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Copper Phytoextraction Using *Phyllostachys pubescens*

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Abstract: The *Phyllostachys pubescens* macrophyte, known also as Moso Bamboo, was evaluated in laboratory conditions for its potential to renovate copper-polluted soil. Pot experiments were conducted to determine *Phyllostachys pubescens'* growth, tolerance and phytoextraction potential capacity to restore copper-contaminated soil in Mediterranean conditions. Data collected evidenced that the *Phyllostachys pubescens* evolution rate was 0.47 cm/day on average, with a 1.644 mm/d irrigation flow. Moso Bamboo tolerance was tested over a twelve-week irrigation period, while adding copper-polluted water. Copper removal from soil was 51.4% and the quantity of copper per gram of root/rhizome was equal to 1.18 mg Cu/g, while the amount of copper per gram of stem/leaves was 0.50 mg Cu/g, after 12 weeks. The conducted laboratory experiments show that environmental restoration using the phytoextraction technique, and using *Phyllostachys pubescens*, should be considered for the restoration of copper-contaminated soils.

Keywords: *Phyllostachys pubescens*; tolerance; contamination; heavy metals; phytoextraction

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1. Introduction

Increasing concentrations of copper (Cu) as an environmental pollutant are cause for concern [1]. Copper is one of the most used metals and has with multiple advantageous applications, but it may also become toxic to aquatic life in excess quantities [2]. Copper is found in the environment at minimal concentrations; its average concentrations in the earth's crust and in topsoils, as indicated by several authors, stay at the limits of 14–68 mg/kg. The presence of copper in soils can be attributed to both natural and anthropic sources [3].

Copper is an important micronutrient for plants, and it performs essential functions in biological and physiological activities, such as photosynthesis, protein synthesis, and respirational functions [4–6]. Overexposure to metals, and Cu specifically, can cause brain and kidney injury, liver cirrhosis, as well as stomach and intestinal disease [7–13]. Copper is an important element in the biological activities of plants, but if absorbed in large quantities, it can have toxic effects. High Cu absorption interrupts cellular functions, such as photosynthesis, respiration and uptake, disrupts transfer protein structures, deactivates enzymes, reduces plant growth, and threatens the survival of plants. Metal-contaminated soils can pose significant risks to biota and to human health. Therefore, it is of great importance to take measures to treat these soils [14–24].

Cu-polluted soils can be cleaned by many different techniques. In situ techniques are now favored because they are less costly and environmentally disturbing [25,26].

High concentrations of copper are very toxic to plants and for this reason, many species have adapted by developing an effective system to counteract toxicity; this is based

principally on the development of multiplexes [26]. Consequently, most Cu-tolerant plant species show a strategy typical of excluders, while the mechanism involved in the serious accretion of Cu by plants is very sporadic [26,27].

In this context, phytoextraction could be an appropriate alternative; this method uses plants with great metal-storing potential and their associated microbes to remove, reduce or separate toxic substances from the environment to renovate polluted sites [28–35]. Phytoextraction depends on the specific plant species, the concentration of contaminants in the soil, the duration of the treatment, and the environmental conditions. The efficacy of phytoextraction as a possible environmental cleaning solution is dependent on heavy metal bioavailability, soil characteristics, heavy metal speciation, and the plant's capacity to absorb metals and accumulate aboveground components [36]. Specifically, the efficient uptake and transport of contaminants by plant roots to the above-ground parts of the plant are critical for the success of phytoextraction. However, factors such as a low plant biomass, slow growth rates, and limited root depth can hinder phytoextraction. Additionally, the success of phytoextraction is largely dependent on the ability of the contaminated soil to support plant growth, and the availability of nutrients required for plant growth and metal uptake [31,33,36,37]. For the evaluation of phytoextraction potential, the bioconcentration factor index (BCF) and the translocation factor (TF) are fundamental [36]. The BCF is defined as the ratio of the concentration of a contaminant in a plant to its concentration in the soil or water, and it reflects the plant's ability to accumulate the contaminant. A high BCF indicates a high potential for phytoextraction, in which plants remove the contaminant from the soil or water and accumulate it in their tissues. The TF, on the other hand, is the ratio of the contaminant concentration in the shoot of the plant to its concentration in the root, and represents the plant's ability to translocate the contaminant from the roots to the aerial parts [38,39]. The absence of the secondary production of pollution, the low cost and the ability to restore the properties of the soils make phytoextraction the best alternative to the physico-chemical methods of remediation usually used [40–42]. Furthermore, through the management of biomass-containing contaminants, it is possible to increase the sustainability of the process by recovering metals, energy and soil. Technologies such as phytomining, pyrolysis or anaerobic digestion can be used to obtain bioenergy, timber, cellulose, biochar and even biofortified fodder products in essential trace elements [43–45].

