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CHROMIUM PHYTOEXTRACTION USING PHYLLOSTACHYS PUBESCENS (MOSO BAMBOO)

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Novelty Statement

Moso Bamboo has shown a great adaptability of growing in Mediterranean semi-arid conditions

Moso Bamboo tolerance to contaminated water has been registered up to 125 mg/L of chromium.

Cr Phytoextraction from soil was found to be significant -43%-and Cr is accumulated mostly in the roots

Abstract

In this study, a bamboo species, the Phyllostachys pubescens – Moso Bamboo (*MB*) -, was selected for its heavy metals accumulation and translocation potential to restore Chromium (Cr)-contaminated soil. In order to evaluate the *MB* Chromium growth, tolerance and the potential for phytoremediation using *MB* to restore Cr-contaminated soil, pot experiments were carried out in simulated Mediterranean conditions in a laboratory, in a controlled environment, at a temperature of 20°C. The results showed that *MB* growth rate was 4.28 cm/week on average, with an irrigation flow of 1.644 mm/d. *MB* tolerance was tested over a 12-week irrigation period with the addition of Cr-contaminated water. Cr removal from soil was 43 % starting from a Cr content of approx. 200 mg/kg dry weight (dw) and the quantity of Cr per gram of root and rhizome was equal to 1.31 mg/g dw, while the quantity of Cr per gram of stem and leaves was equal to 0.86 mg/g dw, after 12 weeks. Pot experiments confirm that phytoremediation using plants such as *MB* provides an alternative approach for handling Cr-contaminated soil.

Keywords: Bamboo growth; Tolerance; Chromium removal; Metals translocation; Phytoextraction.

1. Introduction

Worldwide, there is an increasing concern about Chromium (Cr) as an environmental pollutant because of its gradual increase to toxic levels in the environment as a result of various industrial (e.g. tannery) and agricultural activities. In fact, due to its wide anthropogenic use in industry, the Cr environmental contamination is increased in the last years (Shanker et al. 2005; Ranieri and Swietlik 2010; Van Lienden et al. 2010; Ciudin et al. 2014; Ragazzi et al. 2014; Petrella et al. 2016a; Petrella et al., 2016b; Ranieri et al. 2020a).

Cr occurs essentially in three oxidative conditions Cr(0), Cr(III), and Cr(VI), which are the most constant forms of Cr. The forms of Cr(III) and Cr(VI) are the most preeminent in soils and water, since Cr(0) is the metallic form. Cr(VI) presents elevated oxidizing potential, high solubility and movement across the membranes in existing biological systems and in the environment. It is a dominant nuisance, a human hazard and it is also noxious to many plants, aquatic faunas and biological organisms (Oliveira 2012). Chromium is also required as a nutrient for human metabolism with consume of 100 µg daily, while excessive exposure in particulate of Cr(VI) has negative effects on human respiratory system (Ranieri, 2012; Capodaglio et al., 2016; Budiawan et al. 2017; Giuliano et al, 2021; Das et al. 2021).

Cr is noxious 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 Cr in plants is less than 1 µg/g, under normal situations (Oliveira, 2012). In soil, generally, Cr represents a combination of both Cr(III) and Cr(VI). Cr accumulates mainly in roots and usually only a small part translocated to the shoots (Oliveira 2012). Otherwise Cr(VI) has been found to stimulate microbial growth for concentrations up to about 25 mg/l, while Cr(III) stimulate microbial growth for concentrations up to about 15 mg/l (Gikas and Romanos 2006).

Due to the large use of Cr in industrial processes, the release of Cr has been regulated around the world (Choppala et al. 2013). In particular in the European Union, the maximum discharge limit in the aquatic environment is 1 and 5 mg/l for Cr(VI) and Cr(tot), respectively (Vaiopoulou & Gikas 2020).

Cr contaminated soils can be remediated by various methods. In situ methods are currently preferred because are less expensive and environmentally disruptive. In fact, these methods have the advantage of not involving the movement of contaminated materials to treatment sites eliminating risks of secondary contamination and hence the impact on food chain and ecosystem (Gikas 2014; Al-Bataina et al. 2016; Ranieri et al. 2020b; Prasad et al. 2021).

In this context, biotechnology offers phytoremediation techniques as a suitable alternative. Phytoremediation is an in-situ remediation technique, economically feasible and environment-friendly, that uses plants with exceptional metal-accumulating capabilities and their associated microorganisms in order to remove, degrade or isolate toxic substances from the environment to restore contaminated sites (Zayed & Terry, 2003; Yoon, 2006; Muraje 2009; Bosire 2014; Favas et al., 2014; Were et al. 2017; Sunitha et al. 2017). Phytoremediation success largely depends on the characteristics of the plant to be utilized and the contaminants present in the ecosystem. It is one of the best alternatives to conventional physicochemical remediation technologies, which produces secondary pollution, are highly expensive and can deteriorate soil fertility (Ali et al. 2013; Mahar et al. 2016; Muthusaravanan et al. 2018; Ranieri et al. 2020b).

