EFFECTS ON GROWTH, PRODUCTIVITY AND BIOMASS QUALITY OF *MISCANTHUS* X *GIGANTEUS* OF SOILS CONTAMINATED WITH HEAVY METALS

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ABSTRACT: The aim of this work was to study the effects of different heavy metals on growth, productivity and biomass quality of *Miscanthus* x *giganteus*. The plants were placed in pots, where of soils were contaminated, differently, with Cd, Cu, Ni, Pb, Zn, Hg and Cr. For each metal, two levels of contamination were tested. The results lead to the conclusion that in terms of the productivity and in what concerns the height of the plants, there were significant differences among the plants obtained in pots with the different heavy metals contamination. Cd, Cu and Hg were the metals that negatively affected, more significantly, the productivities obtained. In contrast, *Miscanthus* showed high tolerance to Ni, Pb, Zn and Cr soil contamination. There was a significant influence of those heavy metals contamination on the uptake and distribution of phosphorus in the *Miscanthus* biomass, but not on nitrogen content. Ash content was also influenced by soils heavy metals contamination. Overall, *Miscanthus* is able to remove and accumulate heavy metals from contaminated soils, but the highest proportion of those metals taken up by plants remain in the rhizome and roots.
Keywords: miscanthus, bioremediation, biomass composition

1 INTRODUCTION

In the context of the European *Miscanthus* Productivity Network, *Miscanthus* x *giganteus* was introduced in Portugal in July 1990 [1]. *Miscanthus* is a promising crop, yielding high quality lignocellulosic material for both energy and fiber production [2]. It is characterized by relatively high yields, low moisture content at harvest, high water and nitrogen use efficiencies and an apparently low susceptibility to pests and diseases [2]. The main objective of this Network, was to generate information about the potential of *Miscanthus* as a non-food crop in Europe. Subsequently, several studies have already been conducted at UBiA, in the Faculty of Sciences and Technology of the New University of Lisbon, to evaluate the capacity of *Miscanthus* to phytoremediate soils contaminated with heavy metals, namely soils contaminated with sewage sludge [3, 4, 5]. Following this context, the main objective of this work was to study the effects of different heavy metals on growth, productivity and biomass quality of *Miscanthus* x *giganteus,* in order to access if there are specific patterns of toxicity involved in each metal metabolism and translocation in the plants.

2 MATERIALS AND METHODS

At the beginning of April 2000, plants of *Miscanthus* x *giganteus* were placed in pots (2 plants per pot), containing a clay and alkaline soil contaminated, differently, with Cd, Cu, Ni, Pb, Zn, Hg and Cr. For each metal, two levels of contamination were tested, L_1 and L_2, in a duplicate trial. The levels of contamination tested were chosen according to the decree 176/96, that establish the limit-values for the application of Cd, Cu, Ni, Pb, Zn, Hg and Cr, in soils. The low level of contamination, for each metal, was ½ of the limit-value, and the high level of contamination was ⅔ of that limit-value. A control test was also carried out. Table I shows for each metal, the levels of contamination tested. For the heavy metal contamination of the pots, saline solutions were used. P-fertilizer (10 g $P.m^{-2}$), K-fertilizer (14 g $K.m^{-2}$) and N-fertilizer (6 g $N.m^{-2}$) were equally applied to all the pots. In order to compensate water deficit of the soil and to prevent water stress, irrigation with a 1.2 dm^3 of tap water was applied three times a week. All the water leached out of the pots was collected and added to the following irrigation.

Table I: Levels of contamination tested ($g.m^{-2}$) for each metal studied.

Metal	L_1	L_2
Cd	0.0075	0.010
Cu	0.6	0.8
Ni	0.15	0.2
Pb	0.75	1.0
Zn	1.5	2.0
Hg	0.005	0.0067
Cr	0.225	0.3

At the end of the growing season (December), the plants were harvested and the following biometric parameters were determined: height of the plants and number of tillers per plant. To determinate the productivity of the biomass, the total aerial dry weight and the total below-ground dry weight (rhizomes and roots) was determined. The quality of the biomass (aerial and below-ground) was analysed taking in consideration the following parameters: ash content, nitrogen content, phosphorus content and Cd, Cu, Ni, Pb, Zn, Hg and Cr content.

The chemical analysis were performed according to the following procedures: a) ash content: by calcination at 550ºC for two hours, in a muffler furnace; b) nitrogen content: by the Kjeldahl method; c) phosphorus content: by the ascorbic acid method, after digestion of the sample; d) metals: by atomic absorption after digestion of the ashes with nitric acid.

3 RESULTS AND DISCUSSION

3.1 Biometric Parameters

Table II presents the effect of the heavy metal contamination on the plant height and on the number of tillers per plant, in comparison with the control.