Plant species have been chosen based on their ability to accumulate various metals in their organs, particularly copper (up to 1000 mg per kg in dry matter), as well as on their BCF, when these values are more than 1.0 [46–49]. Furthermore, for high-efficacy, fast-growing and high-biomass-emerging plants are necessary [50]. The plants proposed for Cu extraction must be tolerant to pollutants such as *Phyllostachys pubescens* (PP); this is also known as Moso bamboo, and is a fast-growing, large, and highly productive bamboo species. PP is widely cultivated for its edible shoots, timber, and various industrial uses; it also possesses a wide-ranging root apparatus and produces a high yield of biomass in the presence of elevated levels of heavy copper mass [20,21]. Macrophytes with great root biomass levels, such as PP, are suitable options for phytoextraction [23–51]. PP belongs to the grass family, or Graminae (Poaceae), which has the ability to survive even within the harshest soil and climatic environments, even though the use of phytoextraction can be limited by harsh climatic conditions [52]. It possesses a variety of benefits associated with other plants, such as fast growth, high biomass production and the vigorous capability to adapt to different conditions [20].

The features of PP, such as a large biomass formation and a high Cu tolerance, are valuable qualities for phytoextraction, but the efficacy of plants for the removal of Cu from soils and their antioxidative system responses under Cu stress are not well known [20,21].

PP biomass production can be very abundant, and PP has a good ability to adapt to various environments [52]. PP is characterized by a quick harvest time (4–5 years), and numerous applications, such in equipment, construction supplies, and ornaments. Further, it is recognized as the most favorable species for CO_2 capture [53,54] and has a high mean

aboveground CO_2 capture value (8.130 ± 2.150 Mg ha^{-1} y^{-1}) [55]. PP grows up to its full size within two months, with a 15.0 m elevation [56].

The aim of this study was to assess the ability of PP to tolerate copper contamination and to assess the phytoextraction potential of Cu-polluted soil in a Mediterranean climate. Laboratory experimentations were therefore used to determine PP phytoextraction and tolerance under Cu stress.

2. Material and Methods

Considering the reports in the literature on the accumulation and translocation potential of PP [20,21,23,53–55], an experimental investigation was conducted in the laboratory to assess its efficacy in the remediation of Cu-contaminated soils. The laboratory test was conducted in controlled conditions. The first step was the adaptation test, in which PP's growth was evaluated in Mediterranean environmental conditions [23]. The test was conducted with only one PP plant placed in a pot with a diameter (D) of 0.25 m and a height (h) of 0.20 m. The pot had a horizontal superficial area of 0.049 m^2 and a capacity of 0.01 m^3. It was filled with a combination of blond, brown peat, natural vegetable conditioner and organic substance. The pH was 6.90. The total soil mass was 4 kg and it belonged to the order Histosols, according to the taxonomy classification. The soil density was equal to 250 kg/m^3. In the soil, carbon and nitrogen were, respectively, approximately 20.0% and 1.0% of the dry weight. Tap water was used for irrigation with the following chemical characteristics: bicarbonate, 269.0 mg/L; calcium, 30.70 mg/L; potassium, 27.80 mg/L; magnesium, 9.30 mg/L; nitrate (N), 8.10 mg/L; phosphate (P), 1.20 mg/L; and fluorides, 1.0 mg/L.

The quantity of water for watering was determined on the basis of a precipitation regime of 600 mm/year, which is a regime very similar to the average annual precipitation experienced in the Mediterranean regions [57]. Therefore, given the respected rainfall regime and the pot radius, a continuous irrigation rate of 1.644 mm/day = 0.0805 L/day was utilized. Plant traits such as stem height, leaf length, and rhizome size were measured during the experimental trials using a millimeter ruler and a professional camera (Nikon D50).

The PP tolerance was evaluated by determining its growth with irrigation, using a solution of 125.0 mg of Cu/L. With the aim of polluting the pot soil, tap water was supplemented with Cu using a diluted solution of $CuSO_4 \cdot 5H_2O$, forming an aqueous solution of 125.0 mg of Cu/L [58]. The procedures for measuring the plant traits were conducted using the same method employed for the growth test.