Plants that accumulate high concentrations of metals in their tissues are called hyperaccumulators. According to Baker and Brooks (1989) 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 mg Cr/kg (0.05%) in dry leaf tissue can be considered hyperaccumulators (Baker et al. 2000).

For phytoextraction, model plants should possess a wide root apparatus with high yield of biomass in presence of elevated heavy metals mass (Chen et al., 2015a). Macrophytes that produce great root biomass such as Moso Bamboo (*MB*) are possibly suitable alternatives for phytoextraction (Gerhardt et al. 2009; Ranieri et al., 2021). *MB* is part of grass family, Graminae (Poaceae) with the aptitude to persist even in the worst soil and climatic conditions, however, the use of phytoremediation may be restricted by severe climatic conditions (Song et al. 2013). It has various benefits related to other plants such as rapid growth, elevated biomass yield and robust ability to acclimatise to various environments (Chen et al., 2015a).

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. Further, it is known as the most promising species for carbon sequestration (Chen et al., 2016; Zhou et al., 2011) and has a high mean aboveground carbon sequestration value (8.13 ± 2.15 Mg ha^{-1} $year^{-1}$) (Yen 2014). Moso bamboo grows rapidly, reaching its maximum size within 2 months, with an average height of 15 m (Bian et al. 2017).

The aim of this research was to investigate the ability of *MB* for enhancing the phytoremediation of Cr-contaminated soil in a typical Mediterranean climate. In order to evaluate its suitability to restore Cr-contaminated sites, pot experiments were carried out to study mechanism of phytoextraction and tolerance of *MB* under Cr stress.

2. Material and Methods

In this study, Moso Bamboo (*MB*) species (*Phyllostachys pubescens*) was selected for its heavy metals accumulation and translocation potential to restore Cr-contaminated soil.

Preliminary tests were carried out in a laboratory, in a controlled environment, for evaluating *MB* growth with irrigation in Mediterranean conditions. In fact, adaptation tests were necessary to evaluate *MB* growth in climatic conditions different from optimum climatic conditions for its growth (i.e. tropical conditions). *MB* showed a good adaptability in Mediterranean conditions (Ranieri et al., 2020a). The experiment was carried out with only one *MB* plant allocated in a pot with a diameter (D) of 25 cm and a height (h) of 20 cm. The pot had a horizontal surface of 490 cm^2 and a volume of 10 l. It was filled with a mixture of blond, brown peat, natural vegetable conditioner and organic substance. The pH was 6.9. The total soil mass was 4 kg and soil density was equal to 0.25 kg/l.

In the soil, carbon and nitrogen were, respectively, approximatively 20% and 1% of dry weight. For the irrigation, it was used tap water with the following chemical characteristics: bicarbonate 269 mg/l; calcium 30.7 mg/l; potassium 27.8 mg/l; magnesium 9.3 mg/l; nitrate (N) 8.1 mg/l; phosphate (P) 1.2 mg/l; fluorides 1 mg/l.

In terms of water requirements, the quantity of irrigation water, was calculated based on the

rainfall regime of 600 mm/year that is a rainfall regime close to annual mean precipitation in Mediterranean regions (Ranieri, 2003; Gorgoglione et al., 2016; Kalimeris et al., 2017). Therefore, given the considered rainfall regime and the pot diameter, a constant watering rate of 1.644 mm/day = 0.0805 l/day was used.

After *MB* tolerance test, his capacity of Cr phytoextraction has been evaluated in the pot where the Cr concentration in soil was homogenous and equal to 200 mg Cr/kg dw.

For the analysis, the *MB* plant was also separated into its components: roots, rhizomes, stems and leaves. The total biomass analyzed was approx. 1.5 kg.

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 to a constant weight. Successively, they were ground to a particle size of 0.2 mm and homogeneously mixed samples of 0.5 g of plant materials were allocated in desiccators.

The digestion of the 0.5 g plant material samples was carried out by using 7 ml of concentrated nitric acid: 1 ml hydrochloric acid, HCI, (7:1) in a closed system. The closed system was an oven equipped with a quartz power system (1800 W) containing a sealed vessel. In the closed vessel system, the soil sample and the acid are added to a vessel made of a fluorocarbon polymer (PFA/TFM). The vessel was equipped with an extraction fume system. The clear liquid, after cooling the vessel, was diluted to 50 ml in acid-washed vials.

Dried ground soil samples of 1.5 g, aqua regia, a mixture of 20 ml concentrated HNO3 and HCl, 70% in a ratio of 1:4 were transferred to the 100 ml digesting tubes covered by a funnel. Then, digestion at 160 °C was carried out in a fume chamber using a digestion block which was heated until about 4 ml was left in the tube.

