Phytoextraction by Moso Bamboo Under High Level Chromium Stress in Mediterranean Conditions

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ABSTRACT

In this study a bamboo species, *Moso Bamboo (MB*) – *Phyllostachys pubescens* – has been selected for its heavy metal accumulation capacity and translocation potential to restore Cr-contaminated soil. Experiments have been conducted so to evaluate the capability of *MB* to remove Cr from soil, growing under Mediterranean conditions, irrigated with water containing 180 mgCr/L, at flow rate of 600 mm/year.

The soil has been contaminated by the irrigation water. When the concentration of Cr in soil reached 300 mgCr/kg, Cr phytoextraction by MB from soil at the same irrigation rate of 600 mm/year with uncontaminated water has been evaluated. Cr removal from soil was approx. 42% after 6 weeks and 60.7% after 12 weeks, starting from a Cr content in soil of approximately 300 mg/kg. *MB* growing in Cr contaminated soil has shown Cr concentration per gram of dry biomass in aerial parts greater than the underground parts of the plants. After 12 weeks of cultivation, the quantity of Cr in roots and rhizome was measured as 1.79 mg/g, while in stems and leaves as 2.49 mg/g. Results shown a bioconcentration factor of 0.77, 0.65, 0.18, 0.08, after 6 weeks and 0.64, 0.98, 0.53, 0.26 after 12 weeks for roots, rhizomes, stems and leaves, respectively and a translocation factor equal to 0.23 and 0.11 after 6 weeks and 0.83 and 0.40 after 12 weeks, for stems and leaves, respectively.

1. INTRODUCTION

Many Heavy Metals (HM) are essential for plant growth, however, excessive levels of either essential or non-essential metals, such as Chromium (Cr), are toxic to plants, causing a wide range of deleterious effects (Eleftheriou et al., 2015). Lately, an increase in Cr levels to the environment has been monitored, due to increased use in industrial applications, such electroplating, catalysts production, refractory steel production and metallurgical applications. (Shanker et al., 2005; Ranieri and Świetlik, 2010; Van-Lienden et al., 2010; Ciudin et al., 2014; Ragazzi et al., 2014; Petrella et al., 2016; Ranieri et al., 2020a). HM can cause serious problems in the ecosystems and human health due to the bioaccumulation in the food chains of plants and animals (Nabulo et al., 2010; Petruzzelli et al., 2016; Giuliano et al., 2021). Exposure to Cr, may cause toxic, genotoxic, teratogenic, and mutagenic effects in living organisms (Dixit et al., 2015; Sarwar et al., 2017). Indeed, Cr has gained global attention due to the large use in industrial processes (Choppala et al., 2013, Capodaglio et al., 2016) that brought strict regulation on Cr discharge to the environment on a global level. Particularly in the European Union, the maximum concentration of this element in industrial wastewaters discharged into the aquatic environment is 1 and 5 mg/L for Cr(VI) and Cr(tot), respectively (Vaiopoulou and Gikas, 2020).

Cr is one of the most abundant elements on the earth's crust, naturally occurring in widespread mafic-ultramafic complexes (Becquer et al., 2003) and have a number of oxidation states ranging from Cr(0) to Cr(VI). Cr(III) and Cr(VI) are the most stable forms of Cr in nature (Srivastava et al., 2021) and most preeminent in soils and water. Being Cr(VI) more toxic than Cr(III) (Shankar and Venkateswarlu, 2011; Jiang et al., 2019), the goal is to promote the reduction of Cr(VI) to Cr(III) (Koósz et al., 2008). The distribution of Cr in the environment depends on the presence of oxidizing or reducing compounds, redox potential, the formation of Cr(III) complexes or insoluble Cr(III) salts, the kinetics of the redox reactions, pH, and the total chromium concentration (WHO, 2020; Srivastava et al., 2021). Cr(VI) presents elevated oxidizing potential, high solubility and movement across the membranes in existing biological systems and in the environment. It is a human hazard and it is noxious to many plants, aquatic faunas and biological organisms (Oliveira, 2012; Tumolo et al., 2020), however, at low levels it may function as a trace element for microorganisms (Gikas and Romanos; 2006). Many HM ions such as Cr ions may accumulate in algae, aquatic plants, invertebrates and fishes, often in hazardous levels, and inhibit or completely suppress their growth (Muthukumaravel and Rajaraman, 2013; Prasad et al. 2021). Chromium's toxic carcinogen effects may cause death to animals and humans in particular with ingestion of food and water, inhalation of airborne particulates, and contact with numerous manufactured items containing Cr compounds. However, at low concentrations Cr(III) functions as an essential component of a balanced human and animal diet (Zayed and Terry, 2003; Budiawan et al., 2017; Cabral-Pinto et al., 2020; Das et al., 2021). In nature, Cr concentration in soil depend on the bedrock and range between 10–100 mg/kg with an average concentration of 60 mg/kg (Kabata-Pendias, 2010; Alloway, 2012).

Chromium is toxic for agronomic plants at approx. 0.5 to 5.0 mg/L in nutrient mixture and 5 to 100 mg/g in soil, whereas concentration of Chromium in plants is less than 1 μg/g, under normal conditions (Oliveira, 2012) but there are some plants that can remodulates its genetic and transcriptional regulation for better adaptation (Srivastava et al., 2021). However, Cr accumulation in plant tissues and in the environment depends on climate conditions and Cr speciation (Prasad et al., 2021). Studies on plants have indicated that Cr mainly accumulates in roots, followed by stems and only small amounts of Cr are translocated to leaves (Oliveira, 2012).

Cr is not toxic to all organism in low concentrations and can also be used by many bacteria for their growth. In activated sludge, due to the high diversity of the microorganisms present in the process, some species may adapt to high concentrations of HM (Mulama et al., 2020; Vaiopoulou and Gikas, 2012; Gikas and Romanos, 2006) and have the capabilities to transform contaminants and to reduce their toxicity (Ancona et al., 2020; Hu et al., 2020; Sanjay et al., 2020). In particular, Cr(VI) has been found to stimulate microbial growth for concentrations up to about 25 mg L^{-1}, while Cr(III) stimulate microbial growth for concentrations up to about 15 mg L^{-1} (Gikas and Romanos, 2006). However, high levels of Cr have been found to damaged soil bacterial diversity (Zhang et al., 2021). Both rhizobacteria and mycorrhizal fungi are integral part of the rhizosphere biota and plant roots exudates provide nutrition for microbiological activity in the rhizosphere, which in turn stimulate plant growth (Khan, 2005). In according with CEPA, 2007, the estimated acceptable level of Cr in the soil to preserve environmental safety and human health is 64 mg/kg.

