptake potential remains largely unexplored. As such, the copper uptake of PGB was evaluated under varying environmental conditions, namely initial copper concentration, water hardness and pH.Six-month old propagules were planted in artificially contaminated water in order to determine the copper uptake after 16 days of treatment. Using a Box-Behnken design of experiment, it was found that both initial copper concentration and pH have significant and proportional effects on copper uptake. However, due to possible speciation and/or competition, the optimum copper uptake occurred at 20 ppm Cu and pH 5 (as opposed to 7). A mathematical equation, bearing an $R^2 = 0.7097$, was constructed as a possible model for copper uptake of PGB to understand when PGB phytoremediation is most effective. At copper concentrations lower than 3.81 ppm, higher pH is beneficial to copper uptake and vice versa. Overall, phytoremediation using D. asper or PGB is effective especially at low pH and elevated copper concentrations.

1 Introduction

The Philippines is home to 421 rivers. Due to various anthropogenic activities, 50 of these can no longer sustain aquatic life. One known cause for river death is elevated levels of heavy metals, specifically copper [1]. High copper concentrations in rivers and surrounding soil have been known to cause widespread death of aquatic organisms, and until these concentrations are returned to their natural state, it is not possible for aquatic life to survive in these bodies of water [2].

Many technologies such as dredging, filtration, adsorption, electrocoagulation and phytoremediation are available and continually being developed for the removal of copper from contaminated water [3-6].

Phytoremediation involves growing specific plants in an area to remove pollutants whether by absorption, stabilization or some other mechanism [7]. It is eco-friendly, passive, non-invasive, inexpensive, and not labor intensive. However, it is relatively slow and is often limited by the maximum pollutant concentration the local flora can survive in [8]. In theory, it should be applied to light or moderately polluted areas that do not require immediate results wherein acceptable levels are achieved within ten years [7]. In reality, however, this criterion does not take into account the environmental state of the contaminated site. Pre-existing conditions

such as the copper concentration, water hardness and pH of the river may have an effect on the copper uptake of phytoremediators, and hereby lies a gap in scientific knowledge.

In order to provide a clearer understanding of when phytoremediation will be effective, it will be assumed that the initial copper concentration, water hardness and pH affect the copper uptake of Philippine giant bamboo. This study will then verify this hypothesis.

2 Materials and methods

2.1 Philippine giant bamboo

Some plant species have been proven to sequester high concentrations of heavy metals in stems and leaves, but they often lack the biomass to make the amount of absorbed pollutant significant [9]. Philippine giant bamboo (*Dendrocalamus asper*) were used because of their sufficient biomass and fast growth rate of around 3 to 10 cm per day [10]. Six (6) month old propagules were sourced from the Department of Environmental and Natural Resources - Ecosystems Research and Development Bureau (DENR - ERDB).

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2.2 Hydroponic solutions

After sufficient washing, the bamboo plants were planted in 3-liter hydroponic solutions made from AR grade $CuSO_4 \cdot 5H_2O$, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, Simple Nutrient Addition Program (SNAP) solution, and distilled water. SNAP was jointly developed by the Bureau of Agricultural Research and the University of the Philippines Los Baños as a complete nutrient solution for hydroponic plant growth even without circulation of liquid. It contains calcium, nitrogen, potassium, phosphorus, magnesium, sulfur, iron, manganese, boron, zinc, copper, chlorine and molybdenum [11]. The hydroponic solutions also contained at most 3 mL of 2.4 M NaOH in order to increase the pH. Note that the hydrates of magnesium sulfate and calcium chloride were added in an equimolar manner in order to vary the water hardness, but the values are presented as $CaCO_3$.

2.3 Statistical design

Using Box-Behnken design, the hydroponic solutions contained varying initial copper concentration, water hardness and pH. Consequently, for each factor, a minimum, a maximum and a midpoint are necessary. The initial copper concentrations used were based off the highest reading in the Meycauayan-Marilao-Obando river system, a dead river with elevated copper concentrations (3.14 ppm [12]), that of the acid mine drainage found in the Britannia Beach Mine wherein copper leachate resulted in the death of multiple rivers (20 ppm [13]) and the midpoint (11.57 ppm). Those of water hardness were taken as 0 (nutrient solution only), 90 (moderate water hardness) and 180 ppm as $CaCO_3$ (limit between hard and very hard water) [14]. The pH levels were then chosen because metal uptake is maximized between pH 5 and 7 [15].