The process was repeated by adding a further 20 ml of aqua regia and allowed to evaporate to a volume of about 5 ml. Successively, 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 prior to analysis of total Cr. During the experiments, light and atmospheric moisture were regulated and constant and air temperature was constant and equal to 20 °C. All the digested samples were analysed for levels of total Cr using inductively coupled plasma optical emission spectrometry (ICP-OES).

Statistical analyses

The samples were analyzed in triplicate, and the data obtained was then reported as mean ± standard deviation. In order to evaluate statistically significant differences among values, all data wet and dry weight measurements, Cr content of plant tissues (roots, rhizomes, stem, and leaves), were analyzed using one-way ANOVA and post-hoc Tukey's test ($p < 0.05$). A two-way repeated-measures ANOVA was used to analyze the relationship between plant height and stem diameter growth, treatment, and time (Xiao et al. 2021).

3. Results and Discussions

Bamboo species have a survival rate of 100% for soil concentration around 100 mg Cr/kg dry weight (Were et al. 2017) and for lower metal exposure (< 100 mg/kg dry weight), 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) but it is supposed to be a valuable phytoremediation material for Cr-contaminated soil up to 200–300 mg Cr/kg dry weight.

MB growth test were carried out to verify the ability of the plants to adapt to a climate different from that of their natural habitat. The entire duration of the test was carried out in a laboratory-controlled environment, in which the following parameters were constantly monitored: soil pH (6.9), light exposure (14 h light and 10 h dark), temperature (20°C), and optimal irrigation volume (1.64 mm/d for pots 1,2 and 4.93 mm/d for pots 3,4).

The interpolation curve was: h = 4.71 (weeks) + 36.86 with $R^2 > 0.99$ for the Pot 1 (Figure 1).

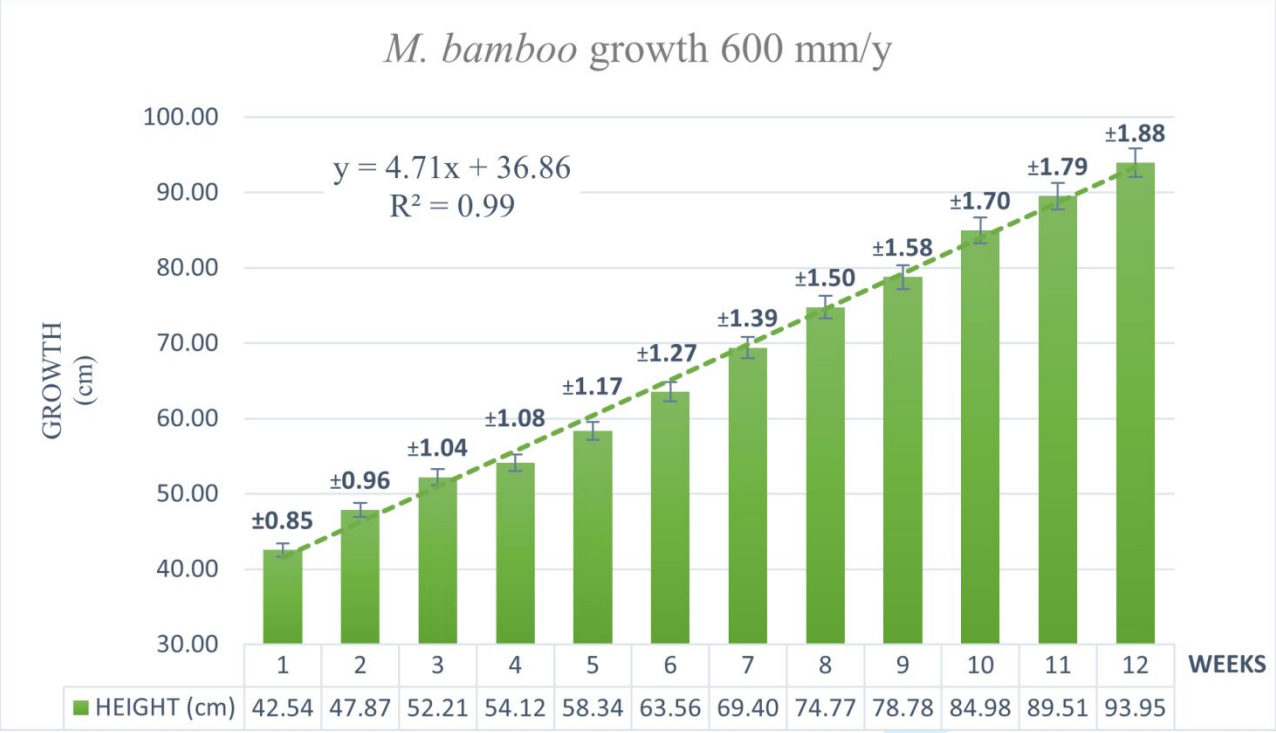

Figure 1 – MB growth with 600 mm/y.