In general, in-situ remediation processes are preferred for the remediation of Cr contaminated soils, as they are less expensive and environmentally disruptive. In fact, the aforementioned processes are advantageous, as they do not require the transportation of contaminated materials to treatment sites, thus reducing the risk of secondary contamination and hence the impact in the food chain and in ecosystem (Gikas and Ranieri, 2014; Al-Bataina et al., 2016; Ranieri et al., 2020b).

Biotechnology offers phytoremediation techniques as a suitable alternative, compared to much more invasive modern methods to remove Cr contaminated soils (Jiang et al., 2020; Go et al., 2021; Ranieri et al., 2021). Phytoremediation is an in-situ remediation technique, economical and with low environmental impact, that uses plants and their associated microorganisms to remove, degrade or isolate toxic substances from the environment to restore contaminated sites. Phytoremediation may be applied for the removal of metals, metalloids, inorganic compounds, radioactive chemical elements, petroleum hydrocarbons, pesticides and herbicides, explosives, chlorinated solvents and industrial organic wastes, and others (Zayed and Terry, 2003; Yoon et al., 2006; Murage, 2009; Bosire, 2014; Favas et al., 2014; Sunitha et al., 2014; Were et al., 2017). It is one of the best alternatives to conventional physicochemical remediation technologies, which may cause secondary pollution, are highly expensive and can deteriorate soil fertility (Ali et al., 2013; Chandra et al., 2015; Mahar et al., 2016; Muthusaravanan et al., 2018). A plant may be used for phytoremediation treatment if it exhibits low transfer of metal from roots to aerial part, is resistant to the presence of HM, is capable of accumulating high level of HM, and possesses rapid growth rate (Haq et al., 2020). Plants that accumulate high concentrations of metals are called hyperaccumulators (Baker and Brooks, 1989). Specifically, plants that accumulate more than 1000 μg/g of nickel in dry leaves, more than 100 mg Cd/kg (0.01%) or more than 500 mgCr/kg (0.05%) in dry leaf tissue can be considered hyperaccumulators (Baker et al., 2000).

Phytoremediation success largely depends on the characteristics of the plant to be utilized and the contaminants present in the ecosystem. For phytoremediation, ideal plants should possess an extensive roots system with maximum production of biomass in presence of high concentration of HM (Chen et al., 2015a). Fast-growing plants that produce large roots biomass such as bamboo are potentially appropriate remediate HM from soil (Gerhardt et al., 2009). However, phytoremediation is a process affected by climatic conditions (Song et al., 2013; Chen et al., 2015a).

Moso bamboo (MB) is recognized as a phytoremediation plant, as it possesses high growth rate, while it is highly tolerant in chromium stress (Chen et al., 2015a). *MB* has numerous advantages compared to other plants such as quick growth, high biomass production and strong ability to adapt to different environments (Chen et al., 2015a). The extraction of HM by the plants, known as phytoextraction, has been suggested as a gentle, in-situ remediation strategy for contaminated soils (Wieshammer et al., 2007). Plants such as *MB* (Figure 1), can accumulate high levels of HM in their aboveground biomass without showing toxicity symptoms (Baker et al., 2000).

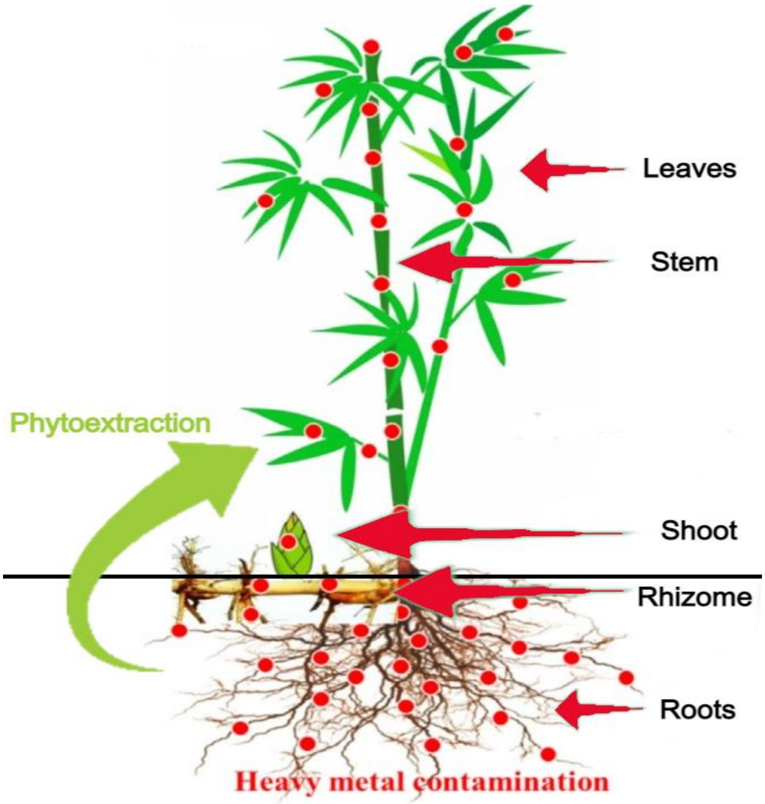

Figure 1 –Distribution of HM in bamboo plants (Bian et al., 2020).

MB is characterized by high biomass productivity, ease in cultivation, extensive competitive ability, short cutting time (4–5 years), and multiple uses such as for furniture, building materials, and decoration. Also, it is known for carbon sequestration (Chen et al., 2016; Zhou et al., 2011) and has a high mean aboveground carbon sequestration value (8 ± 2.15 Mg ha^{-1} $year^{-1}$) (Yen, 2014). *MB* grows rapidly, reaching its maximum size within 2 months, with an average height of 15 m. (Bian et al., 2017).

