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Influence of lead (Pb) on *Jatropha curcas* L. growth under hydroponic conditions

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Abstract: The contamination of the environment with pollutants, like heavy metals from human activity, has caused the loss of agricultural land. One possible solution could be the utilization of phytoremediation technique with particular plant, capable of absorbing the contaminants from soil. *Jatropha curcas* L., an important tropical and sub-tropical plant for biodiesel production, has the capacity to grow~~n~~ in marginal land, compromised for food cultivation. The experiment was conducted in hydroponic conditions with the objective to evaluate the response and growth parameters of juvenile plants grown in presence of different Pb levels (0-100-200 mg/L) in the media. It was possible to investigate the interaction between some mineral element (Ca, Mg, Zn and Fe) and lead, and evaluate some tolerance indicators. Results showed that the plants were able to grow in presence of high concentration of Pb and to accumulate large amounts of heavy metal in the roots, followed by the stems and leaves. However, *J. curcas* subjected to Pb treatment demonstrated stunted growth and an alterations in mineral elements contents. The results suggest that J. curcas may tolerate the levels of Pb imposed, but there is low translocation of heavy metal to areal tissues, within the time period of analysis.

Keywords: Jatropha curcas *L.; phytoremediation; accumulation; hydroponic culture; heavy metal*

Introduction

Global industrialization and human activity have caused widespread contamination of ecosystems with persistent pollutants (Lado *et al.*, 2008; Clemens, 2006). Heavy Metals (HM) are one of the major ecological problems both for the environment and human health (Halim *et al.*, 2003; Lasat, 2000). Lead (Pb) is a non-essential element for living organisms, but has major effects on the physiological and growth processes of plants, as well as nutrients assimilation (Sharma and Dubey, 2005; Patra *et al.*, 2004).

Conventional remediation techniques are too expensive and can be applied only to small areas (Gamalero *et al.*, 2009). A possible solution is phytoremediation based on capacities of some plants, such as "Hyperaccumulator plants", to absorb the contaminants from the soil at levels 100-fold greater than non-accumulator plants (Lasat, 2000; Kumar *et al.*, 1995). In these plants, the metals are translocated and accumulated in the areal parts, which are subsequently removed by harvesting (Lasat, 2002; Huang and Cunningham, 1996; Tang *et al.*, 2009).

Jatropha curcas (Euphorbiacea), is a multipurpose, small perennial tree, native to Central America, currently widespread in a vast array of environments, from arid to humid areas, in all tropical countries (Jongschaap *et al.*, 2007 and Kumar and Sharma, 2008). Not only there is interest in *J. curcas* for biodiesel production, due to the high concentrations of oil (45%) within the seeds (Achten *et al*, 2008), but also for its capacity to grow in marginal land, which are already compromised for food cultivation (Jongschaap *et al.*, 2007). An additional advantage of *J. curcas* cultivation, may reside in its potential as hyperaccumulator of heavy metals (Chang *et al.*, 2014; Liang *et al.*, 2012).

Only few experiments have been performed to investigate the effects of heavy metals on the *J. curcas* growth and its accumulation capacity. A study of Shu et al. (2011) compare the effect of different concentration of Pb treatment on the growth of seedling and cutting of *J. curcas* in contaminated soil. The work demonstrating the low translocation of Pb to root to areal tissues and that the accumulation of Pb is concentration-dependent. In another work (Liang *et al.*, 2012), retardation of growth and physiological processes inhibition in *J. curcas* were revealed after seedling exposition to polluted soil with different level of Pb, for 30 days. There are many others papers on heavy metals and *Jatropha curcas*. Please see the following: Ahmadpour P. *et al.* (2010) and Bernabe-Antonio A. *et al.* (2015).

No information is available about the accumulation of heavy metal in the seed and oil of *J. curcas*. In general, the concentration of Pb in areal parts of plant decreases as the distance from the roots increases. However, the localization of the contaminant in different tissues of plant is dependent of the species but in general, the presence of Pb in the vegetable organs tends to decrease in the following order: root>leaves>stem>inflorescence>seed (Sharma and Dubey, 2005).

This preliminary study aim was to evaluate the *Jatropha curcas* seedling growth in response to Pb presence in the nutrient solution and to investigate the interaction between heavy metal and nutrient elements such as Ca, Mg, Fe, Zn. The hydroponic system was chosen because is easier to manage and for a rapid evaluation of interaction between the contaminant and the nutrient and growth parameter, without the interference of soil particle.

Materials and Methods

Seed sources and preparation

The seeds of *J. curcas*, were collected the same year of the experimentation (2011) in a plantation placed in the village of Genotò (coordinates 13°33'10.44" N; 13°49'12.94" W) in Tambacounda region in the eastern part of Republic of Senegal and, until its use, they were stored under vacuum in a dark room. To avoid fungal contamination, which may reduce the germination and influences the trial, the seeds were sterilized, firstly soaked in 70% ethanol solution for 30 seconds, then dipped in 2.5% NaClO for 15 minutes and finally rinsed with sterile distilled water. The germination occurred on sterile sand and the seedlings were placed in growth chambers at 26 °C, with a 16 hour photoperiod for 10 days. Eighteen-day old seedlings of *J. curcas* were transplanted into pots containing quartz sand as inert substrate. The Pb was added to the hydroponic solution after 20 days of acclimation. The sand and the substrate were previously sterilized at 105 °C for 72 hours.