Afterwards, in the same pot with a Cu concentration of 100 mg of Cu/kg dry weight, the phytoextraction factor was assessed. The biomass analyzed was equal to 0.755 kg and was divided into roots, rhizomes, stems and leaves. Each part was washed with tap water to remove soil particles and other debris, and then rinsed with deionized water. The macrophyte tissues were cut into little fragments, which were then dried at 70 °C to a stable weight. Then, they were triturated to a particle size of 0.2 mm and the samples were homogeneously mixed. After this, 0.50 g of plant material was placed in the desiccators.

The samples were then digested with acid etching, using 7.0 mL of concentrated nitric acid, HNO_3: 1 mL hydrochloric acid, and HCl, (7:1) in a closed structure. The closed system was an oven endowed with a quartz power system (1900 W), containing a sealed bottle. In the closed system, the soil sample and the acid were added to a bottle made of a fluorocarbon polymer. The bottle had a fume extraction system. The clear liquid, after cooling the bottle, was diluted to 50.0 mL in acid-washed vials. Dried soil samples of 2 g, aqua regia, an amalgam of 25 mL of concentrated HNO_3 and HCl, with 70.0% in a ratio of 1:4, was transferred to the 100.0 mL digesting tubes covered by a funnel. Then, digestion at 155 °C was conducted in an emission chamber using a digestion block, which was heated until about 5 mL was left in the cylinder. The procedure was repeated by adding another 25 mL of aqua regia and evaporating this to a volume of about 5 mL. Subsequently, membrane filters (10 μm) were used to filter the solution and the filtrate was

brought to a volume of 25.0 mL with deionized and distilled water before analyzing the total Cu. Throughout the duration of the experiments, light and atmospheric humidity were controlled, and the air temperature was maintained at 20.0 °C. Then the concentration of copper in the previously treated plant tissues was analyzed using inductively coupled plasma optical emission spectrometry.

Two main factors were applied for the assessment of the phytoremediation potential of a plant: bioconcentration factors and Translocation Factors [36]. BCF and TF were calculated using the following formulas:

- BCF = Cu contents in all tissues/Cu in soil
- TF = Cu in upper sections/Cu in root and rhizome

Statistical Analyses

The data deriving from the analysis have been reported as mean ± standard deviation. The experiment was repeated three times with modifications made as minimally as possible. With the aim of evaluating the substantial variance in the data about the Cu soil concentration and the data about the copper in the plant organs, one-way ANOVA and post-hoc Tukey's tests were conducted ($p < 0.050$). Additionally, a two-way repeated-measures ANOVA was utilized to measure the affinity between the plant height and the organ growth, treatment, and time [59].

3. Results and Discussions

By carrying out the growth test, the ability of the plant to adapt to a climate different from that of its origin was evaluated. The test was performed in a controlled environment in which the following parameters were kept constant: soil pH (6.9), light exposure (14 h light and 10 h dark), temperature (20 °C), and optimal irrigation flow (1.644 mm/d). In these conditions, the growth tendency was evidenced in Figure 1, where the interpolation line was as follows: h (cm) = 3.39 (weeks) + 53.56 with $R^2 > 0.99$.