The aim of the present study is to investigate the ability of *MB* to be used as a phytoremediation plant for the extraction of Cr from contaminated soils containing up to 300 mg/kg Cr contaminated soil, in typical Mediterranean climate (Kalimeris et al., 2017). *MB* does not represent a native plant of the Mediterranean area, so, in order to evaluate its suitability to restore Cr-contaminated sites, pot experiments were carried out in a controlled environment, at the laboratories of the University of Bari (Italy), to assess the plant tolerance *MB* under Cr stress and his capacity and mechanism of phytoextraction.

2. MATERIAL AND METHODS

The bamboo specie *MB* – *Phyllostachys pubescens*, was selected to examine its HM accumulation and translocation potential to restore Cr-contaminated soil. The experiments were carried out in a laboratory under controlled environmental conditions (at 20°C), so as to simulate Mediterranean climate. Preliminary tests were carried out for evaluating *MB* growth with a constant watering rate of approx. 600 mm/year for 12 weeks. Adaptation tests were necessary to evaluate *MB* growth in these climatic conditions, which were different from the optimum climatic conditions for *MB* growth (i.e., tropical conditions). In accordance with Ranieri et al., 2020a, *MB* showed good growth in the selected climate of approx. 4.56 cm/week with an irrigation flow of 600 mm/year.

In the laboratory, *MB* plants were planted in two equal pots with a diameter (D) of 25 cm and a height (h) of 20 cm. Every pot had a horizontal surface of 490.6 cm^2 with a volume of 10 L; all pots were sealed at the bottom, so all the water was retained within the pots. Each pot was filled with 4 kg a soil mixture of blond, brown peat, natural vegetable conditioner and organic substance. The density of the soil mixture was 0.25 kg/L, the pH 6.9, while the carbon and nitrogen content were respectively 20% and 1% of dry weight, respectively. The height of *MB* plant in pot 1 was initially equal to 93.7 cm and equal to 178.2 cm in pot 2.

Tap water with the following chemical concentration was used as irrigational water: bicarbonate 270 mg/L; calcium 30.9 mg/L; potassium 27.7 mg/L; magnesium 9.5 mg/L; nitrate (N) 8 mg/L; phosphate (P) 1.2 mg/L; fluorides 1 mg/L. The quantity of irrigation water for the pot experiments, was calculated based on rainfall intensity of 600 mm/year, which is a rainfall pattern close to annual mean precipitation in Mediterranean regions. Therefore, given the considered rainfall regime and the diameter of the pot, a constant flow rate of 1.644 mm/day = 0.0805 L/day was used. The irrigational water was contaminated with $K_2Cr_2O_7$, at a concentration of 180 mgCr/L (APHA, 1998).

For the analysis, *MB* plant was also separated into its components: roots, rhizomes, stems and leaves. In order to remove soil particles and debris, each component was washed in tap water and rinsed with deionized water. The plant organs were separated into small pieces, and they were dried at 75 °C until a constant weight. Successively, they were ground to a particle size of approximately 0.2 mm and samples of 0.5 g of plant materials were homogeneously mixed and placed in desiccators. Pot 1 was used as control to compare the *MB* growth and tolerance with pot 2. Phytoextraction and Cr distribution analysis were performed only on pot 2. The total *MB* biomass in the two pots was approx. 3 kg with 23% DW.

0.5 g of dry plant matter was digested in closed system using a mixture of concentrated nitric acid and hydrochloric acid at a ratio of 7:1. The closed system consisted of an oven equipped with a quartz power system (1800 W) containing a sealed pot. In the closed pot system, the soil sample and the acid are added to a pot made of a fluorocarbon polymer (PFA/TFM), equipped with an extraction fume system. When the pot had reached room temperature, the clear liquid was transferred into acid-washed vials and distilled water was added to a final volume of 50 mL. Following the above, 1.5 dried ground soil samples and 20 ML of aqua regia (a concentrated HNO_3 and HCl 70% at a 1:4 ratio) were transferred in 100 mL digesting tubes covered with a funnel. The mixture was digested in a fume chamber at 160 °, to a final volume of about 4 mL.

The above process was repeated by adding a further 20 mL of aqua regia and allowed to evaporate to a final volume of about 5 mL. Successively, before analysing total Cr, membrane filters (10 μm) were used to filter the solution and the filtrate was made up to a volume of 25 mL with de-ionized and distilled water. Cr concentration was determined in every digested sample, using an inductively coupled plasma optical emission spectrometry (ICP-OES) analyser.

In this study, an experiment was carried out to evaluate *MB* growth under Mediterranean conditions, during 12 weeks of irrigation with uncontaminated water and with water containing Cr at concentration of 180 mgCr/L.

MB growth performance was assessed in the two pots when irrigated with Cr contaminated water at concentration of 180 mgCr/L. The growth rate has been evaluated measuring weekly the height of the plant. The distance covered by a single element, cluster or group of stems of the bamboo plant growing from a common underground rhizome system, was recorded for each measurement using computer software.

During the tolerance test, after 12 weeks, from 1217 mgCr supplied into each pot, a slight quantity, approximately 20 mgCr per pot had been discharged with drainage water (< 5% of the total flow).

In order to evaluate the statistical differences among the measured parameters, all data were analysed using SPSS 18.0 software. Wet and dry weight measurements, Cr content of plant tissues (roots, rhizomes, stems, and leaves), were analysed using one-way ANOVA and post-hoc Tukey's test (p\0.05). A two-way repeated-measures ANOVA was used to investigate the relationship between plant height, stem diameter growth, treatment, and time (Xiao et al., 2021).

3. RESULTS AND DISCUSSION

The results of the experiment that has been carried out to evaluate *MB* growth under Mediterranean conditions, during 12 weeks of irrigation with uncontaminated water are reported in Figure 2.