It is known that Cr has a toxic effect that can alter physiological and metabolic pathways of some organism, there are some plants that can remodulates its genetic and transcriptional regulation for better adaptation (Srivastava et al. 2021). Bamboo tolerance in Cr contaminated soils can be assessed by measuring the plant growth in a soil contaminated by a Cr solution. In this study, *MB* tolerance was assessed by measuring its growth with irrigation with a solution of 125 mg Cr/l. In fact, to contaminate the soil of the pot, tap water was added with Cr by a solution of K2Cr2O7 forming an aqueous solution of 125 mg Cr/l (APHA, AWWA 1998).

In order to evaluate growth performance, the height of the bamboo plant was measured using a ruler,

each week for a period of 12 weeks. By measuring weekly, the variations in length, the growth rate has been evaluated. Figure 2 shows the results. 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. During the tolerance test approx. 210 mgCr was lost as drainage water from the bottom of the pot and approx. 46 mgCr were absorbed by the plant. It was possible to note that, at the concentration of irrigation water 125 mg Cr/l, even if the growth rate was considerably reduced, the bamboo plant still maintained his vegetative functions. Moreover, the bamboo plant did not show any evidence of malformation and not significant damages to the plant tissues were observed. The interpolation curve for the pot was h = 0.78·(weeks) + 93.40 with R2 > 0.99.

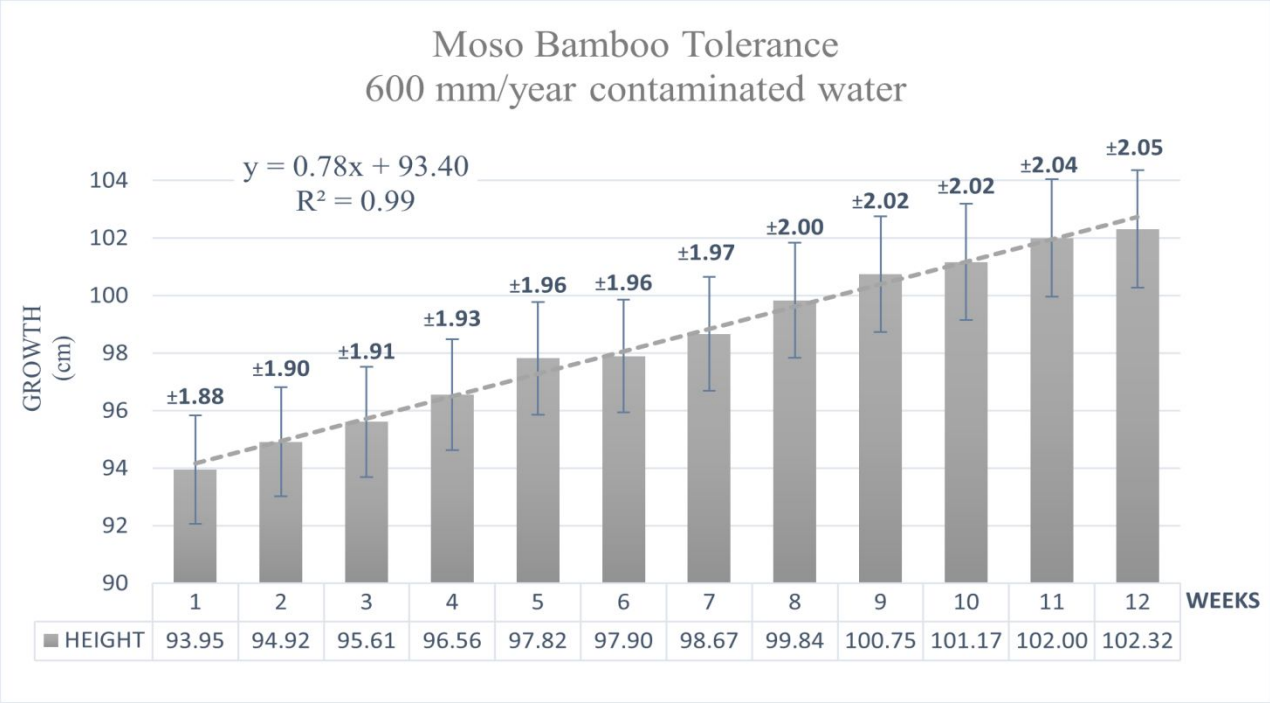

Figure 2 - MB tolerance: growth with 600 mm/year contaminated water with 125 mg Cr/l.

