

Phytoremediation using *Phyllostachys pubescens* (Moso Bamboo) to reduce the risk of chromium exposure

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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 Cr-contaminated soil.

In order to evaluate the potential for phytoremediation using MB to restore Cr-contaminated soil, pot experiments were carried out in simulated Mediterranean conditions, in laboratory, in a controlled environment, at a temperature of 20°C.

Cr removal from soil was 43 % starting from a Cr content of approx. 200 mg/kg Dry Weight 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.

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. 2016; Ranieri et al. 2020a).

Chromium 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).

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) is revealed the most toxic form, as it 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 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 Chromium 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. Literature results show that, independently from

tested Cr form, Cr mainly accumulates in roots, followed by stems and leaves and only small amounts of Cr are translocated to leaves (Oliveira, 2012).

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 (Favas et al., 2014; Were et al. 2017; Zayed & Terry, 2003; Sunitha et al., 2017; Bosire, 2014; Muraje, 2009; Yoon, 2006).

Phytoremediation success largely depends on the characteristics of the plant to be utilized and the contaminants present in the ecosystem.

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).

However, phytoremediation is a time-limited technology, in fact, its wide use has been restricted by climatic conditions (Chen et al, 2015a; Song et al., 2013).

MB is part of grass family, Graminae (Poaceae) with the aptitude to persist even in the worst soil and climatic conditions (Rojo et al., 2000; Roxas, 2012; Chua et al., 2019).

MB, for example, is recognized as phytoremediation plant due to production of huge biomass and high tolerance in stressed environment (Chen et al., 2015a).

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).

The aim of this research was to investigate the ability of MB for enhancing the phytoremediation of chromium-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 antioxidatives in MB under Cr stress.

Material And Methods

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

The experiment was carried out in simulated Mediterranean conditions, in laboratory, in a controlled environment at a temperature of 20°C. Preliminary tests were carried out, in laboratory, 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., 2020).

A *MB* plant was 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 cm² 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, approximately 20% and 1% of dry weight.

For the irrigation, it was used tap water with the following chemical characteristics:

- 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.

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. Therefore, given the considered rainfall regime and the diameter of the vessel of the sample, a flow of 1648 mm/day = 0.0805 l/day was used.

The *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 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, HCl, (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 constants 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).

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.

Bamboo tolerance in chromium contaminated soils can be assessed by measuring the plant growth in a soil contaminated by a chromium solution. In this study, *MB* tolerance was assessed by measuring his growth with irrigation with a solution of 100 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 100 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 1 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 by using a computer aided drawing.

It was possible to note that, at the concentration of irrigation water 100 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 $h = 0.7778 \cdot (\text{weeks}) + 93.404$ with $R^2 = 0.9913$.

ANALYSIS OF Cr LEVELS IN THE PLANT ORGANS

Total Cr levels, expressed in milligram per kilogram of dry weight for each sample, for rhizosphere soils, roots, rhizomes, stems and leaves were determined, respectively, after 6 and 12 weeks of growth.

The distribution of Cr in bamboo tissues, respectively after 6 and 12 weeks, is reported in Figure 2 and Figure 3 shows the relative percentages. Similar percentages of chromium distribution in the different plant tissues were observed after 6 and 12 weeks.

Inside the plant matters, roots accumulated the maximum concentration of Chromium representing a phytostabilization/phytodegradation potential of the MB.

As the ratio of Cr mass recollected per plant section mass is concerned, the quantity of Cr per gram of root and rhizome and the amount of Chromium per gram of stem and leaves were assessed. The concentrations were reported in milligram per gram of dry weight (mg/g dw) of the corresponding samples after six and twelve weeks of growing phase.

The amount of Chromium per gram of root and rhizome was 0.96 mg/g dw, and the amount of Cr per gram of stem and leaves was 0.5 mg/g dw, after six weeks.

The amount of Chromium per gram of root and rhizome was 1.31 mg/g dw, and the quantity of Cr per gram of stem and leaves was 0.86 mg/g dw, after twelve weeks. Data are reported in Figure 4.

it is assumed that MB retains Cr mainly in the rhizome-root apparatus by limiting translocation in the aerial plant including leaves. (Vernay et al. 2007; Shahid et al. 2017).

Cr PHYTOEXTRACTION FROM THE SOIL

The phytoextraction capacity of bamboo and the soil chromium content after 13 weeks for irrigation with 600 mm/year contaminated water are reported in the Figure 5. The residual level of Cr in the soil after 13 weeks is 114 mg/kg dry weight.