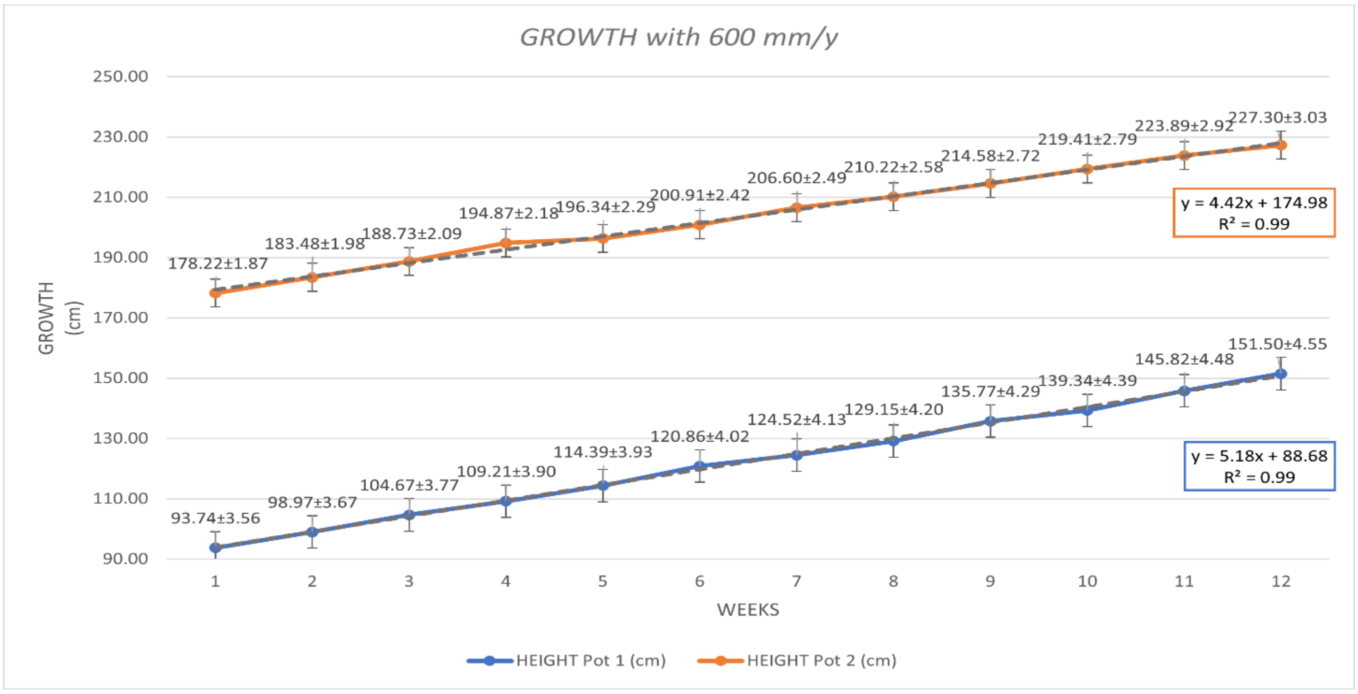

Figure 2 – MB Growth irrigated with 600 mm/y of uncontaminated water.

The height of each bamboo plant was measured in each pot using a ruler, each week for a period of 12 weeks. As shown in Figure 2 the MB growth in mediterranean rainfall simulation was noteworthy in both pots and with a similar rate.

Subsequently tolerance tests to chromium were performed on both pots.

The results of the experiment that has been carried out to evaluate *MB* growth under Mediterranean conditions, during 12 weeks of irrigation with water containing Cr at concentration of 180 mgCr/L are reported in Figure 3.

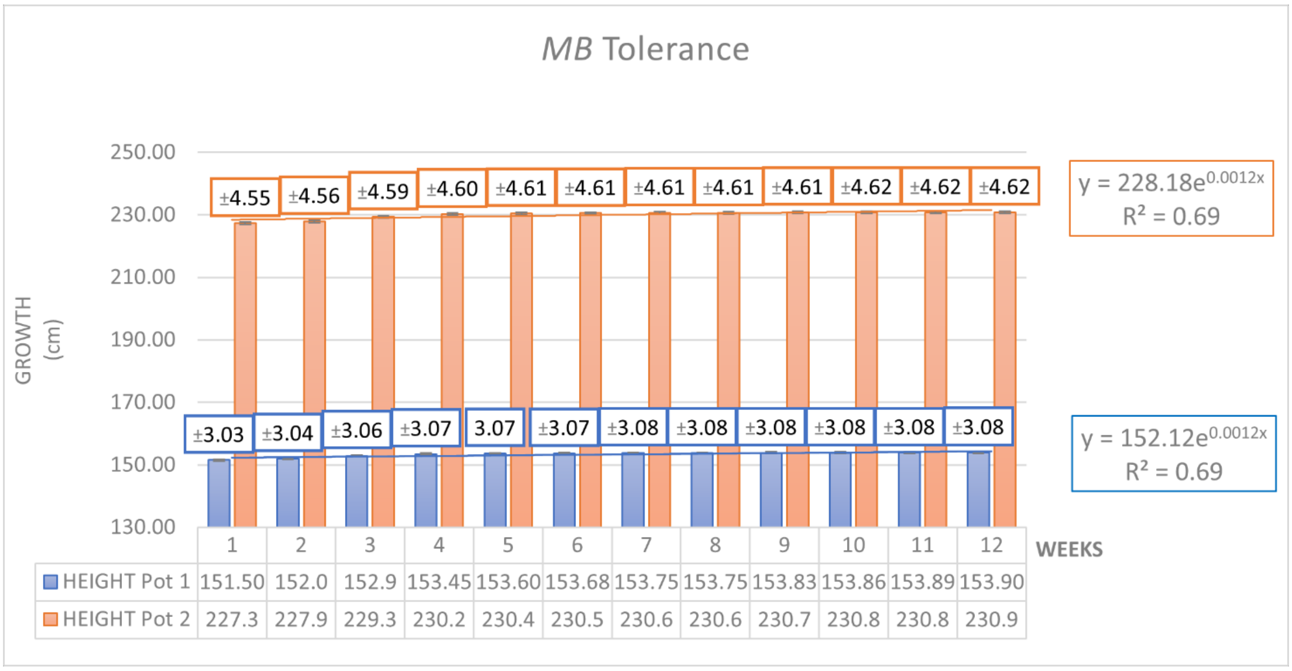

Figure 3 – Tolerance of *MB* irrigated with water containing 180 mg Cr/L (numbers on frame shown the standard error).