Cr Phytoextraction from The Soil

Cr phytoextraction has been evaluated in the pot starting from a homogenous Cr concentration in soil equal to 200 mg Cr/kg dw. The phytoextraction capacity of bamboo and the soil Cr content after 12 weeks for irrigation with 600 mm/year contaminated water are reported in the Figure 3. The residual level of Cr in the soil after 12 weeks is 114 mg/kg dry weight and approx. 26 mgCr was lost as drainage water from the bottom of the pot and approx. 241 mgCr were absorbed by the plant.

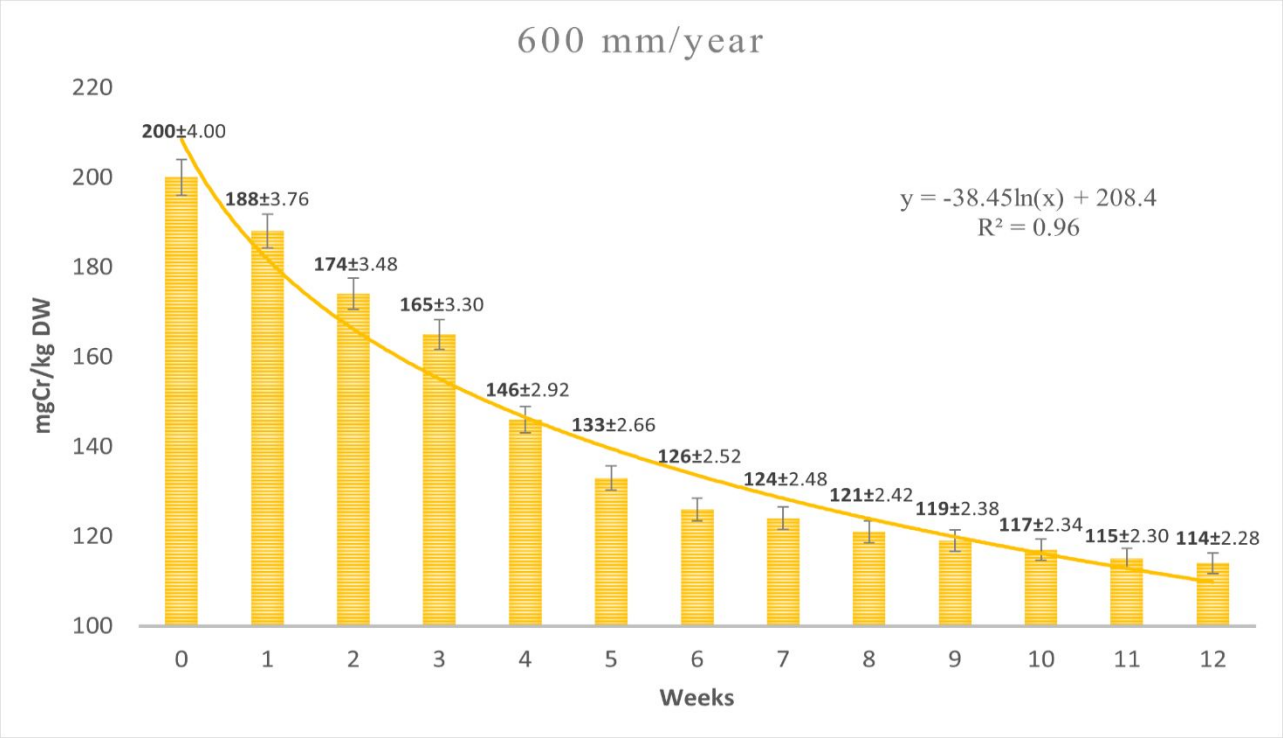

Figure 3 - MB phytoextraction after 12 weeks.

The interpolation curve for the pot was [mg Cr/kg dw] = -38.45ln·(weeks) + 208.4 with R2 = 0.961. Cr removal from soil was 43 %, so it should be possible to extract from soil, in full scale plants, up to 15 kgCr/ha.

The capacity of phytoextraction should be even higher if the soil should have not revealed humic acids inside; Cr tends to form bonds with them, limiting the extraction by decreasing its bioavailability (Carvalho-Pereira et al. 2015; Kalčíková et al. 2016).

Some intercrops can effectively increase the heavy metals accumulation capacity in plant, improve the environmental quality of contaminated soil and the content of nutrients (Bian, et al. 2021) as organic acids released by *MB* roots exudates, in particular oxalic, malic and lactic (Chen, et al. 2016).

Some experiments showed that Moso bamboo intercropped with S. plumbizincicola change the distribution of heavy metals extracted from soils, affected by the planting pattern and not only remediated contaminated soil but also provided high biomass productivity as reported in Tab. 1 (Bian et al. 2017).