Table II: Height of the plants (cm) and number of tillers per plant, at the end of the growing period*.

Treatments	Plant height	No Tillers
Control	102	3
Cd	80 (-22)	2 (-50)
Cu	43 (-58)	1 (-67)
Ni	85 (-17)	3 (-17)
Pb	101 (-1)	4 (33)
Zn	106 (4)	4 (33)
Hg	107 (5)	3 (0)
Cr	115 (12)	3 (-17)
Average without control	91 (-11)	3 (-12)

*Values within brackets represent the effect of the different heavy metals on plant height and tillers number, expressed as the relative reduction or increase in comparison with control (%).

The reaction of *Miscanthus* plants to heavy metal toxicity can be, globally, characterized by a reduction of the plant height and of the tillers number per plant (Table II). However, this reaction was significantly different among different heavy metals. Cd, Cu and Ni were the metals that affected more negatively those parameters. The other metals didn't affect significantly those parameters.

3.2 Biomass Productivity

Table III shows the *Miscanthus* biomass productivity (aerial and below-ground) obtained under different treatments applied.

Table III: Shoot and rhizome + root dry weight ($g.m^{-2}$), of the different treatments, at the end of the growing period*.

Treatments	Shoot dry weight	Rhizome + Root dry weight
Control	366	988
Cd	245 (-33)	1247 (26)
Cu	180 (-51)	709 (-28)
Ni	368 (1)	925 (-6)
Pb	522 (43)	1236 (25)
Zn	458 (25)	1088 (10)
Hg	344 (-6)	647 (-35)
Cr	406 (11)	917 (-7)

*Values within brackets represent the effect of the different heavy metals on biomass productivity, expressed as the relative reduction or increase in comparison with control (%).

As shown is Table III, shoot dry weights were more negatively affected by Cd and Cu contamination, and rhizome and roots were more negatively affected by Cu and Hg contamination. In contrast, shoot dry weights were positively affected by Pb and Zn contamination, and the rhizome and roots were positively affected by Cd and Pb contamination.

3.3 Biomass Quality

Tables IV, V and VI show the effects of the different heavy metals treatments on the composition of the *Miscanthus* biomass, in terms of the ash, nitrogen and phosphorus content.

Table IV: Ash content of shoot and rhizome + root (% dry matter), of the different treatments*.

Treatments	Shoot	Rhizome + Root
Control	6.9	5.4
Cd	8.0 (16)	7.9 (45)
Cu	8.9 (29)	6.7 (23)
Ni	7.3 (6)	5.7 (5)
Pb	6.1 (-12)	4.0 (-27)
Zn	6.3 (-9)	4.4 (-19)
Hg	7.0 (2)	3.8 (-31)
Cr	7.2 (4)	4.9 (-10)

*Values within brackets represent the effect of the different heavy metals on the ash content, expressed as the relative reduction or increase in comparison with control (%).

Table V: Nitrogen content of shoot and rhizome + root (% dry matter), of the different treatments*.

Treatments	Shoot	Rhizome + Root
Control	0.31	0.44
Cd	0.27 (-13)	0.37 (-17)
Cu	0.40 (27)	0.43 (-2)
Ni	0.32 (3)	0.49 (10)
Pb	0.32 (3)	0.48 (8)
Zn	0.29 (-8)	0.49 (10)
Hg	0.31 (-2)	0.46 (3)
Cr	0.29 (-8)	0.45 (2)

*Values within brackets represent the effect of the different heavy metals on the nitrogen content, expressed as the relative reduction or increase in comparison with control (%).

Table VI: Phosphorus content of shoot and rhizome + root (% dry matter), of the different treatments*.

Treatments	Shoot	Rhizome + Root
Control	0.065	0.083
Cd	0.066 (2)	0.076 (-8)
Cu	0.078 (20)	0.097 (16)
Ni	0.065 (0)	0.107 (29)
Pb	0.055 (-15)	0.086 (3)
Zn	0.055 (-16)	0.089 (7)
Hg	0.080 (23)	0.084 (1)
Cr	0.076 (17)	0.096 (16)

*Values within brackets represent the effect of the different heavy metals on the phosphorus content, expressed as the relative reduction or increase in comparison with control (%).

According to these results, Cd and Cu contamination induced a higher ash content in the biomass of *Miscanthus*. Roots and rhizomes from the pots contaminated with Pb, Zn and Hg presented lower ash content than control. The other metals didn't affect this parameter.

Overall, contamination of the soils with heavy metals didn't influence the nitrogen content of *Miscanthus*.

However, shoots of *Miscanthus* obtained in the pots contaminated with Cu, showed higher nitrogen content than the control and roots and rhizomes of *Miscanthus* obtained in the pots contaminated with Cd, showed lower nitrogen content than control.