The interpolation curve for the pot was $y = -38.45 \ln \cdot (\text{weeks}) + 208.4$ with $R^2 = 0.961$.

Cr removal from soil was 43 % starting from a Cr content of approx. 200 mg/kg Dry Weight. 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).

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 100 mg Cr/l solution utilized for irrigation. In fact, it was possible to note that, at the concentration of irrigation water 100 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.

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. Chromium accumulation was found to concentrate the most in the roots indicating a phytostabilization/phytodegradation potential of the plant: 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 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.

Declarations

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Authors Contributions

The contribution of the authors: E.R., B.C., A.C.R. is parithetic

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Competing Interests

The Authors declare no conflict of interest

Availability of data and materials

Not applicable

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Figures

Moso Bamboo Tolerance 600 mm/year contaminated water

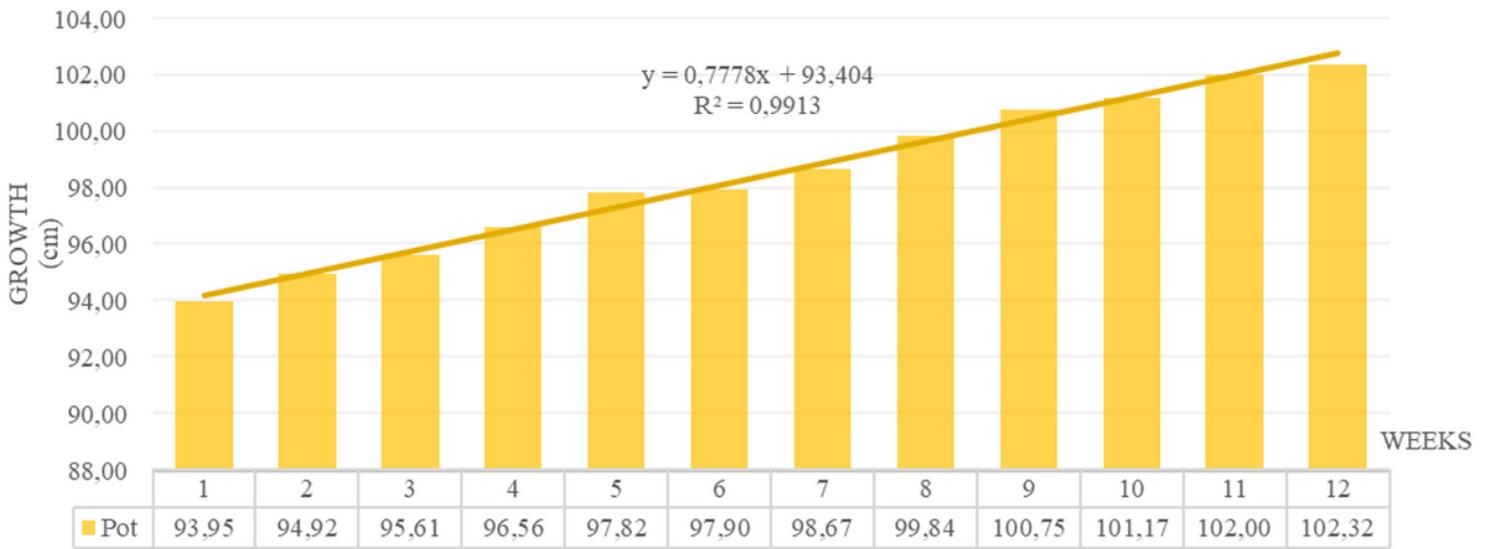


Figure 1

MB tolerance: growth with 600 mm/year contaminated water.