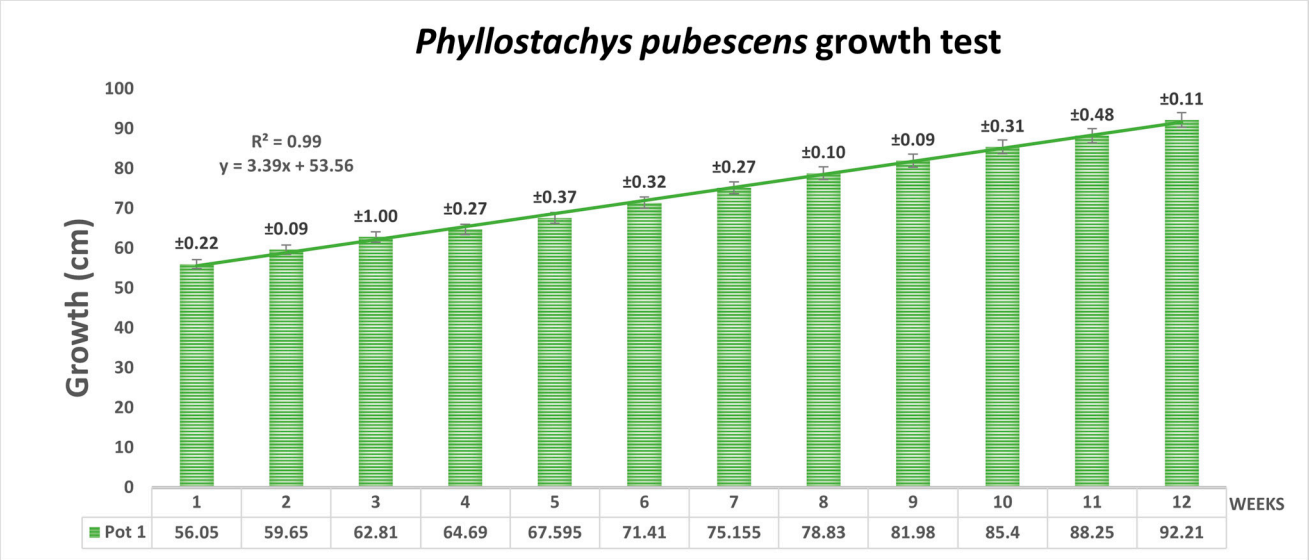

Figure 1. Evaluation of PP growth over 12 weeks with 600 mm/y of irrigation.

For a soil concentration of 100 mg Cu/kg, the PP has a rate of survival of 100% [33]; for a lesser metal exposure (<100 mg per kg), plant growth is not blocked in the experiments [21,60–62]. PP does not survive in metal-polluted soils with more than 300 mg per kg [20] of Cu, but it is a significant phytoextraction macrophyte for Cu-polluted soil up to 200–300 mg Cu/kg.

The presence of copper in certain quantities can lead to alterations in the metabolism and physiology of organisms [2]. Therefore, tolerance tests were performed with Cu-

polluted water irrigation. PP tolerance in Cu-polluted soils can be evaluated by determining the plant growth in a soil contaminated by a Cu solution of 125 mg of Cu/L. To assess growth, PP plant height was quantified utilizing a ruler, every week for twelve weeks. From the changes in the distance, it was possible to derive the growth rate. Figure 2 illustrates the experimental data. For each measurement, the length and the distance from a single element, and the cluster or group of bamboo plant stems growing from a common underground rhizome system were recorded. In accordance with [20], PP can tolerate a content up to 300.0 mg kg^{-1} of Cu, and the biomass production of PP is initially higher with increasing Cu levels in the soil until the level of 50.0 mg kg^{-1} Cu; then, it reduced with the use of all the added increments of Cu in the pot. In the tolerance test, approximately 26.0 mg of Cu was lost as drainage water from the base of the pot and approximately 419.0 mg of Cu was absorbed by the plant. It can be seen that, despite the concentration of irrigation being 125.0 mg of Cu/L, the growth rate decreased; thus, PP still preserved its vegetative functions. Furthermore, no signs of malformation and injury to the plant tissues were detected. The interpolation line was h = 0.654·(weeks) + 91.246 with $R^2 > 0.99$.