The interpolation curve of *MB* growth for the pot 1 was h = $152.12e^{0.0012x}$ with $R^2 = 0.69$, while for the pot 2 was h = $228.18e^{0.0012x}$ with $R^2 = 0.69$ showing in both cases the *MB* tolerance to chromium contamination (Figure 3), maintaining its vegetative functions

The experiments revealed that during the above trials, even if the plant growth rate was considerably inhibited, the bamboo plant still maintained its vegetative functions, while it was not exhibited evidence of malformation neither significant damage to the plant tissues.

In the bamboo HM are distributed in the cell wall, vacuole, and cytoplasm and in excessive concentrations they cause excessive stress and damage to bamboo plants. Intercropping, fertilization, exogenous amendments, and endogenous substances to increase the Bioconcentration Factor (BCF) and Translocation Factor (TF), contribute in reducing the plant stress, compared to monoculture conditions (Bian et al., 2020).

MB can survive and absorb many HM through its strong roots system under high levels stress (Liu et al., 2015). The plant roots system is the first organ to be polluted and can influence shoot physiology and growth (Zhang et al., 2014).

For soil concentration around 100 mgCr/kg dry weight (Were et al., 2017) and for lower metal exposure (< 100 mg/kg dry weight), bamboo species have a survival rate of 100% and plant growth is not inhibited in pot experiments (Michaud et al., 2008; Collin et al., 2013; Chen et al., 2015b; Liu et al., 2015). *MB* does not survive in metal contaminated soils with more than 300 mg/kg dry weight (Chen et al., 2015a). However, MB may be used for the phytoremediation of Cr-contaminated soils containing up to 200–300 mgCr/kg dry weight. It is noted that biotic stresses, such as global climate change, salinity, HM, extreme temperature, etc. may limit plant growth (Hasanuzzaman et al., 2020).

Experiments carried out in plants cultivated in soil containing 1000 mg/kg Cr(VI), indicated that the stem growth of 94% of tested plant species was drastically reduced (Lukina et al., 2016), confirming that Cr excess can cause many plant metabolic changes and reduce their growth (Kumar et al., 2019). However, in some plants, Cr at a low concentration (0.05–1.0 mg/L) may enhance growth and yield, especially in crops, however, it has not been proven that Cr is an essential element for plant growth (Oliveira, 2012; Prasad et al., 2021).

3.1. Cr levels in the plant organs

The capacity of *MB* to accumulate HM in its tissues has been documented in a number of hydroponic experiments, that shown Cu(II) accumulation of 340 mg/kg, 60 mg/kg and 23 mg/kg in roots, stems and leaves respectively, with an initial Cu(II) concentration in soil of 600 mg/kg (Monferrán et al., 2012; Chen et al., 2015a).

Furthermore, it has been found that after 12 months of cultivation, MB can reduce the quantity of Pb and Cr in soil by 42.57% and 86.86%, respectively (Braud et al., 2009; Mulama et al., 2020). Other experiments have shown about 44% Cr removal after 12 weeks of cultivation, in soil initially containing approximately 105 mg/kg dw (Ranieri et al., 2020a).

High level of Cr in soil, limit the seed germination rate of some plant species, such as *Cucumis melo L. Hibiscus esculentus*, *Triticum aestivum* and *Echinochola colona,* delays the growth of two freshwater plants, *Lemna minor*, and *Pistia stratiotes* and inhibits the seed germination of *Salvia sclarea L.* (Bassi et l., 1990; Corradi et al., 1993; Rout et al., 2000; Akinci et al., 2010; Amin et al., 2013; Riaz et al., 2019; Srivastava et al., 2021). Cr exposure affects the shoot length and shoot growth of *Helianthus annus L.*, *Allium cepa*, *Citrus aurantium L., Camellia sinensis, Myriophyllum spicatum* (Chandra and Kulshreshtha, 2004; Fozia et al., 2008; Nematshahi et al., 2012; Ding et al., 2019; Shiyab et al., 2019) and causes negative effects on roots growth in *Pistia stratiotes, C. sinensis, T. aestivum, Oryza sativa L., Pisum sativum* (Sundaramoorthy et al., 2010; Rodriguez et al., 2011; Tang et al., 2012; Ghani et al., 2015; Kakkalameli et al., 2018; Srivastava et al., 2021). The translocation of Cr from roots to the aerial parts of the plant comes up to the leaf. Cr (III) and Cr(VI) toxicity affects the total leaf area and can cause a decrease in leaf size, reduction in leaf number, wilting, chlorosis and in some cases necrosis of the leaf as shown in *O. sativa, Brassica oleracea*, *Lolium perenne L.*, *Saccharum officinarum*, *Phaseolus vulgaris, Prosopis laevigatar* (Poschenrieder et al., 1993; Chatterjee et al., 2000; Radha et al., 2000; Vernay et al., 2007; Buendía-González et al., 2010; Sundaramoorthy et al., 2010).

In this work total Cr levels, expressed in milligram per kilogram of dry weight for samples of roots, rhizomes, stems and leaves were determined after 6 and 12 weeks of growth. The average distribution of Cr in bamboo tissues is reported in Figure 4 and shows the relative percentages as: 46% for roots, 39% for rhizomes, 10% for stems and 5% for leaves after 6 weeks and 26% for roots, 41% for rhizomes, 22% for stems and 11% for leaves after 12 weeks. Within the plant tissues, roots and rhizomes accumulated the highest concentration of Cr indicating a phytostabilization potential of the *MB*.