Copper uptake was used to quantify phytoremediation as this takes into account the change in liquid concentration and liquid volume, along with the mass of the plant. Simply put, it is the loss in mass of copper per unit plant. Mathematically, this is defined by **Eq. 1** below.

$$CU = \frac{C_o V_o - CV}{m} \qquad (1)$$

where *CU* is copper uptake at time, C_o is the initial concentration of copper in the liquid, V_o is the initial volume of the liquid, *C* is the final concentration of copper in the liquid, *V* is the final volume of the liquid, and *m* is the dried mass of the plant

After 16 days, aliquots were taken from the hydroponic solutions. These were then filtered to remove particles, before being vaporized in an Atomic Absorption Spectrophotometer (Shimadzu AA-6300). A calibration curve was then constructed using 0.05, 1, 5, 10 & 20 ppm Cu solutions made from copper sulfate pentahydrate with the proportional amount of SNAP solution. The spectrophotometer then yielded the final copper concentrations of the liquids. The plants were

then dried at 65°C for 3 days and then weighed on an analytical balance.

3 Results and discussion

3.1 Analysis of copper uptake

It was observed that all of the hydroponic 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 volume and the mass of each individual plant (the average is 108.55 $\pm$ 37.42 g), copper uptake was calculated using **Eq. 1** (see **Table 1**).

Table 1. Copper uptake of *D. asper* at varied initial copper concentration, water hardness and pH

Plant #	Run #	Initial Copper Concentration (ppm)	Water Hardness (ppm $CaCO_3$)	pH	Copper Uptake (mg Cu/g dried biomass)
1	13	3.14	0	6	0.0356
2	9	20	0	6	0.3410
3	5	3.14	180	6	0.0423
4	3	20	180	6	0.2765
5	18	3.14	90	5	0.0267
6	17	20	90	5	0.7596
7	1	3.14	90	7	0.0334
8	10	20	90	7	0.3197
9	4	11.57	0	5	0.5504
10	14	11.57	180	5	0.1889
11	11	11.57	0	7	0.1653
12	15	11.57	180	7	0.1799
13	7	11.57	90	6	0.2051
14	6	11.57	90	6	0.3082
15	8	11.57	90	6	0.2437
16	12	11.57	90	6	0.1949
17	16	11.57	90	6	0.2409
18	2	11.57	90	6	0.2049

These were then inputted into DesignExpert version 10 and forced into a quadratic equation containing three types of terms: (1) the independent terms: initial copper concentration (represented by A), water hardness (B) & pH (C), (2) their interaction terms: AB, AC & BC, and (3) the square of each independent term: A², B² & C². After eliminating insignificant terms (p-value greater than 0.05), it was determined that water hardness is insignificant, while initial copper concentration, pH and their interaction, are significant to the copper uptake of Philippine Giant Bamboo (see **Table 2**).

Table 2. ANOVA table for the significant factors

Source	Sum of Squares	df	Mean Square	F value	p-value Prob > F
Model	0.44	3	0.15	14.86	0.0001
A Initial [Cu]	0.30	1	0.30	31.05	<0.0001
C pH	0.082	1	0.082	8.36	0.0119
AC	0.049	1	0.049	5.01	0.042
Residual	0.14	14	9.805E-003		
Cor Total	0.57	17			

3.2 Empirical relationship between copper uptake, initial copper concentration and pH

Excluding water hardness, the statistical significance of the experimental factors confirms the hypothesis that environmental conditions such as initial copper concentration and pH may have an effect on the phytoremediation capabilities of Philippine giant bamboo. In order to better understand how these factors alter copper uptake, significant terms were fitted to an appropriate linear equation with an adjusted r² value of 0.7097. Considering that plants are involved and that there is inherent randomness between plants in absorbing copper, this shows that there is a strong relationship between copper uptake and the independent variables.