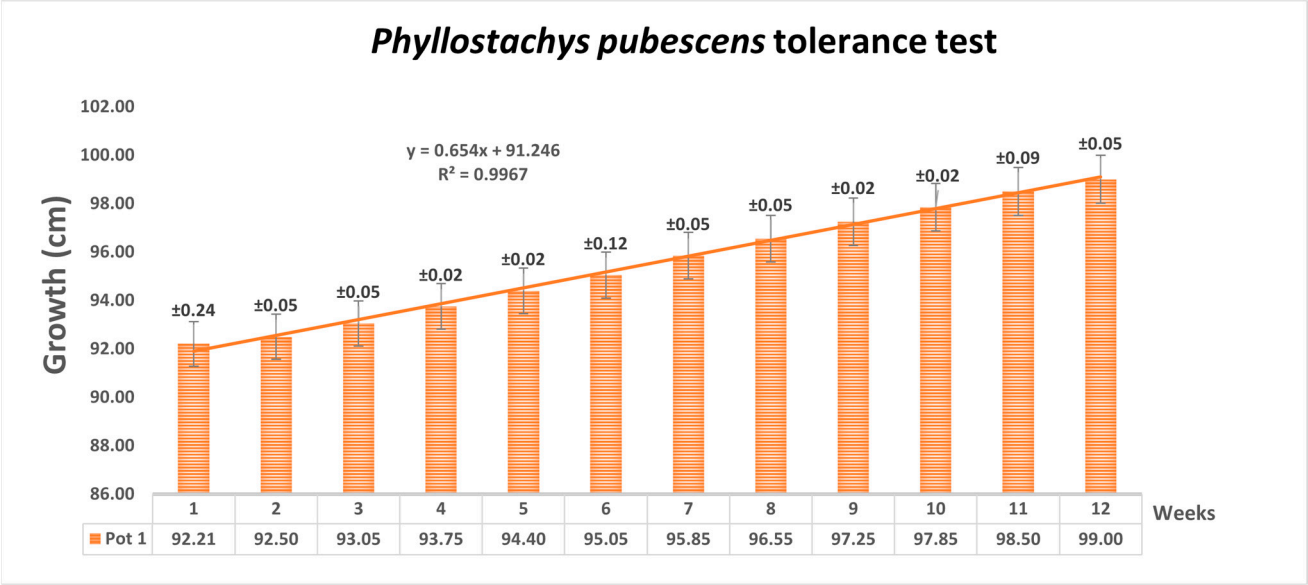

Figure 2. Evaluation of PP tolerance: during 12 weeks growth with 600 mm/year Cu-polluted water (125 mg Cu/L).

3.1. Cu Soil Removal

Cu removal was evaluated using the phytoextraction test, in which the soil contained 100 mg of Cu/kg and was irrigated by simulating a rainfall regime of 600 mm/year with unpolluted water. During the phytoextraction test, negligible drainage water was observed and no significant copper content was lost.

The PP phytoextraction results and the soil Cu concentration after a twelve-week interval are illustrated in the Figure 3.

The remaining Cu in the soil after six weeks was 77.15 mg per kg of dry weight, and approximately 91.0 mg of Cu was absorbed by the PP. After twelve weeks, the Cu content was 48.80 mg per kg of dry weight, and approximately 205.0 mg of Cu was absorbed by the PP.

The extraction of Cu happened according to similar studies [3], in a soothing way, with no radical alterations in the soil, and with no significant effects on the biological balance and the permanency of the ecosystem. It is probable that growing plants bring only the most soluble Cu forms, which could pose a threat to the environment or could get percolated from the soil [3]. The interpolation Cu phytoextraction curve for the pot was [mg Cu/kg dry weight] = $-0.0601 \cdot (\text{weeks})^2 + 100.6$ with $R^2 = 0.99$. The Cu elimination from soil was approximately 51.0%.

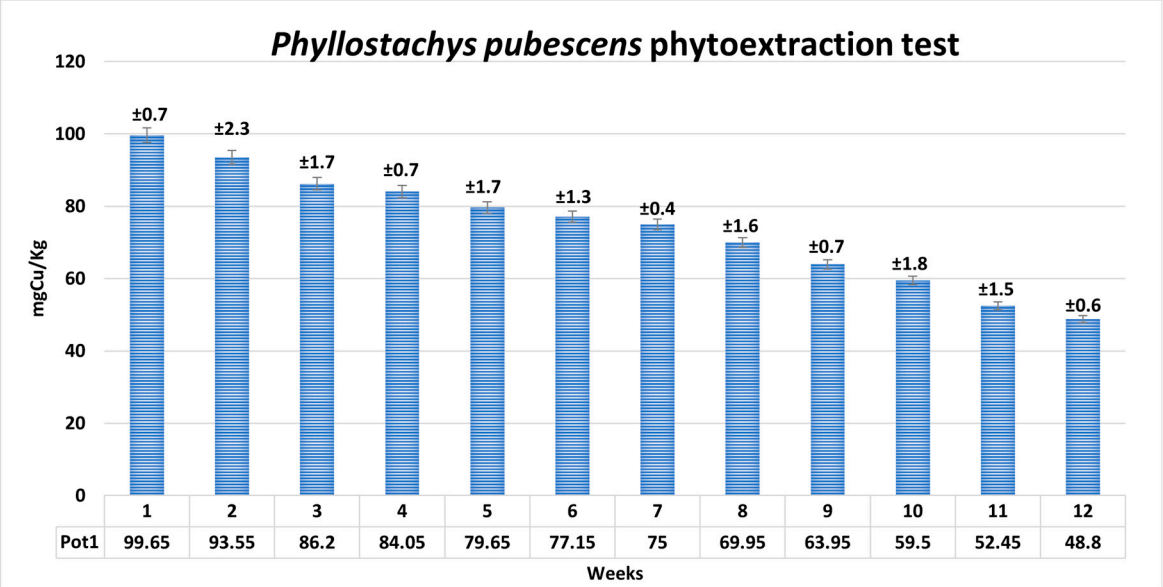

Figure 3. Evaluation of phytoextraction capacity of PP in 12 weeks with 600 mm/year.

