EFFECTS ON GROWTH, PRODUCTIVITY AND BIOMASS QUALITY OF KENAF 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 kenaf. Several varieties of kenaf were studied: Tainung 2, Everglades 41, Gregg, Dowling, SF 459 and G4. The plants were placed in pots, whose soils were contaminated. Several heavy metals (Cd, Cu, Zn, Hg and Cr) were separately studied, in order to access if there were specific patterns of toxicity involved. For each metal, one level of contamination was 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 contaminated with the different heavy metals. Zn was the metal that more negatively significantly affected the productivities obtained. In contrast, kenaf showed high tolerance to Hg, Cd, Cu and Cr soil contamination. Mineral matter accumulation was also influenced by heavy metals soils contamination. Overall, we may conclude that kenaf is able to remove and accumulate heavy metals from contaminated soils. Nevertheless, the highest proportion of metals taken up by plants is present in the shoots.
Keywords: kenaf, bioremediation, biomass composition

1 INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a short day, annual, herbaceous plant producing high quality cellulose. It is a member of the Malvaceae family as well as cotton and okra, and is endemic to Africa [1]. The entire plant can be used to produce pulp for the paper industry. Lower quality paper can be made from the short wood fibres of the inner core, while high quality paper can be made from the long fibres of the bark [2]. Kenaf, as a high yielding plant, is also a potential energy crop when used as a whole for this purpose. The residues from different industrial processes can, as well, be utilized as energy sources [1].

In the scope of the project Biomass Production Chain and Growth Simulation Model for Kenaf (Biokenaf), supported by the European Union, several studies with kenaf are being conducted at UBiA, in the Faculty of Sciences and Technology of the New University of Lisbon. In order to generate information on the potential of kenaf as a non-food crop in Europe, the main purpose of this work was to evaluate the capacity of kenaf to phytoremediate soils contaminated with heavy metals. To do so, the effects of different heavy metals on growth, productivity and biomass quality of kenaf were studied.

2 MATERIALS AND METHODS

At the end of July 2004, 5 seeds per pot of different kenaf varieties were placed in pots containing fertile soil. The varieties of kenaf studied were Tainung 2 (T), Everglades 41 (E), Gregg (G), Dowling (D), SF 459 (S) and G4. P-fertilizer (6 g $P_2O_5.m^{-2}$), K-fertilizer (12 g $K_2O.m^{-2}$) and N-fertilizer (15 g $N.m^{-2}$) were applied to all the pots. In order to compensate water deficit of the soil and to prevent water stress, irrigation with a 0.3 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. 10 days after sowing, the soil was contaminated, separately, with Zn, Cu, Cr, Cd and Hg, with three replications, to each variety.

The levels of contamination tested were chosen according to the guidelines for the definition of contaminated sites in Ontario [3]. Those guidelines establish the remediation criteria for agricultural land uses. For each variety a control test was also carried out. Table I presents for each metal, the level of contamination tested. For the heavy metal contamination of the pots, saline solutions were used.

Table I: Levels of contamination tested ($mg.kg^{-1}$) for each metal studied and salt used for the contamination

Metal	Contamination	Salt
Zn	600	$ZnCl_2$
Cu	150	$CuCl_2.2H_2O$
Cr	8	$K_2Cr_2O_7$
Cd	3	$Cd(NO_3)_2.4H_2O$
Hg	10	$HgCl_2$

At the end of the growing season (October), the plants were harvested and the following biometric parameters were determined: height of the plants and LAI (Leaf Area Index, LI-3100 Area meter). The seed germination (%) was also determined for each variety and for each pot. To determinate the productivity of the biomass, the total aerial dry weight and the total below-ground dry weight (roots) was determined. The quality of the biomass (aerial fraction and roots) was analysed taking in consideration the following parameters: ash content and Zn, Cu, Cr, Cd, and Hg contents. The chemical analyses were performed according to the following procedures: a) ash content: by calcination at 550ºC for two hours, in a muffler furnace; b) metals: by atomic absorption after digestion of the ashes with nitric acid.