Distribution of Cr in plant organs
 6 weeks = 175 mgCr 12 weeks = 287 mgCr

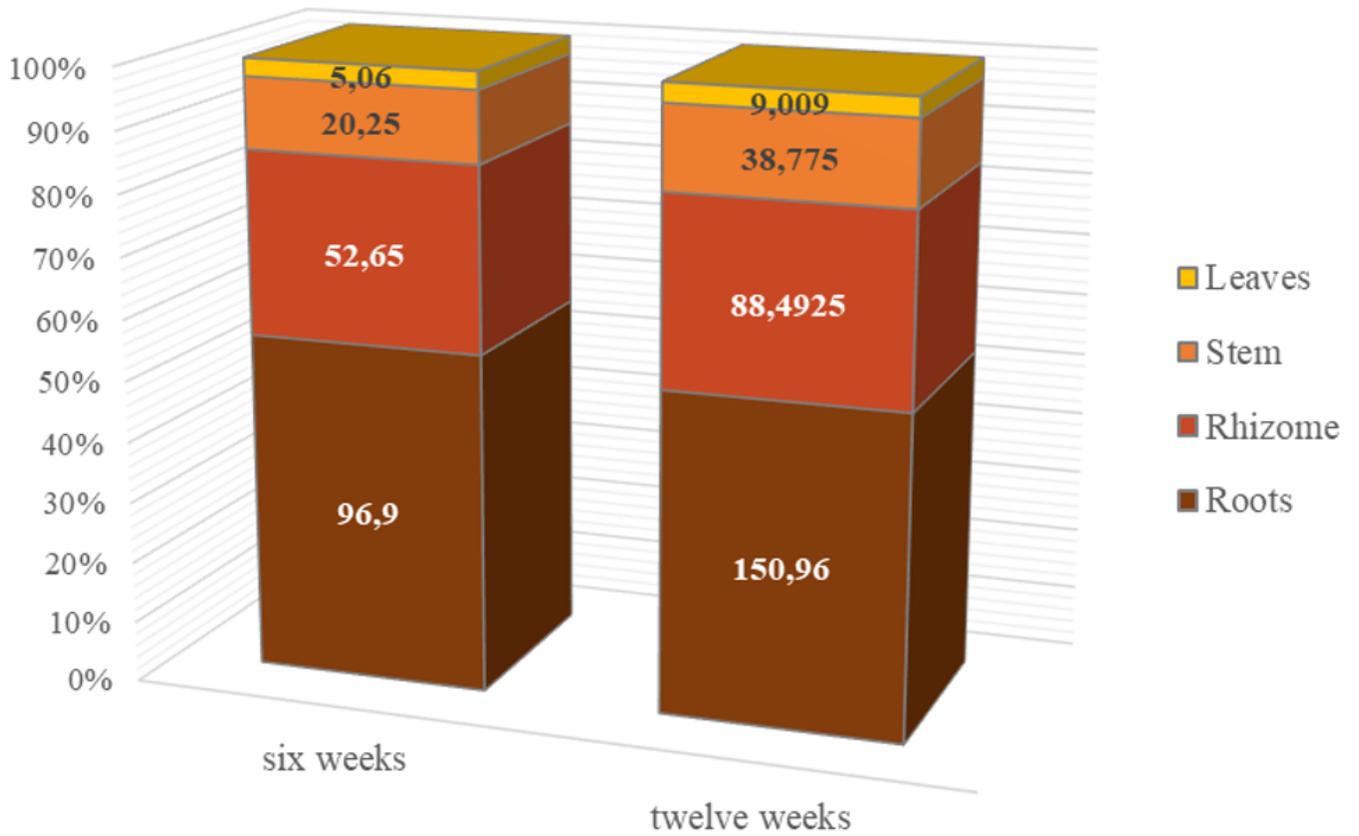


Figure 2

Chromium (mg and %) absorbed by plant organs after 6 weeks.