3.2. Analysis of Cu Levels in the Plant Organs

The total Cu levels of the plant tissues measured after six and twelve weeks, respectively, showed an accumulation of 510.0 mg of Cu and 624.0 mg of Cu (Figure 4). The distribution of Cu in the plant tissues after twelve weeks is also shown in Figure 4 and indicates the relative percentages of content. PP, in another study, has shown that it can extract copper from the soil [63].

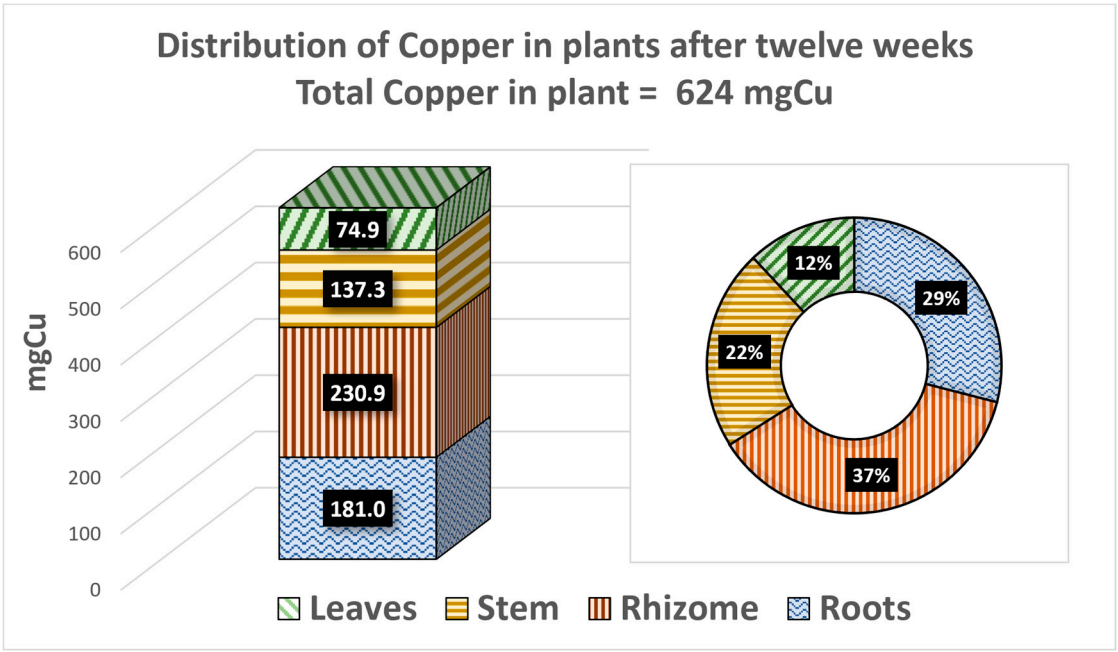

Figure 4. Cu (mg and %) absorbed by plant tissues after twelve weeks.

The amounts of Cu per gram of root and rhizome, and the quantity of Cu per gram of stem and leaves were evaluated. Figure 5 illustrates the amount of Cu per gram of root–rhizome and stem–leaves after six and twelve weeks (mg Cu/g).

The quantity of Cu per gram of root–rhizome was 1.14 mg of Cu/g, and the quantity of Cu per gram of stem and leaves was 0.48 mg of Cu/g, after 6 weeks. The quantity of Cu per gram of root–rhizome was equal to 1.18 mg of Cu/g, and the amount of Cu per gram of

stem–leaves was 0.50 mg of Cu/g, after 12 weeks (Figure 5). Data seem to be elevated and similar to the mean value of the Cu concentrations in leaves (429.0 mg per kg), but much higher than the values in the stems on average [3]. According to some authors [26,64,65], the roots' Cu concentration was higher than that measured in the stems or leaves for the control and 200.0 mg kg^{-1} of Cu treatment. On the contrary, other studies [1] report stem–leaves Cu concentrations higher than those in the roots.