Tab. 1 - Biomass of plant tissues and accumulation of Cu, Zn, and Cd in the plants of Monocolture Moso bamboo (MM), intercropped with Sedum plumbizincicola (IMS) and with only S. plumbizincicola (Bian et al. 2017).

Pattern ha^{-1})	Scientific name	Items	Biomass (t ha^{-1})	Cu (kg ha^{-1})	Zn (kg ha^{-1})	Cd (kg
MM	*Phyllostachys pubescens*	Stems	16.68 ± 4.86	0.29 ± 0.09	26.21 ± 7.64	0.07 ± 0.02
		Branches	3.17 ± 0.92	0.07 ± 0.02	5.70 ± 1.65	0.01 ± 0.00
		Leaves	1.95 ± 0.57	0.05 ± 0.01	3.64 ± 1.06	0.01 ± 0.00

IMS	*Phyllostachys pubescens*	Aboveground	21.79 ± 6.35	0.41 ± 0.12	35.55 ± 10.35	0.09 ± 0.03
		Stems	22.21 ± 4.66	0.28 ± 0.06	49.55 ± 10.40	0.12 ± 0.02
		Branches	4.00 ± 0.84	0.14 ± 0.03	10.42 ± 2.19	0.02 ± 0.00
		Leaves	2.48 ± 0.52	0.07 ± 0.02	5.51 ± 1.16	0.02 ± 0.00
	Sedum plumbizincicola	Aboveground	28.69 ± 6.02	0.50 ± 0.10	65.48 ± 13.74	0.15 ± 0.03
		Roots	0.44 ± 0.05	0.01 ± 0.00	1.96 ± 0.23	0.02 ± 0.00
		Leaves	0.64 ± 0.20	0.01 ± 0.00	3.59 ± 1.14	0.03 ± 0.01
		Whole plant	1.08 ± 0.25	0.02 ± 0.01	5.55 ± 1.38	0.05 ± 0.01

Analysis of Cr Levels in the Plant Organs

Total Cr levels, expressed in milligram per kilogram of dry weight for each sample, for, roots, rhizomes, stems and leaves were determined, respectively, after 6 and 12 weeks accumulating, respectively, an overall amount of 175 mg Cr and 287 mg Cr in all organs (Figure 4). The distribution of Cr in bamboo tissues, respectively after 6 and 12 weeks, is also reported in Figure 4 and showing the relative percentages of content. Moso Bamboo (Phyllostachys pubescens) exhibited also a definite trend of absorbing Chromium from soil according to similar experience where lead, copper and chromium were extracted from different contaminated soil and climatic conditions (Mulama, et al. 2020).