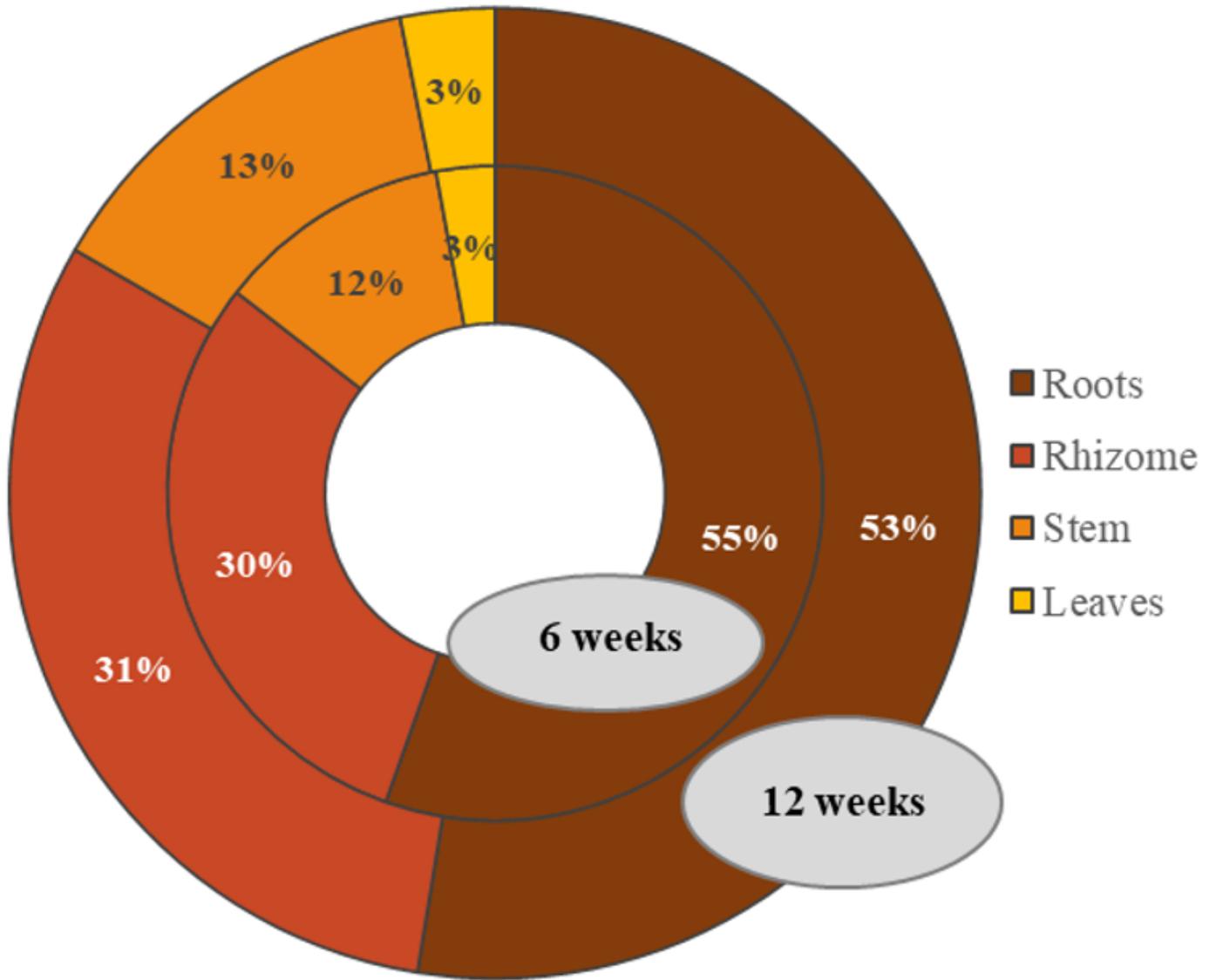


Figure 3

Chromium (mg and %) absorbed by plant organs after 12 weeks.