There was a significant influence of heavy metals contamination on the uptake and distribution of phosphorus in the *Miscanthus* biomass. In comparison with control, *Miscanthus* obtained from pots contaminated with Cu and Cr, presented a higher content of P. Contamination with Hg, induced a higher accumulation of P in the shoots and contamination with Ni, induced a higher accumulation of P in the roots and rhizomes. Pb and Zn contamination induced a lower accumulation of P in the shoots.

Table VII shows the heavy metal accumulation in *Miscanthus* biomass with the increasing heavy metal concentrations in the soil.

Table VII: Heavy metals concentration (mg.kg^{-1} dry matter) of shoot and rhizome + root, in comparison with the control (values in brackets).

Treatment		Shoot		Rhizome + Root	
Cd (L_1)	[Cd]	<DL	(<DL)	0.044	(<DL)
Cd (L_2)		0.013		0.116	
Cu (L_1)	[Cu]	6.57	(6.18)	5.67	(4.07)
Cu (L_2)		5.86		5.38	
Ni (L_1)	[Ni]	1.29	(2.10)	1.21	(1.13)
Ni (L_2)		2.05		1.80	
Pb (L_1)	[Pb]	2.26	(1.21)	0.85	(0.42)
Pb (L_2)		0.84		1.69	
Zn (L_1)	[Zn]	75.4	(81.8)	16.1	(19.7)
Zn (L_2)		62.2		20.8	
Hg (L_1)	[Hg]	<DL	(<DL)	0.009	(<DL)
Hg (L_2)		0.004		0.023	
Cr (L_1)	[Cr]	2.57	(2.05)	3.07	(0.65)
Cr (L_2)		2.70		12.1	

DL – detection limit

According to the results presented in Table VII, heavy metals accumulation in rhizome and roots followed the increasing heavy metal concentrations of the soil, for all the metals studied. Only in the Zn treatment (L_1) the amount of zinc in the rhizome and roots were lower than in the control. In comparison with control, the accumulation of Cd, Pb, Hg and Cr were those where increase was most significative.

But heavy metals accumulation in the aerial fraction did’t follow the same pattern. Cd, Hg and Cr induced an increase in the accumulation of those metals in the shoots. In contrast, Ni and Zn induced a decrease in the accumulation of those metals in the shoots. For L_1 level of Cu and Pb, shoots presented a higher accumulation in these metals than control, but for L_2, the accumulation was lower than control. However, the differences observed between shoots from contaminated pots and shoots of control are not statistically significant.

According to these results, it is possible to conclude, that the proportion of the heavy metals taken up by plants remaining in the rhizome and roots is higher. This was in agreement with a number of other reports, which indicated that metals accumulated more in the below-ground fraction of *Miscanthus* than in the abovegroung fraction [3, 4, 5].

3.4 Effect of Cd

Miscanthus showed high tolerance to Cd toxicity in terms of the productivity of the below-ground fraction of the plant. Effectively, although Cd concentration in rhizome and roots increased with concentrations of Cd in the soil, the rhizome and roots dry weight increased also. Such tolerance might be due to multiple mechanisms, such as complexation or precipitation of the toxic metal, sequestration of the metal in certain compartments of the cell or development of a metabolic system efficient even in the presence of a high concentration of the metal [6]. Those mechanisms may prevent Cd interference with sensitive sites of cellular metabolism of rhizomes and roots. In contrast, *Miscanthus* showed low tolerance to Cd toxicity in terms of the biometric parameters and in terms of the aerial biomass productivity. The increased accumulation of Cd in shoots with increasing Cd concentrations in the soil induced a reduction in plant height and shoot dry weight. Cd contamination influenced significantly the ash content of the biomass, that was higher than in the control. Cd contamination also influence the uptake of nitrogen from the soil. The N-content of rhizomes and roots were lower than in control, although in the aerial biomass no differences were observed. Cd didn’t influence phosphorus accumulation.

3.5 Effect of Cu

Miscanthus presented low tolerance to Cu toxicity in terms of the growth parameters and in terms of the biomass productivity. The increased Cu addition to the soil led to a sharp reduction in plant height and shoot, rhizome and root dry weight. An increase of Cu concentration in the biomass was also observed, except in the L_2 level of contamination where Cu concentration was lower than in the control. Maybe that at higher levels of Cu contamination, the plant has a resistance mechanism that exclude the translocation of the toxic metal to the aboveground fraction of the plant. Accumulation of Cu in the biomass showed a synergy with the uptake of nitrogen and phosphorus. In fact, N-content and P-content increased in the biomass, in comparison with the control. Ash content was also significantly higher in the biomass obtained from the Cu contaminated pots than from control.