More specifically, **Eq. 2** shows that increased initial copper concentration and pH have inherently positive effects on copper uptake; however, when both are present in higher levels, copper uptake is hindered.

$$CU = -0.33 + 0.1025 * C_o + 0.0504 * pH - 0.0132 * C_o * pH \quad (2)$$

3.3 Effect of initial copper concentration on copper uptake

Of the various abiotic factors, the initial copper concentration of the solution seems to have the greatest effect on the copper uptake of the plant, and is directly related to the copper uptake of *D. asper*. This is in line with the findings of Chen et al. **[16]** where Moso bamboo, *Phyllostachys pubescens*, were planted both in pot and hydroponics. At the highest tested concentration of 600 mg/kg Cu, copper absorption was maximized – most of which was stored in root vacuoles. However, this was accompanied by adverse effects such as inhibited enzymatic activities and altered thylakoids to the extent that the treatment almost killed the plants.

One proposed model for this phenomenon is known as the intensity/capacity model. Capacity refers to the amount of copper present in the environment, while intensity refers that which is readily available for plant uptake. More specifically, this refers to copper that is dissolved in aqueous solution. The more copper in the liquid solution, the more likely it is for the bamboo to uptake copper **[17]**. However, it is possible that at higher pH, although the same amount of copper is present

(capacity), less copper is readily available as free ions in the liquid (intensity).

3.4 Effect of pH on copper uptake

It can be seen from **Eq. 2** that at copper concentrations higher than 3.81 ppm, the copper uptake actually decreases with increasing pH, while at concentrations below 3.81 ppm, it is actually beneficial to have a higher pH. This breakeven point may explain why findings of various researchers are not in agreement with one another i.e. Willscher et al. **[18]** found that copper uptake is decreased at lower pH due to competition of hydrogen ions with those of heavy metals and increased dissociation of H_2CO_3 on the cell walls, while Ginocchio et al. **[17]** found that plants grown in slightly acidic soils (pH 5.4-6.7) absorbed more copper than those grown in neutral soils (pH 7.0-7.6).

One such theory that attempts to explain this breakeven point is known as the Biotic Ligand Model (BLM). The BLM assumes that plant roots absorb nutrients through various receptors known as biotic ligands. These binding sites may be enzymes such as ATPase that utilizes an ATP pump to transport the copper through the cell membrane or proteins such as the COPT protein which binds with copper to allow entry to the cell. These are then transported from the cytosol to the copper-dependent enzymes and proteins by copper chaperones **[19]**.

The more free copper (Cu^{2+}) is present in the system, the more likely it is for copper to bind with these biotic ligands. At higher copper concentrations, the effect of increasing pH on copper ions may cause a decrease in readily available ions and ion mobility – possibly due to decreased solubility, increased attraction to organic complexes or both **[20]**. On the other hand, competition of H^+ and available mobile copper ions may be the reason why low pH becomes a hindrance to copper uptake at low copper concentrations **[21]**.

Within the limits provided ($3.14 < [Cu]_o < 20$; $5 < pH < 7$), an optimal copper uptake of 0.650 mg copper/g biomass is predicted to occur when the solution is initially at 20 ppm Cu and pH 5. Three additional runs were then performed at said conditions for verification of the optimum. These resulted in copper uptakes of 0.3987, 0.4395 and 0.5488 mg Cu/g plant. Along with the previously measured copper uptake of Run 17, the average copper uptake was determined to be 0.5366 mg Cu/g plant, which is within 82.55% of the predicted value of 0.650.

3.5 Effect of water hardness on copper uptake

With water hardness, there are conflicting evidences on the significance of its effect. Some researchers found that calcium and magnesium ions may compete with copper ions for binding sites. Thus, their presence may inhibit copper uptake and ameliorate toxic effects **[20]**. This was evident in experiments performed on aquatic ferns, rice and grapevine **[22-24]**. The effect of water hardness on the copper uptake of Philippine giant

bamboo, however, seems to be insignificant, similar to findings on microalgae and hornworts [25-26].