3 RESULTS AND DISCUSSION

3.1 Seed germination

Table II presents the seed germination, in all the pots, for each variety.

Table II: Seed germination (%) for each treatment and for each variety

	Varieties					
Treatments	T	E	G	G4	D	S
Control	86	94	74	66	80	0
Zn	80	100	74	60	86	0
Cu	100	94	60	60	94	0
Cr	100	86	74	54	74	0
Cd	94	94	94	34	80	0
Hg	94	100	80	66	90	0
Average without control	94	95	76	55	85	0

As shown in Table II, SF 459 seeds didn't germinate. Those seeds might have been eventually corrupted. G4 was the variety that presented a lower seed germination percentage. Seed germination was determined only to evaluate the emergence of the seeds. Because contamination of the pots was done only after germination of the seeds and emergence of the plants, this was not a factor affected by the heavy metals.

3.2 Biometric Parameters

Tables III and IV presents the effect of the heavy metal contamination on the plant height and on the LAI, in comparison with the control, for each variety.

Table III: Height of the plants (cm), at the end of the growing period, for each treatment and for each variety

	Varieties				
Treatments	T	E	G	G4	D
Control	83	77	73	68	64
Zn	63	62	68	65	66
Cu	81	86	78	72	62
Cr	89	93	77	84	77
Cd	92	94	72	80	79
Hg	84	91	72	81	63
Average without control	82	85	73	76	69

Table IV: LAI (cm^2/cm^2), at the end of the growing period, for each treatment and for each variety

	Varieties				
Treatments	T	E	G	G4	D
Control	4.5	3.5	4.8	3.3	2.5
Zn	2.9	1.2	2.7	3.2	3.2
Cu	4.2	2.1	3.4	2.7	1.3
Cr	3.4	3.4	2.7	2.8	1.9
Cd	3.9	3.3	3.4	2.2	1.5
Hg	4.1	4.2	4.5	4.0	2.9
Average without control	3.7	2.8	3.3	3.0	2.2

The reaction of kenaf plants to heavy metal toxicity can be, globally, characterized by a non-effect or a positive effect on the plant height. Nevertheless, Zn affected negatively this biometric parameter, for every variety studied, except Dowling. In contrast, heavy metal toxicity affected negatively LAI. Only Hg contamination didn't affect Everglades 41, G4 and Dowling LAI, and Zn contamination didn't affect Dowling LAI.

3.3 Biomass Productivity

Tables V and VI show kenaf biomass productivity (aerial and below-ground) obtained under different treatments applied, for each variety.

Table V: Shoot dry weight ($g.m^{-2}$), at the end of the growing period, for each treatment and for each variety

	Varieties				
Treatments	T	E	G	G4	D
Control	571	467	403	380	375
Zn	308	221	310	212	436
Cu	604	432	425	337	406
Cr	614	579	428	498	430
Cd	731	643	637	335	476
Hg	654	833	705	599	564
Average without control	582	542	501	396	462

Table VI: Root dry weight ($g.m^{-2}$), at the end of the growing period, for each treatment and for each variety

	Varieties				
Treatments	T	E	G	G4	D
Control	96	75	92	97	109
Zn	63	29	76	50	121
Cu	101	68	104	94	114
Cr	99	113	104	124	118
Cd	100	147	179	67	112
Hg	136	234	234	164	168
Average without control	100	118	139	100	127

According to results presented on Tables V and VI, Zn was the metal that most significantly negatively affected the productivity of kenaf plants (shoot and roots). Only Dowling variety was not affected (negatively) by this metal. The other metals affected positively the productivity of kenaf plants (shoot and roots). Except G4 root dry weight, that was negatively affected by Cd contamination. Tainung 2 and Everglades 41 were the most productive varieties.

3.4 Biomass Quality

Tables VII-X show the effects of the different heavy metals treatments on the composition of the kenaf biomass, in terms of the ash content and heavy metals accumulation.