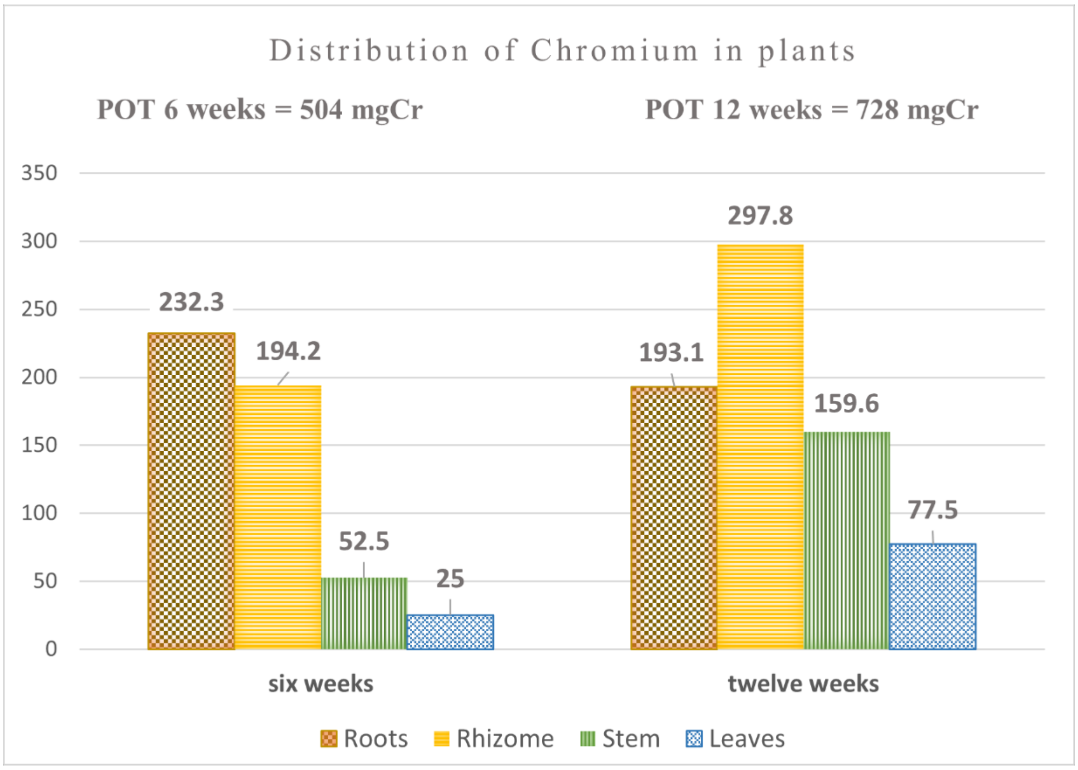

Figure 4 – Chromium (mg) absorbed by various plant parts after 6 and 12 weeks of cultivation.

In some studies, *MB* exhibited a definite trend of absorbing HM such as lead, copper and chromium, from contaminated soil, with roots accumulating the majority of HM, while bioaccumulation is directly dependent on the ambient concentration of the HM in soil and on the leaching rate (Mulama et al., 2020).
Some other species, such as *Diectomis astigiate* can accumulate high quantities of Cr in their roots (about 2371 mg/kg dry weight), while other, such as *Vernonia cinerea* may accumulate approximately 5500 mg/kg dry weight in shoots (Mohanty and Patra, 2020). Also, *Cannabis sativa* and *Allium griffithianum* can accumulate high concentration of Cr in all tissues ranging from 568.33 to 1233.3 mg/kg (Sajad et al., 2020).

In this work it was evaluated the total chromium mass retained per plant organ mass, the quantity of Cr per gram of roots, rhizomes, stems and leaves. The levels were expressed in milligram per gram of dry weight (mg/g) of the respective samples after 6 or 12 weeks of growth period. It was also calculated the BCF and the TF for 12 weeks, in accordance to Ullah et al., 2021. The results are shown in Tab. 1.

$$BCF = \frac{Cr\ content\ in\ plant\ organ}{Cr\ content\ in\ treated\ soil}$$

$$TF = \frac{Cr\ concentration\ in\ aerial\ parts}{Cr\ concentration\ in\ roots}$$

Table 1 – Quantity of Cr, weight of biomass, capacity of Cr extraction, BCF and TF in various organs of *M. bamboo*, after 6 and 12 weeks of cultivation.

Plant part	mgCr (6 weeks)	mgCr (12 weeks)	g biomass (6 weeks)	g biomass (12 weeks)	mgCr/g (6 weeks)	mgCr/g (12 weeks)	BCF (6 weeks)	TF (6 weeks)	BCF (12 weeks)	TF (12 weeks)
Roots	232.3 ± 4.6	193.1 ± 3.9	272 ± 5.4	326.4 ± 6.5	0.85	0.59	0.77	-	0.64	-
Rhizomes	194.2 ± 3.9	297.8 ± 6.0	216 ± 4.3	248.4 ± 5.0	0.9	1.2	0.65	-	0.99	-
Stems	52.5 ± 1.0	159.6 ± 3.2	120 ± 2,4	160 ± 3.2	0.44	1.0	0.18	0.23	0.53	0.83
Leaves	25 ± 0.5	77.5 ± 1.6	35 ± 0.7	52 ± 1.0	0.71	1.49	0.08	0.11	0.26	0.40

It has also been calculated the ratio of the Cr concentration in the aerial parts and the underground parts, after 12 weeks of cultivation:

$$\frac{\text{leaves} + \text{stems}}{\text{roots} + \text{rhizomes}} = \frac{77.5 + 159.6}{193.1 + 297.8} = 0.48$$

The results shown that after 6 weeks of cultivation, the amount of Cr per gram of roots and rhizome was equal to 1.75 mgCr/g (0.85 mgCr/g + 0.9 mgCr/g) and for stem and leaves was equal to 1.15 mgCr/g (0.44 mgCr/g + 0.71 mgCr/g), while after 12 weeks of cultivation, the amount of Cr per gram of roots and rhizome was equal to 1.79 mgCr/g (0.59 mgCr/g + 1.2 mgCr/g) and equal to 2.49 mgCr/g (1 mgCr/g + 1.49 mgCr/g) for stem and leaves. Results are shown in Figure 5.