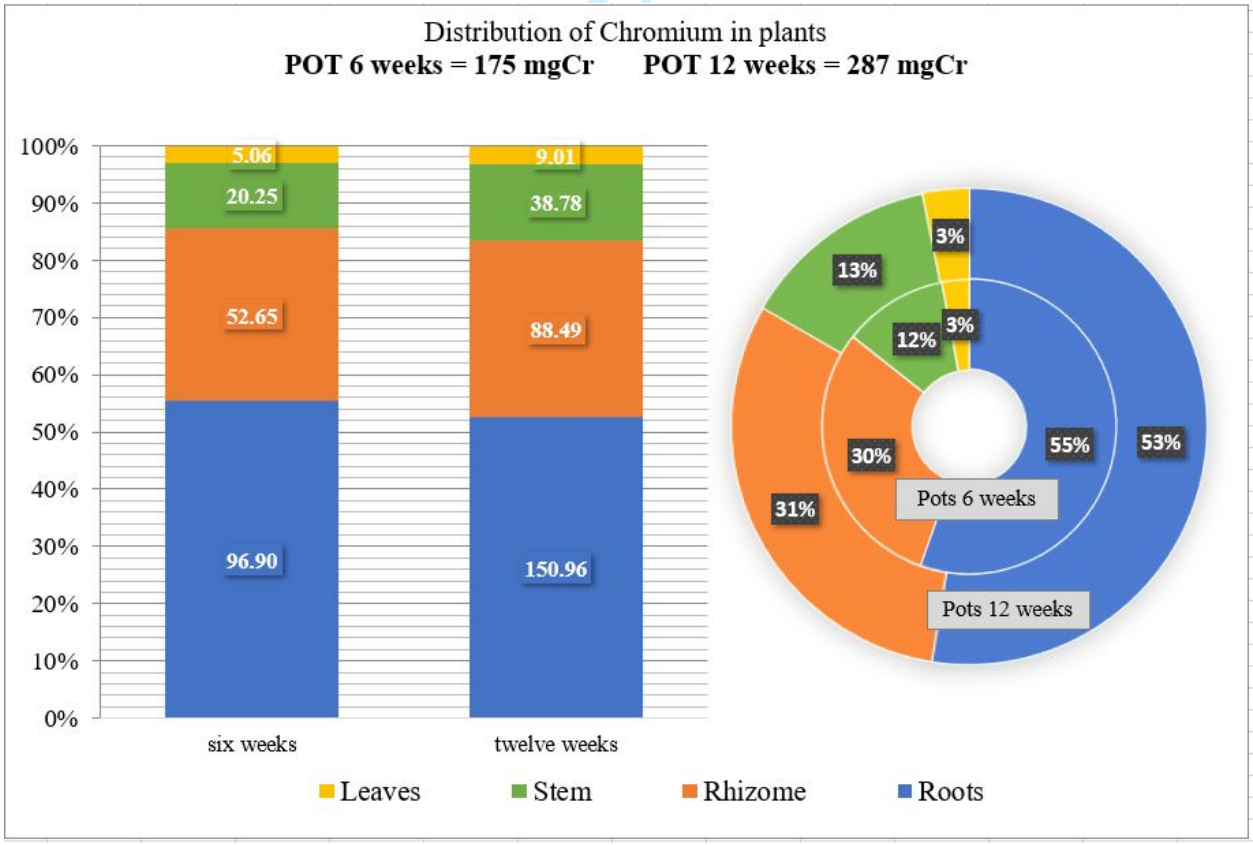

Figure 4 - Cr (mg and %) absorbed by plant organs after 6 and 12 weeks.

As the ratio of Cr mass recollected per plant section mass is concerned, the quantities of Cr per gram of root and rhizome and the amount of Cr per gram of stem and leaves were assessed. Figure 5 shows the quantity of Cr per gram of root/rhizome and stem/leaves after 6 and 12 weeks (mg/g dw).

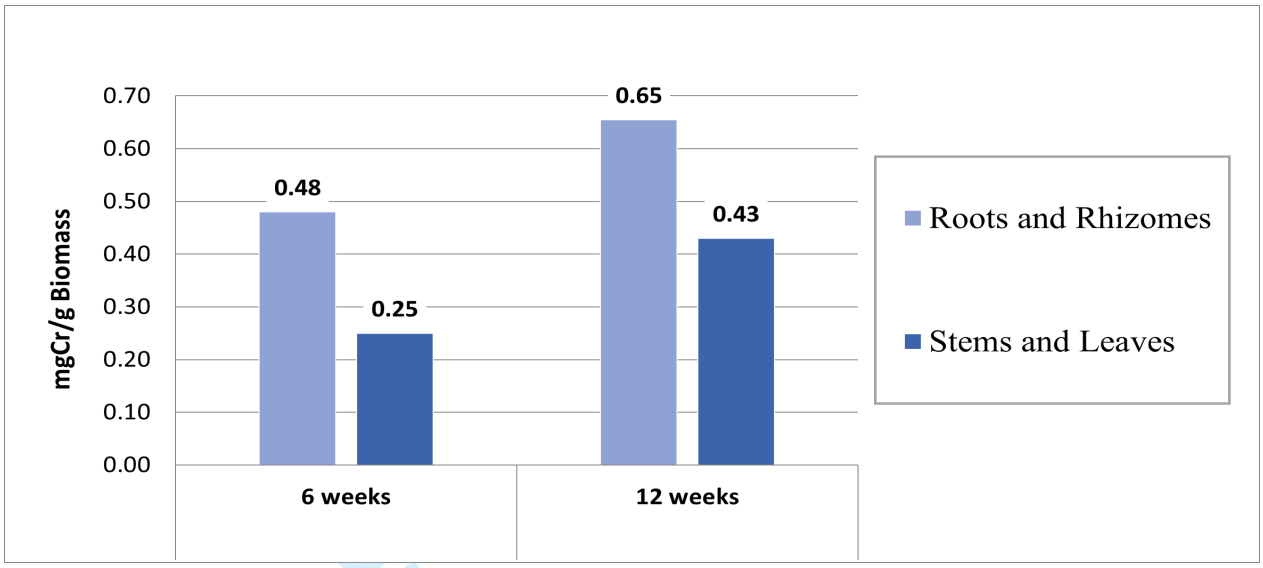

Figure 5 - Quantity of Cr per gram of root/rhizome and stem/leaves after 6 and 12 weeks (mg/g dw).

The amount of Cr per gram of root and rhizome was 0.48 mg/g dw, and the amount of Cr per gram of stem and leaves was 0.25 mg/g dw, after six weeks. The amount of Cr per gram of root and rhizome equal to 0.65 mg/g dw, and the quantity of Cr per gram of stem and leaves was 0.43 mg/g dw, after twelve weeks (Figure 5).

In Tab. 2 are reported the full results collected per each *MB* tissue (Roots, Rhizomes, Stems, Leaves).