Table VII: Ash content of shoot (% dry matter), at the end of the growing period, for each treatment and for each variety

	Varieties				
Treatments	T	E	G	G4	D
Control	8.6	9.0	9.7	9.5	8.4
Zn	10.0	12.3	10.6	12.9	8.8
Cu	8.3	9.7	9.3	10.5	8.4
Cr	8.7	8.2	9.0	9.5	8.2
Cd	8.5	7.9	7.7	10.8	8.6
Hg	7.6	7.2	7.8	8.6	7.9
Average without control	8.6	9.1	8.9	10.5	8.4

Table VIII: Ash content of roots (% dry matter), at the end of the growing period, for each treatment and for each variety

	Varieties				
Treatments	T	E	G	G4	D
Control	7.7	5.5	7.1	8.3	7.9
Zn	7.3	14.3	6.6	10.3	7.6
Cu	8.2	5.8	6.6	8.9	8.8
Cr	7.6	6.4	6.8	9.1	8.7
Cd	7.9	7.2	6.1	11.4	8.6
Hg	6.0	6.6	6.7	10.1	8.8
Average without control	7.4	8.1	6.6	10.0	8.5

Table IX: Heavy metals concentration (mg.kg^{-1} dry matter) of shoot, at the end of the growing period, for each treatment and for each variety, in comparison with the control

		Varieties				
		T	E	G	G4	D
Treatments	Zn	441	992	362	624	303
	Cu	3.9	3.9	0.04	5.8	2.2
	Cr	0.50	0.83	0.46	0.19	<DL
	Cd	8.0	5.2	4.20	11	6.6
	Hg	0.07	0.04	0.04	0.09	0.20
Control	Zn	173	340	105	215	113
	Cu	2.4	3.4	1.2	1.5	0.51
	Cr	0.42	0.32	0.56	0.12	<DL
	Cd	0.41	0.42	0.40	0.22	0.19
	Hg	0.02	0.08	<DL	<DL	<DL

DL – detection limit

Table X: Heavy metals concentration (mg.kg^{-1} dry matter) of roots, at the end of the growing period, for each treatment and for each variety, in comparison with the control

		Varieties				
		T	E	G	G4	D
Treatments	Zn	5.4	6.0	4.5	6.5	5.3
	Cu	0.57	0.47	<DL	0.85	0.44
	Cr	0.10	0.04	0.03	0.02	0.03
	Cd	0.09	0.07	0.09	0.07	0.07
	Hg	<DL	<DL	<DL	<DL	<DL
Control	Zn	0.58	3.3	0.56	0.46	0.62
	Cu	0.02	<DL	<DL	<DL	0.02
	Cr	<DL	<DL	<DL	<DL	<DL
	Cd	0.01	<DL	<DL	<DL	<DL
	Hg	<DL	<DL	<DL	<DL	<DL

DL – detection limit

Globally, contamination of soils with heavy metals didn’t influence the ash content of the aerial biomass of kenaf plants. Nevertheless, Zn contamination induced a higher ash content of the aerial biomass in most of the varieties. Only Dowling aerial ash content was not affected. Cu contamination also induced a higher ash content of the aerial biomass of Everglades 41 and G4. Ash content of the aerial biomass of G4 increased also due to Cd contamination. Heavy metals contamination induced a higher accumulation of metals by the roots of Everglades 41, G4 and Dowling, but not by the roots of Tainung 2 and Gregg.

According to the results presented in Tables IX and X, plants from contaminated pots accumulated and absorbed more heavy metals from soil, than what was obtained in the controls. This was observed for both parts of the plants (aerial and roots). Kenaf accumulated more Zn and Cd than Cr, Cu and Hg. Hg was not detected in the roots, as well as in the control as in the plants obtained in the Hg contaminated soils, for every variety.

According to these results, it is possible to conclude, that the shoots of kenaf accumulated more heavy metals then the roots.