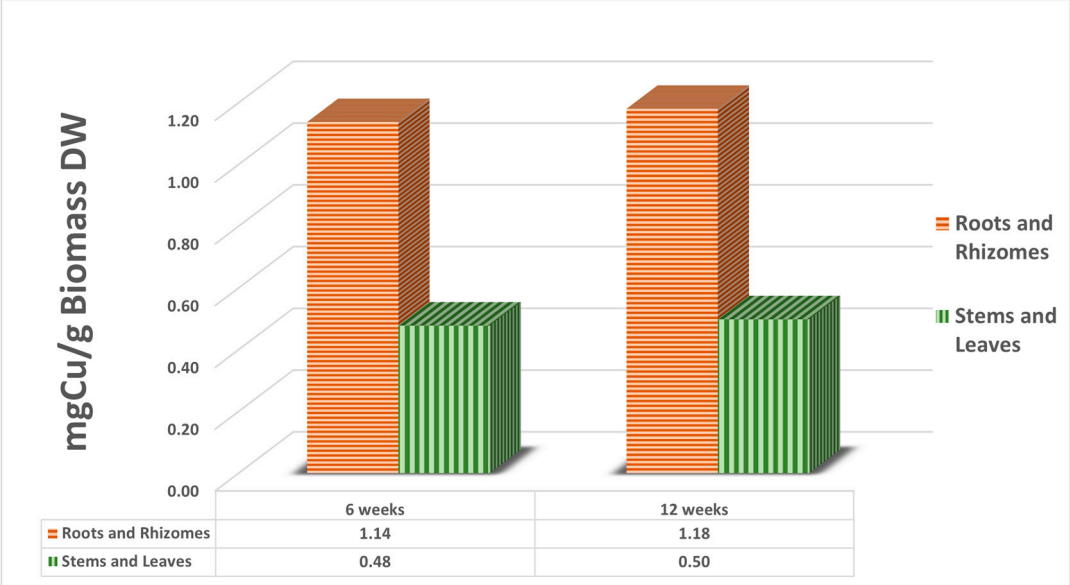

Figure 5. Cu per gram of root/rhizome and stem/leaves after six and twelve weeks (mg Cu/g).

In Table 1 shows the complete results collected for each PP tissue.

Table 1. Copper (mg), g of biomass and the ability to extract Cu in the Moso Bamboo (mg Cu/g) after six and twelve weeks (w.).

Organs	mg Cu (Six w.)	mg Cu (Twelve w.)	g Biomass (Six w.)	g Biomass (Twelve w.)	mg Cu/g (Six w.)	mg Cu/g (Twelve w.)
Roots	26.39	59.5	125.8	163.1	0.58	0.56
Rhizomes	54.72	67.0	170.2	185.8	0.56	0.62
Stems	41.51	50.8	332.5	388.4	0.06	0.07
Leaves	13.46	16.5	30.5	37.6	0.42	0.43

The data show that PP can accumulate a lot of copper in the roots. Copper is spread in the plant's internal membranes and in disproportionate concentrations, can cause stress and injury to plants; this may increase the trend in metal translocation. Some authors [66–70] show that PP retains Cu mainly in the rhizome–root apparatus, thus reducing translocation in the aerial part of the plant. The low transmigration in the stem and leaves may, in this case, reduce the transfer of copper from the plant to the higher trophic levels. As indicated in Table 2, the translocation of Cu in the aerial parts was significant but lower than the growth rate of the PP. Therefore, the translocation factor and bioconcentration factor index were determined.

BCF and TF were computed considering stems–leaves and root–rhizome Cu accumulations using the following expressions [71–77]:

$$BCF = \text{Cu content in all tissues}/\text{Cu in soil} = 624/195 = 3.20$$

$$TF = \text{Cu content in upper sections}/\text{Cu in root and rhizome} = 212/412 = 0.52$$

The bioconcentration factor index value indicates a good capacity to extract the metals from soil and to store them in the plant organs, while the translocation factor value indicates the moderate ability of PP to transfer metal from the roots–rhizome to aerial the organs when compared to other studies (Table 2).

Table 2 reports the translocation and bioconcentration factors of the principal hyperaccumulator species.

Table 2. Translocation and bioconcentration factor of principal hyperaccumulator species.