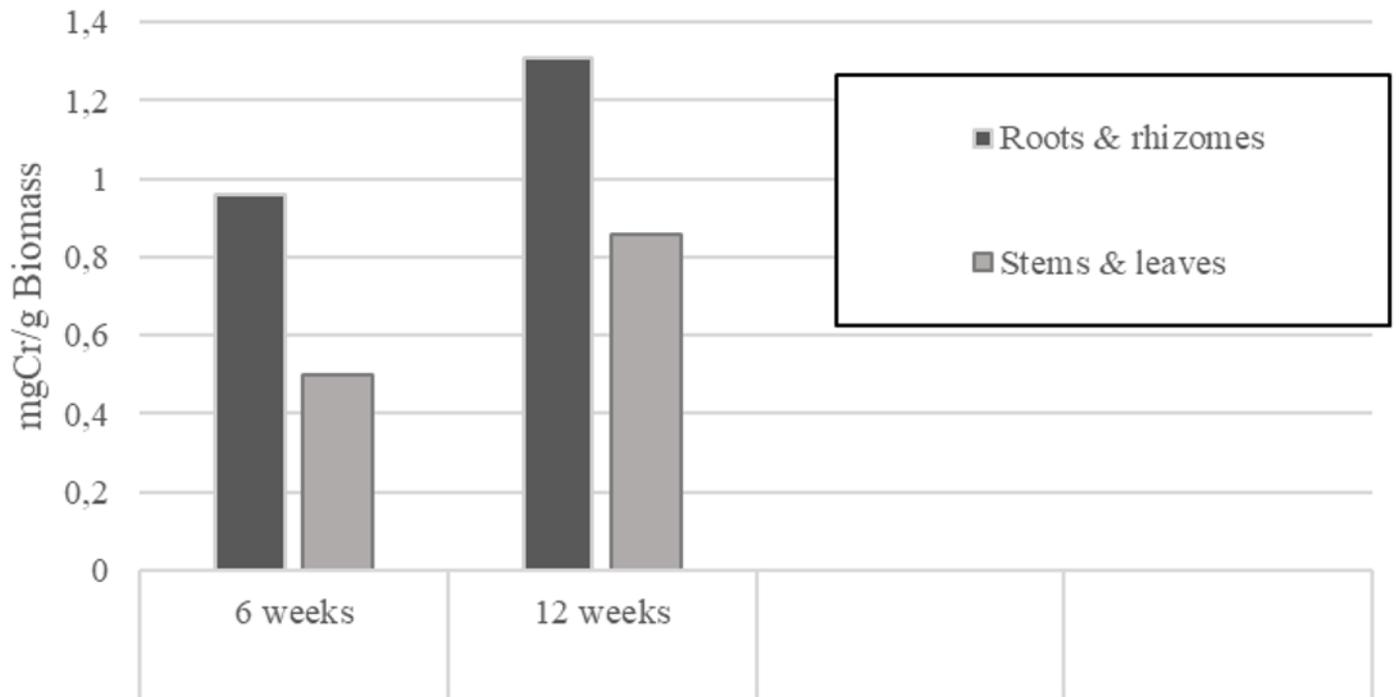


Figure 4

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

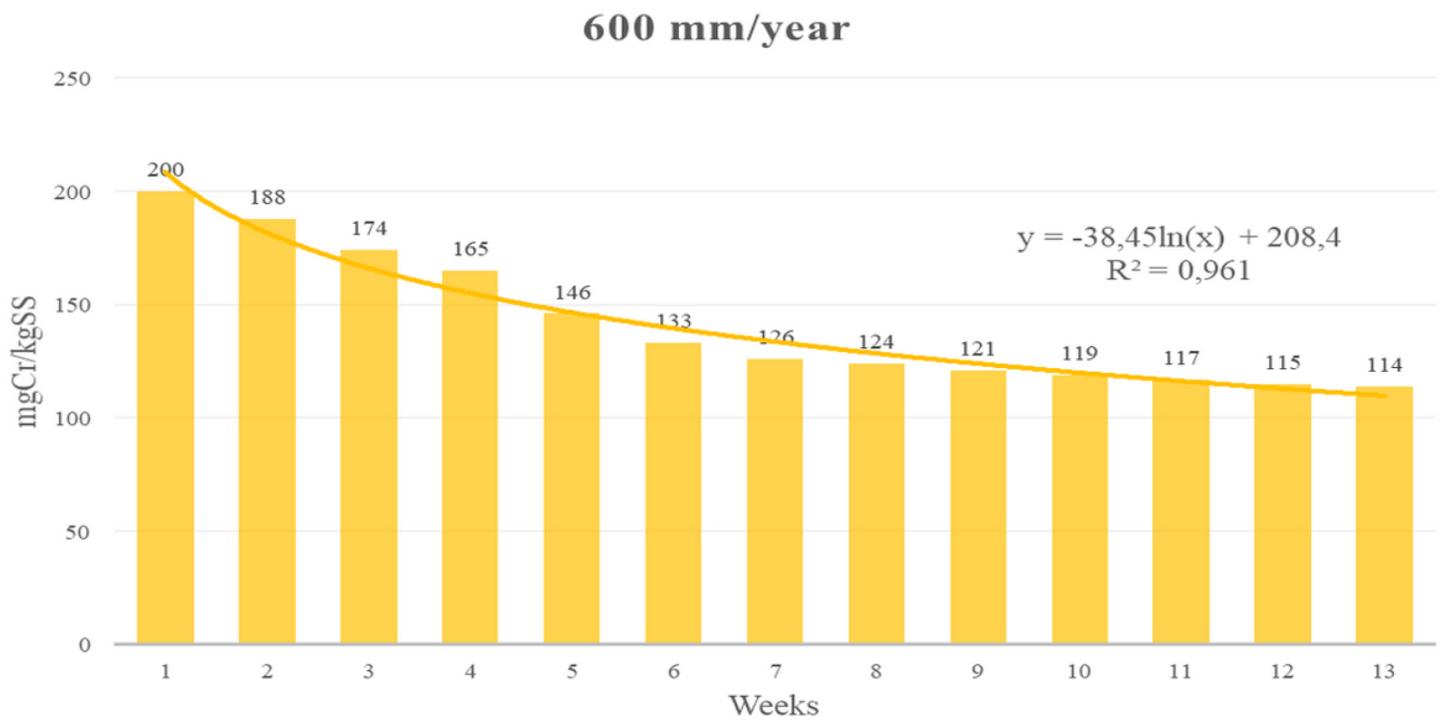


Figure 5

MB phytoextraction: growth corresponding to irrigation with 600 mm/year contaminated water.