3.6 Effect of Ni

Miscanthus showed high tolerance to Ni toxicity in terms of biomass productivity. However, growth parameters were somewhat affected by this metal. This tolerance can be explained by an exclusion mechanism. In fact, with increasing Ni concentrations in the soil, rhizomes and roots showed only a relatively light accumulation of Ni than in the control (not significant), and shoots showed a lower Ni concentration than in the control. The exclusion mechanism can also explain why Ni contamination of the soils didn´t affect the ash content of the biomass and the N-uptake by the plants. P-uptake from the soil by rhizome and roots was higher than in the control showing a synergystic effect with the increase of Ni in the below-ground fraction. But translocation of this excess of P to the shoots was not observed, like what was observed with the Ni.

3.7 Effect of Pb

Miscanthus presented high tolerance to Pb toxicity in terms of the growth parameters and in terms of the biomass productivity. Effectively, although Pb concentration in biomass increased with increasing Pb concentrations in the soil, the plant height was not affected and the biomass dry weight increased. As reported to Cd, such tolerance might be due to multiple mechanisms [6]. Those mechanisms prevented Pb from interfering with sensitive sites of cellular metabolism of *Miscanthus*. However, the accumulation of Pb by *Miscanthus* biomass affected the P content of the aerial fraction of the plants, that was lower than in the control. Pb accumulation also affected ash content of the roots and rhizomes, being significantly lower than in the control. Consequently, Pb accumulation can be responsible for mineral deficiencies. But this lower ash and P content might be also due to the higher productivities observed in the Pb contaminated pots. Pb accumulation didn´t affect the nitrogen content.

3.8 Effect of Zn

Miscanthus showed high tolerance to Zn toxicity in terms of growth parameters and in terms of biomass productivity. This tolerance can be explained by an exclusion mechanism, like for Ni. Effectively, with the increasing Zn concentrations in the soil, rhizomes and roots showed only a somewhat higher accumulation of Zn than in the control, not significant statistically, and shoots showed a lower Zn concentration than in the control. In terms of the nitrogen content, Zn contamination didn't affect this parameter. But, in contrast, ash content of the biomass and P content of the shoots were lower than in the control. This effect might not be due to the Zn contamination of the soils but to the higher productivities observed in these pots, that induce a dilution on the P and mineral contents at the plants biomass.

3.9 Effect of Hg

Miscanthus showed low tolerance to Hg toxicity in terms of biomass productivity. Effectively, with the increasing Hg concentrations in the soil, biomass showed a significant higher accumulation of Hg, and a lower production, in comparison with the control. But, growth parameters were not affected by the Hg contamination. Hg contamination didn't affect the nitrogen content of the biomass. This contamination showed a synergetic effect in relation to the P content in the shoots, that was higher than in the control. In contrast, the contamination induced a lower ash content in the biomass material, in comparison with the control.

3.10 Effect of Cr

Miscanthus showed high tolerance to Cr toxicity, in terms of growth parameters and in terms of biomass productivity. Effectively, although Cr concentration in the biomass increased with higher Cr concentrations in the soil, plant height and shoots, rhizome and roots dry weight were not affected. As explained for Cd and Pb, this tolerance might be due to multiple mechanisms that prevent Cr from interfering with sensitive sites of the cellular metabolism of *Miscanthus* [6]. The accumulation of Cr and P by *Miscanthus* biomass was synergistic. P content of the biomass obtained in Cr contaminated pots was higher than, in the control. Cr accumulation didn't affect ash content and nitrogen content.

4 CONCLUSIONS

Miscanthus showed high tolerance to Ni, Pb, Zn and Cr contamination of soils but low tolerance to Cd, Cu and Hg contamination. This means that *Miscanthus* can be grown in fields contaminated with Ni, Pb, Zn and Cr without a reduction in terms of its productivity, and consequently in its economic value. *Miscanthus* can also be grown in fields contaminated with Cd, Cu and Hg, but only for soil remediation purposes, because economically might not be feasible due to reduction of its productivities.

Miscanthus tolerance mechanisms were apparently different according to toxic metal. For Ni and Zn contamination *Miscanthus* eventually showed an exclusion mechanism as a way to resiste to the metal toxicity. In the cases of the Pb and Cr contamination, this tolerance might be due to other mechanisms, such as complexation or precipitation of the toxic metal, sequestration of the metal in certain compartments of the cell or development of a metabolic system that is efficient even in the presence of a high concentration of the toxic metal.

Overall, *Miscanthus* is able to remove and to accumulate heavy metals from contaminated soils, but the highest proportion of the heavy metals taken up by plants remain in the rhizome and roots. This fact, makes possible the utilisation of the biomass obtained in those contaminated fields, for example for paper pulp production or energy production. This will represent a real contribution to its economical value, in terms of a sustainable agriculture strategy.

Further studies are needed for making clear the interaction among heavy metals and other nutrients like Ca, K, Mg, in their uptake and translocation by *Miscanthus* plants.

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