Species and Content of Cu in mg kg^{-1}	Translocation Factor	Bioconcentration Factor	References
Phyllostachys edulis Content of Cu = 99	Rhizome 0.60 Stems 0.28 Leaves 0.43	Root 0.60 Rhizome 0.36 Stems 0.17 Leaves 0.26	[33]
Phyllostachys praecox Content of Cu = 195	Rhizome 0.91 Stems 0.30 Leaves 1.00	Root 0.47 Rhizome 0.43 Stems 0.14 Leaves 0.47	[33]
Artemisia vulgaris $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4)	Loam 2 = 0.39 Loam 3 = 0.70	Loam 2 = 1.45 Loam 3 = 0.87	[50]
Achillea millefolium $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 2 = 0.61 Loam 3 = 0.16 Loam 4 = 0.59	Loam 2 = 0.61 Loam 3 = 3.94 Loam 4 = 0.37	[50]
Sisymbrium loeselii $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 2 = 0.47 Loam 3 = 0.60	Loam 2 = 1.44 Loam 3 = 1.21	[50]
Thymus kotschyanus $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 2 = 0.08 Loam 4 = 0.08	Loam 2 = 4.84 Loam 4 = 1.57	[50]
Rosa canina $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 3 = 0.71	Loam 3 = 0.73	[50]
Trifolium pratense $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 3 = 1.87	Loam 3 = 0.65	[50]
Hypericum perforatum $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 3 = 1.27	Loam 3 = 0.67	[50]
Tussilago farfara $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 3 = 0.84	Loam = 0.75	[50]
Phleum pratense $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 2 = 0.35 Loam 4 = 0.15	Loam 2 = 4.64 Loam 4 = 1.11	[50]
Sedum caucasicum $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 4 = 0.31	Loam 4 = 0.75	[50]
Astrodaucus orientalis $Cu_{bioavailable}$ = 184.4 (loam 2), 82.4 (loam 3), 894.4 (loam 4).	Loam 4 = 1.19	Loam 4 = 0.12	[50]
Rumex acetosella	1.8–1.9		[78]
Rubia peregrina Cu conc. = 31 to 251 mg kg^{-1}	0.2–0.9		[78]
Phytolacca americana Cu conc. = 134–75	1.8–2.9	0.17	[78]
Chenopodium album Cu conc. = 134–75	5.9		[78]
Conyza albida Cu conc. = 134–75	1.4–2.3		[78]
Rumex induratus Cu conc. = 134–75	2		[78]
Phyllostachys pubescens Cu conc. = 50, 100, 300, 600	Steam = 0.23–0.18–0.22–0.18 Leaves = 0.15–0.09–0.10–0.07	Roots = 0.78–1.41–0.63–0.54 Steam = 0.18–0.26–0.14–0.04 Leaves = 0.12–0.13–0.06–0.04	[21]
Lemna minor Cu conc. = 0.25–0.50–0.75–1.0		0.360 0.191 0.335 0.432	[12]

Table 2. *Cont.*

Species and Content of Cu in mg kg^{-1}	Translocation Factor	Bioconcentration Factor	References
Azolla filiculoides Cu conc. = 0.25–0.50–0.75–1.0		1.600 0.903 1.038 0.954	[12]
Daucus carota Cu conc. = Inceptisol Entisol, 198.6–91.3	0.6–3.2		[79]
Phyllostachys pubescens Cu conc. = 100 mg kg^{-1}	0.18 = Stem 0.09 = Leaves	1.41 = Root 0.26 = Stem 0.13 = Leaves	[20]

According to Chen et al. [20], the concentration of Cu in Moso bamboo can attain levels of 340, 60, and 23 mgkg^{-1} in the roots, stem and leaves, respectively.

The application of 300 mg kg^{-1} of Cu significantly affected the growth of Moso bamboo, as the biomass decreased sharply compared with 100 mg kg^{-1} of Cu treatment. This may be due to a high exchangeable or available Cu concentration in the soil after the application of more than 300 mg kg^{-1} of Cu in soil [20].

The noteworthy Cu increase in its organs demonstrates PP's adeptness for phytoextraction, which is still to be confirmed in a full-scale environment [2,80].

4. Conclusions

PP has evidenced, during the experiment, a good response when up to 125 mg of Cu/L solution is used as irrigation flow. In this period, PP growth speed was considerably reduced, but PP still maintained its vegetative functions without showing any sign of irregularity and no significant injuries to the plant organs were detected. Cu phytoextraction investigations were then implemented and the Cu elimination from soil after twelve weeks was 51.4%, after starting with a Cu content of 100 mg per kg. The data analyzed show that the upper parts of PP have a low Cu concentration, which increases with time. The Cu mass was noteworthy and it was concentrated mostly in the roots–rhizomes, indicating the phytoextraction capability of the macrophyte. The root–rhizome after the experiments contained up to 1.18 mg of Cu/g and 0.50 mg of Cu/g for the stem–leaves after twelve weeks.

Other studies and experiments in full scale environments are essential to further validate the findings of this research. PP should be a valuable option for removing Cu from polluted soil.

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