3.5 Effect of Cd

Kenaf showed high tolerance to Cd toxicity in terms of the height of the plant and in terms of the productivity. It was verified a general increase in height values and decrease in LAI values of all de varieties when compared with the controls. A significant increase was also verified for the dry weight’s values (except for G4). Contamination with Cd contamination of the soils induced an increase of the mineral matter of the aerial biomass of G4 and of the roots biomass of Everglades 41, G4 and Dowling.

Plants obtained from the contaminated pots accumulated Cd significantly in comparison with the values obtained for the control.

3.6 Effect of Cu

Kenaf presented high tolerance to Cu toxicity in terms of the height of the plant and in terms of the biomass productivity. The height of the contaminated plants didn’t present any significant variation in comparison with control, but it was verified the opposite for LAI, were the values obtained were significantly lower than the values from the control. The addition of Cu to the soil didn't interfere significantly with the dry weight (aerial and roots). Ash content didn't present significant differences between the biomass obtained from the Cu contaminated pots and from control. Only the Dowling's roots and G4's shoot presented significantly higher ash content in comparison with the controls.

Plants obtained from the contaminated pots accumulated Cu significantly in comparison with the values obtained for the control. Gregg was the only variety that was not able to accumulate Cu significantly.

3.7 Effect of Zn

Kenaf showed a very low tolerance to Zn toxicity in terms of growth parameters and in terms of biomass productivity. Dowling was the only variety that showed high tolerance to Zn toxicity. Due to the contamination of the pots with Zn, ash content of the aerial biomass of all the varieties increased significantly. Zn contamination also induced an increase of the mineral matter accumulation in the roots of Everglades 41 and G4.

Plants obtained from the contaminated pots accumulated Zn significantly in comparison with the values obtained from the control. Zn is the heavy metal whose concentration is higher in the kenaf plants.

3.8 Effect of Hg

Kenaf showed high tolerance to Hg toxicity in terms of the height of the plants and of the biomass productivity. It is observed a significant increase in what concerns the dry weight of all varieties in comparison with the control. In terms of growth parameters, the plants contaminated with Hg presented higher values of height and LAI than the control.

Hg contamination didn't influence the mineral matter accumulation by the aerial fraction of kenaf plants. In contrast, due to the Hg contamination, roots of Everglades 41, G4 and Dowling accumulated more minerals than control. Kenaf shoots accumulated more Hg from the Hg contaminated soils than control. The only exception was Energlades 41. But, roots didn't show any accumulation of this metal from the soil. The presence of this metal in the aerial fraction of the plant suggests that all the accumulated metal by the roots was translocated to the aerial fraction.

3.9 Effect of Cr

Kenaf showed high tolerance to Cr toxicity, in terms of the height of the plant and in terms of biomass productivity. The height of the plants contaminated with Cr was, for all varieties, greater than the control. In terms of LAI, the values obtained indicate a significant reduction of this parameter in the contaminated plants in comparison with the control. The dry weight of the plants contaminated with Cr presented higher values in comparison with the control. Cr contamination didn't affect the accumulation of mineral matter by the aerial fraction of the kenaf plants. This contamination induced an increase of ash content of the roots of Everglades 41, G4 and Dowling.

Kenaf biomass accumulated more Cr in the plants obtained from Cr contaminated pots than from the controls. But this difference was not significant.

4 CONCLUSIONS

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

Overall, kenaf is able to remove and to accumulate heavy metals from contaminated soils, but the highest proportion of the heavy metals taken up by plants is present in the shoots. This fact limits the number of possible utilizations of the biomass obtained in contaminated fields.

Further studies are needed to make clear the interaction among heavy metals and other nutrients like Ca, K, Mg, in their uptake and translocation by kenaf plants.

Since kenaf is an energy crop, it can be utilized for energy production and its heavy metals accumulated by the shoot will remain on the ashes. This represents a contribution to its economical value, in terms of a sustainable agriculture strategy.

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6 REFERENCES

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