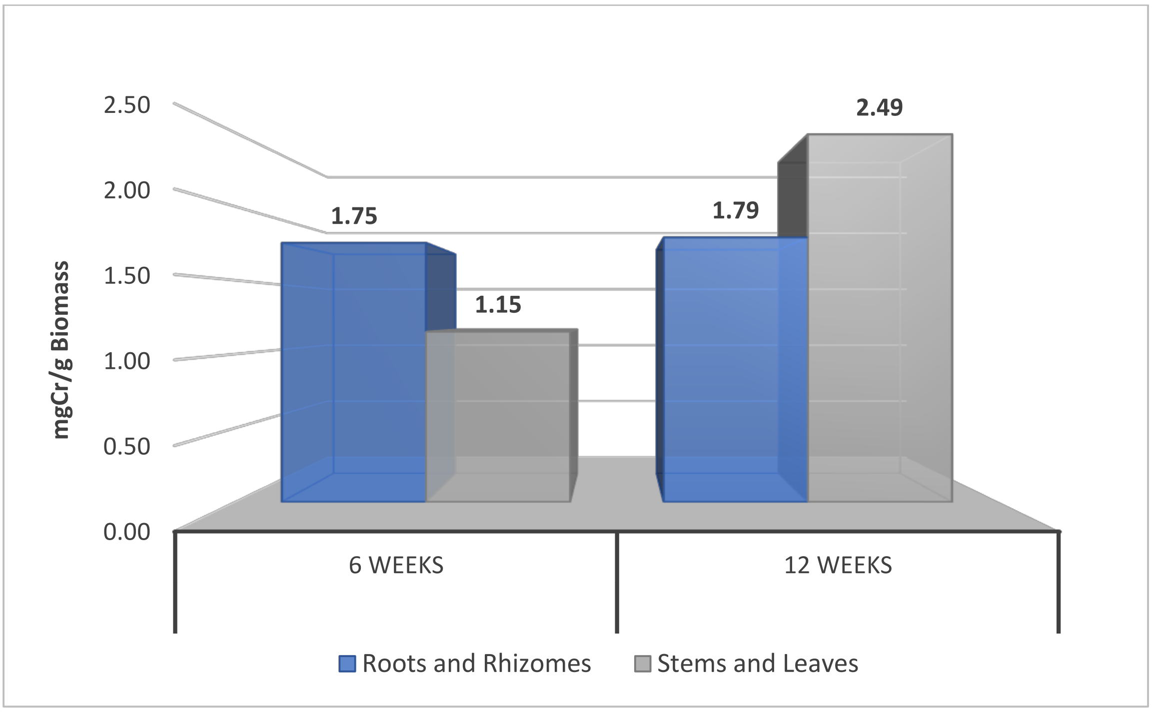

Figure 5 – Quantity of Cr per gram of roots/rhizomes and stems/leaves after 6 and 12 weeks of cultivation (mg/g).

It has been found that *MB* accumulates Cr mainly in the rhizome-roots organs, thus restraining transport in the aerial parts including leaves, which results to reduced transportation of Cr to the animal and human food chain (Vernay et al., 2007; Shahid et al., 2017). In fact, in the first 6 weeks the high Cr concentration in the rhizome and roots and the low translocation in the stems and leaves reduce chromium transfer from the plant to the highest trophic levels. After 12 weeks the accumulation in stems and leaves per gram of biomass is greater than roots and rhizome, due to the translocation of the first 6 weeks and the lower ratio between concentration and the biomass of the leaves. The findings confirm the great capacity of bamboo species to remove HM from soil and stabilize it in the underground parts of the plant (Tab. 2).

Table 2 – Capacities of various bamboo species y to accumulate HM in their tissues.

Species	Heavy Metal	Concentration in soil (mg/kg)	Rhizome mg/kg	Roots mg/kg	Stem mg/kg	Leaves mg/kg	Shoots mg/kg	Rhizosphere soil mg/kg	Reference
M. bamboo	Cu	600	-	340	60	23	-	-	Chen, (2015a)
M. bamboo	Zn	400	-	8642	2012	1039	1443	-	Chen, (2015b)
M. bamboo	Cu	174	-	34	32	27	-	-	Mulama, (2020)
M. bamboo	Pb	101	-	24	21	10	-	-	Mulama, (2020)
M. bamboo	Cr	137	-	15	8	6	-	-	Mulama, (2020)
B. blumeana	Cr	2326	-	628	-	-	148.2	560	Were, (2017)
B. bambos	Cr	2300	-	509	-	-	546.9	1171.2	Were, (2017)
B. vulgaris	Cr	2107	-	971	-	-	21.3	636.4	Were, (2017)
D. asper	Cr	2332	-	736	-	-	62.2	453.9	Were, (2017)
D. birmanicus	Cr	2324	-	431	-	-	88.1	553.3	Were, (2017)
D. membranaceus	Cr	2187	-	808	-	-	56.4	622.3	Were, (2017)
M. bamboo	Pb	400	-	4283	482	149	-	-	Liu, (2015)
M. bamboo	Cd	120	-	159	132	46	-	-	Li, (2016)
M. bamboo	Cu	99	36	59	17	26	-	-	Bian, (2017)
M. bamboo	Zn	3608	1468	1645	1571	1867	-	-	Bian, (2017)
M. bamboo	Cd	11	4.4	4.6	4.1	4.4	-	-	Bian, (2017)

3.2. *Moso bamboo* phytoextraction

Chromium phytoextraction depends on the specific hyperaccumulator-contaminant interaction (Tu et al. 2004; López-Luna et al. 2009). The contaminants uptake occurs in the roots and transported to the aerial parts of the plant, followed by harvesting the plant biomass for safe disposal (Prasad and Freitas, 2003; Kotrba et al., 2009; Mahar et al., 2016; Yadav et al., 2018). Only when the plants have accumulated the maximum amounts of contaminants and have reached the optimum growth, the aerial parts may be harvested for the removal of the HM from the contaminated soils (Jabeen et al., 2009; Jadia and Fulekar, 2008; Yadav et al., 2018).

Phytoextractive plants can stabilize Cr(VI) in the soil through their roots and their above-ground tissues and can reduce it to Cr(III) (Gadd, 2001). Plants used for phytoextraction to remove HM from soils must exhibit rapid growth, abundant roots system, high tolerance level against metallic contaminants, increased potential for metal accumulation, wide geographic distribution, easy cultivation, resistance to pathogens, well adapted to prevailing climatic conditions and repulsion to herbivores to avoid food chain contamination (Yadav et al., 2018).

Our study has been evaluated the capacity of *MB* to reduce soil Cr content starting with approx. 300 mgCr/kg after 6 and 12 weeks with tap water irrigation of 600 mm/year. The residual average concentration of Cr in the soil after 6 weeks was 174 mgCr/kg, while after 12 weeks was 118 mgCr/kg dry weight, as shown in Figure 6.