Tab. 2 –Concentration of Cr, the g of biomass and the capacity of extraction Cr in the Moso Bamboo after 6 and 12 weeks, respectively.

Items	mg Cr (6 weeks)	mg Cr (12 weeks)	g biomass (6 weeks)	g biomass (12 weeks)	mg Cr/g (6 weeks)	mg Cr/g (12 weeks)
Roots	96.9	150.96	170	204	0.57	0.74
Rhizomes	52.65	88.49	135	155.3	0.39	0.57
Stems	20.25	38.78	75	82.5	0.27	0.47
Leaves	5.06	9.01	22	23.1	0.23	0.39

As shown *MB* can accumulate a large amount of Cr, in particular in the root system. Chromium is distributed in the cell wall, vacuole, and cytoplasm and in excessive concentrations they cause excessive stress and damage to bamboo plants and this can enhance the tendency to the metal translocation among the tissues (Bian et al. 2020). Vernay et al. (2007) and Shahid et al. (2017) report that *MB* retains Cr mainly in the rhizome-root apparatus by limiting translocation in the aerial plant including leaves. The high concentration in the rhizome and root and the low translocation in the stems and leaves should reduce, in this case, Chromium transfer from the plant to the highest trophic levels. As shown in Tab. 2 the capacity of Cr translocation in the aerial parts was remarkable but inferior to the *MB* growth rate. Therefore, the Translocation Factor (TF) and Bioconcentration Factor (BCF) were calculated. Aerial and root-rhizome Cr accumulation, bioconcentration factor (BCF), translocation factor (TF) were calculated by following expressions (Gautam et al., 2017; Ullah et al., 2019; Ullah et al., 2021):

BCF = Cr contents in plant organs / Cr contents in treated soil = 287/456 = 0.63

TF = Cr concentration in aerial parts / Cr concentration in root-rhizome = 48/240 = 0.20

These values are quite relevant and similar to the values of translocations reported by Chen et al. (2015a) and Chen et al. (2015b) for Zn in Moso Bamboo where they have shown the capability of Moso Bamboo to accumulate high level of Zn (Tab. 3).

Tab.3 – Zn concentrations in plant tissues of Moso Bamboo (Chen, et al., 2015b).

Zn	Roots	Stem	Leaves	Shoot	
μM	mg/kg	mg/kg	mg/kg	mg/kg	Shoot/root
0	40 ± 11	142 ± 9	27 ± 1	68 ± 4	—
10	2329 ± 319	889 ± 58	490 ± 310	630 ± 185	0.27
25	3920 ± 281	1234 ± 267	425 ± 96a	707 ± 100	0.18
50	5515 ± 1084	1628 ± 85	875 ± 355	1139 ± 227	0.21
100	6758 ± 512	2471 ± 270	1076 ± 333	1594 ± 314	0.24
200	8133 ± 432	1524 ± 292	696 ± 137	1031 ± 221	0.13
400	8642 ± 2550	2012 ± 205	1039 ± 180	1443 ± 147	0.17

The significant Cr accumulation in plant shows the *MB* great capacity of phytoextraction, but this result should be validated also outside the lab conditions in full scale ecosystem (Go et al. 2021; Srivastava, et al. 2021).

4. Conclusions

A pot experiment was carried out in laboratory, in a controlled environment, in simulated Mediterranean conditions, at a temperature of 20°C, in order to evaluate *MB* suitability to restore Cr-contaminated sites.

Tolerance test results have showed a good response of the plant up to 125 mg Cr/l solution utilized for irrigation. In fact, it was possible to note that, at the concentration of irrigation water 125 mg Cr/l, even if the growth rate was considerably reduced, the bamboo plant still maintained his vegetative functions. Moreover, the *MB* plant did not show any evidence of malformation and not significant damages to the plant tissues were observed.

Phytoextraction tests were then performed and Cr removal from soil was 43% starting from a Cr content of approx. 200 mg/kg dry weight. Results show that the aerial parts of the plant exhibited little Cr concentrations but growing with time. Cr accumulation was found to be significant and to concentrate the most in the roots/rhizomes indicating an overall phytoextraction potential of the plant.

The quantity of Cr per gram of root and rhizome was equal to 0.65 mg/g dw, while the quantity of Cr per gram of stem and leaves was equal to 0.43 mg/g dw, after 12 weeks.

Pot experiments show that phytoremediation using *MB* provides an alternative approach for handling Cr contaminated soil. Future experimentations under contaminated field conditions are demanded to further verify the findings of this study.

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Contribution Statement

Ezio Ranieri: Conceptualization and editing; Gianfranco D'Onghia: review and theory; Francesca Ranieri: formal analysis; Barbara Cosanti: writing original draft; Ada Cristina Ranieri:Methodology and bibliography.

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Author Disclosure Statement

The Authors declare no conflict of interest

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