This may be because plants contain different copper-transporting biotic ligands. Some may bind only with cuprous ions, while others with cupric ions. Some ligands may be specific to copper, while others may be non-specific and allow competition [27]. It is possible that for *D. asper*, the biotic ligands for copper may bind with free copper ions and hydrogen protons, but not calcium and magnesium cations.

3.6 Strengths and limitations of the mathematical model

Another way of presenting **Eq. 2** is through a contour plot (see **Fig. 1**). Factor levels that produce a lower copper uptake are depicted in blue (upper left), while those that produce the higher copper uptake values are depicted in red (lower right). A more detailed gradient is provided on the left of the contour plot. It is clearly seen that higher initial copper concentration and lower pH results in an increase in copper uptake.
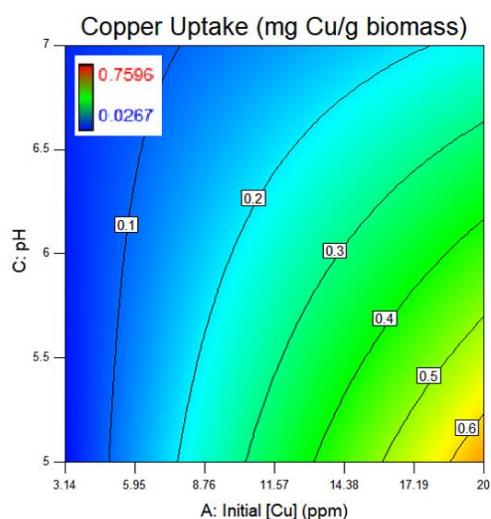

Figure 1. Contour plot of copper uptake with varying initial copper concentration and pH

Using either **Fig. 1** or **Eq. 2**, researchers may easily estimate the copper that will be absorbed by *D. asper* after 16 days of treatment. This may serve as a useful indicator for the effectiveness of phytoremediation given a certain level of copper contamination at a certain pH.

Although the verifying runs managed to achieve 82% of the predicted optimum copper uptake, care must be taken when analyzing data beyond the experimental limits. Further studies are recommended in order to expand the limits of this model in terms of both factor levels and time period. The runs were ended after 16 days as the volume of liquid decreased by 25%. In the future, studies under continuous flow pattern may help avoid this decrease in volume and allow for prolonged experimentation in order to analyze phytotoxic effects. Another limitation for this model is that it was performed in the presence of SNAP to allow for hydroponic testing. Trace metals found in SNAP may cause some differences between laboratory testing and

field implementation. Although this effect was minimized by adding a constant amount of SNAP to each run, the presence of other heavy metals have been shown to either encourage or hinder metal uptake in plants. As such, another limitation is that the model assumes that other pollutants are not present in great amounts [28-29].

As this paper validates the significance of initial copper concentration and pH on the phytoremediation capabilities of *D. asper*, these factors should be taken into account when performing phytoremediation experiments. Plants that were previously excluded from the list of effective phytoremediators may actually uptake a lot of pollutants at an ideal pH. Although optimum copper uptake for Philippine giant bamboo occurs at decreased pH and increased copper concentrations, this may not be true for other plants.

4 Conclusion

The Philippine giant bamboo, *D. asper*, is capable of removing high amounts of copper from artificially contaminated water (up to 0.650 mg Cu/g dried plant at 20 ppm Cu and pH 5), but its effectiveness is greatly affected by the initial copper concentration and pH. Individually, each of these factors promotes copper uptake at increased levels, but together they may compete and hinder remediation. Phytoremediation using *D. asper* is most effective at high copper concentrations and low pH. However, for lower copper concentrations (below 3.81), a neutral pH may be more ideal for *D. asper*. This phenomenon may have been caused by the presence of metal-specific biotic ligands, and it is recommended for future studies to explore this further.

Eq. 2 and **Fig. 1** may be used to make more detailed estimates on copper uptake based on the environmental conditions present in order to ascertain the relative effectiveness of a remediation treatment.

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