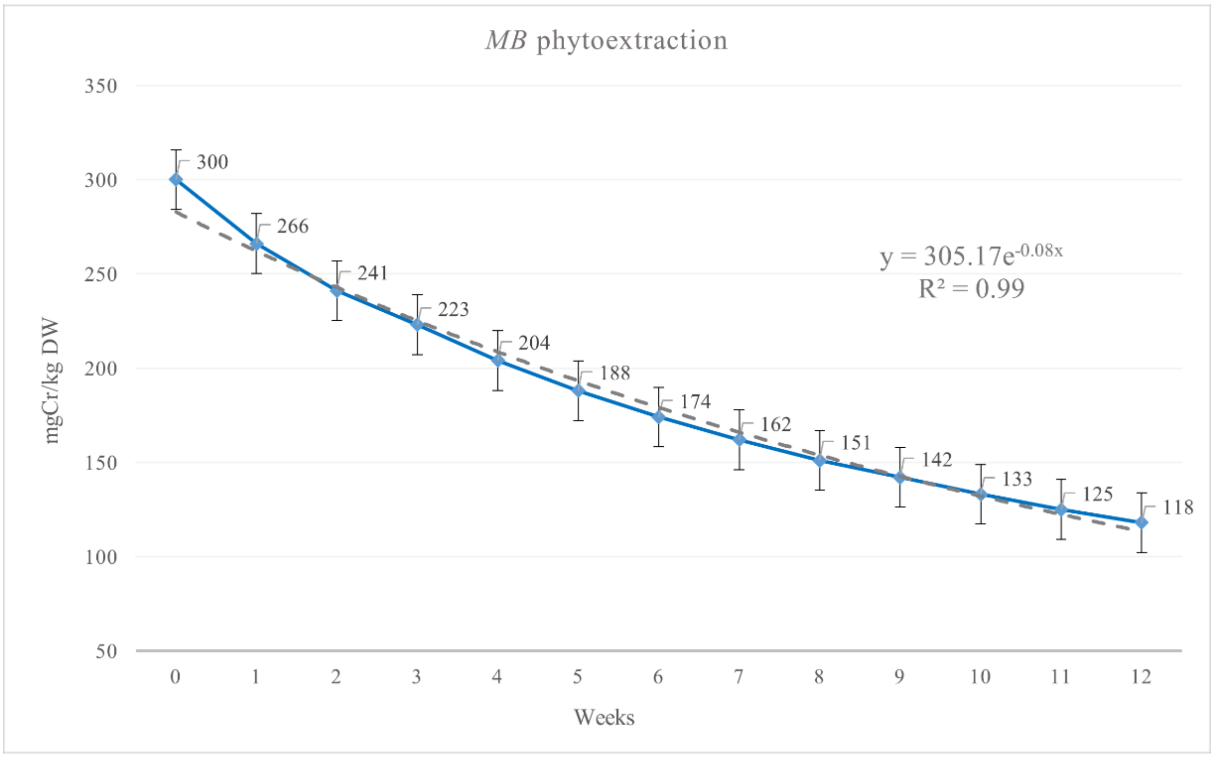

Figure 6 – Cr phytoextraction by *MB* from soil containing 300 mgCr/kg, at irrigation rate of 600 mm/year with uncontaminated water.

The interpolation curve has been calculated as: $y = 305.17e^{-0.08x}$ with $R^2 = 0.99$.

Cr removal from soil was approx. 42% after 6 weeks and 60.7% after 12 weeks, starting with a Cr content of approximately 300 mg/kg dry weight. Due to the presence of humic acids in the soil, the phytoextraction capacity of bamboo should be considerably reduced. In fact, Cr tends to form bonds with humic acids, limiting the extraction by decreasing its bioavailability (Carvalho-Pereira et al., 2015; Kalčíková et al., 2016). During the phytoextraction approximately 20 mgCr have been lost from the soil within the pot with drainage water, while approximately 728 mgCr have been absorbed by the plants after 12 weeks. This observed significant reduction of Cr concentration in the soil indicates the great capacity of phytoextraction.

CONCLUSIONS

In this work, *MB* shown a great capability to grow in Mediterranean conditions with irrigation flow rate of 600 mm/year, containing 180 mgCr/L. The quantity of Cr per gram of *MB* roots and rhizome was equal to 1.75 mgCr/g after 6 weeks and 1.79 mgCr/g after 12 weeks of cultivation, while the quantity of Cr per gram of stem and leaves was equal to 1.15 mgCr/g after 6 weeks and 2.49 mgCr/g after 12 weeks of irrigation. The tolerance test results have showed a good response of plant development at Cr concentrations up to 180 mgCr/L in the irrigational water; even if the growth rate was considerably reduced, the bamboo plant still maintained its vegetative functions. Chromium bioaccumulation was found to concentrate mostly in the underground parts after 6 weeks of cultivation, indicating a phytostabilization potential of the plant, while the aerial parts of MB, after 12 weeks of cultivation exhibited high TF and increased bioaccumulation of Cr. In fact, the BCF was 0.77 for roots, 0.65 for rhizomes, 0.18 for stems, 0.08 for leaves after 6 weeks and 0.64 for roots, 0.98 for rhizomes, 0.53 for stems and 0.25 for leaves after 12 weeks, indicating greater bioaccumulation with at significantly reduced plant growth. TF was 0.23 after 6 weeks and 0.83 after 12 weeks for steams, while 0.11 after 6 weeks and 0.40 after 12 weeks for leaves. Cr removal from soil was approx. 42% after 6 weeks and 60.7% after 12 weeks starting from a Cr content in soil of approximately 300 mg/kg, ending with a concentration in soil of approx. 118 mgCr/kg. Finally, we can suppose that the present results confirm the great capability of *MB* to restore Cr contaminated soils also in Mediterranean conditions. Future experimentations under contaminated field conditions will follow to further verify the findings of this study.

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Yes

Authors Contributions

The contribution of the authors is parithetic

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Competing Interests

The Authors declare no conflict of interest

Availability of data and materials

All data generated or analysed during this study are included in this published article.

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