Nutrient solution and hydroponic cultures

The young plants of *Jatropha curcas* were grown in a hydroponic cultivation system using as nutrient solution a modified Hoagland solution (Tang et al., 2009) containing: 147.5 mg/L $Ca(NO_3)_2{*}4H_2O$; 50.8 mg/L $MgCl_2{\cdot}6H_2O$; 63.1 mg/L KNO_3; 0.15 mg/L H_3BO_3; 0.09 mg/L $MnCl_2{*}4H_2O$; 0.14 mg/L $ZnSO_4{*}7H_2O$; 0.024 mg/L $CuSO_4{*}5H_2O$; 0.012 mg/L $(NH_4)_6Mo_7O_{24}{*}4H_2O$; 10.4 mg/L Fe-EDTA (Fe-$C_{18}H_{20}N_2O_6$). The concentration of Pb as $Pb(NO_3)_2$ added was at 0-100-200 mg/L, hereinafter referred to as level or treatment 0-100-200. To avoid the precipitation of lead phosphate, KH_2PO_4 was removed from the solution over the treatment.

The experiment was performed during 60 days and was prepared in two randomized block designs with three levels of Pb. There were 12 plants for each level per block. The concentration of the heavy metal used was chosen according to the values reported in the literature (Zhao *et al*., 2010), and by taking into account that the statutory limit of Pb soil content in Italy is set at 100 mg/kg soil (D.M.n. 471). The nutrient solution was circulated daily at regular intervals with watering cycles of 15 minutes every 4 hours.

Data collection and plant analysis

Measurements of shoot length (cm), basal diameter (cm) and the number of leaves per plant were performed every 15 days. Four plant samples for each treatment were collected every 20 days for heavy metal concentration determinations in plant tissues. At the end of the experimental trial the remaining plants were subjected to the same treatments mentioned above. The whole plant was separated into roots, stems and leaves and oven dried at 105 °C for 72 hours. The materials were weighed before and after the drying, to obtain fresh weight (FW) and dry weight (DW). The material was ground in liquid nitrogen, and 0,25 g samples were diluted in 9 mL nitric acid and 1 mL deionised water. These samples were mineralised using the MARS (CEM Corporation, Matthews, North Carolina, USA) microwave, according to the manufacturers' protocol for vegetal material. The elements (Pb, Mg, Ca, Fe, and Zn) were determined using the ICP-OES (model IRIS Intrepid II XSP Radial, company Thermo Fisher Scientific, USA).

Three indicators were assessed to evaluate plant tolerance. These were as follows:

The Translocation Factor (TF) was used to evaluate the capacity of *Jatropha curcas* to translocate the heavy metal from the roots to the epigeal parts, in relation to heavy metal content present in the soil (Ghavri and Singh, 2010; Zhi-xin *et al*., 2007). The value was obtained by measuring the ratio in concentration in the areal tissues and that in the roots, respectively, with the heavy metal content expressed as mg kg $^{-1}$.

The Tolerance Index (TI) was the ratio of variable measured (such as plant height and diameter) in plants grown on contaminated soil and control plants grown on uncontaminated soils, and was expressed as a percentage (Yadav *et al*., 2009; Ghavri and Singh, 2010).

The Concentration Index (CI) was determined to observe the effect of metal stress on the nutrient balance (Ca, Fe, Mg, Zn) inside the plant tissues. The CI was the ratio between a nutrient concentration in treated plants and the concentration of the same nutrient in control plants (Ghavri and Singh, 2010).

Statistical analysis

All data obtained from the study were previously tested for their homogeneity and normal distribution and therefore subjected to statistical analysis of variance (ANOVA), performed using the Systat 9 Software, and by adopting GLM (General Linear Model). The Bonferroni test was carried out for the significance value obtained from the ANOVA. The Pearson coefficient was calculated for the correlation analysis.

Results and discussion

Lead Concentration and accumulation in plants

High concentrations of 356 mg/kg, 1533 mg/kg and 60395 mg/kg of lead were detected at the end of the trial in *Jatropha curcas* respectively in leaves, stems and roots when the plants were cropped in Pb level 200. Roots were the first tissues of accumulation followed by stems and leaves, as already found in literature (Majid *et al.*, 2012; Sharma and Dubey, 2005). Despite the elevate quantity of Pb in roots low quantity of pollutant was translocate in areal tissue (Fig. 1a). However, plants growth at level 200 has higher capacity to translocate Pb to stems as compared to level 100.

High Pb concentration in plant root (37145 mg/L), was observed already at the 20th day (Date 1). Nevertheless, at the 40th day from transplant (Date 2) begin to increase the amount of the contaminant translocated to stems (Fig. 1b). The data were corroborated by the experimentation of Shu *et al.* (2011) in which also with higher level of Pb in the substrate, roots were the first tissue of accumulation and the translocation to areal tissue was slow. Hydroponic system allow an accumulation of Pb at levels 100 and 200, 60 times greater than soil system of Shu *et al.* (2011) proof.

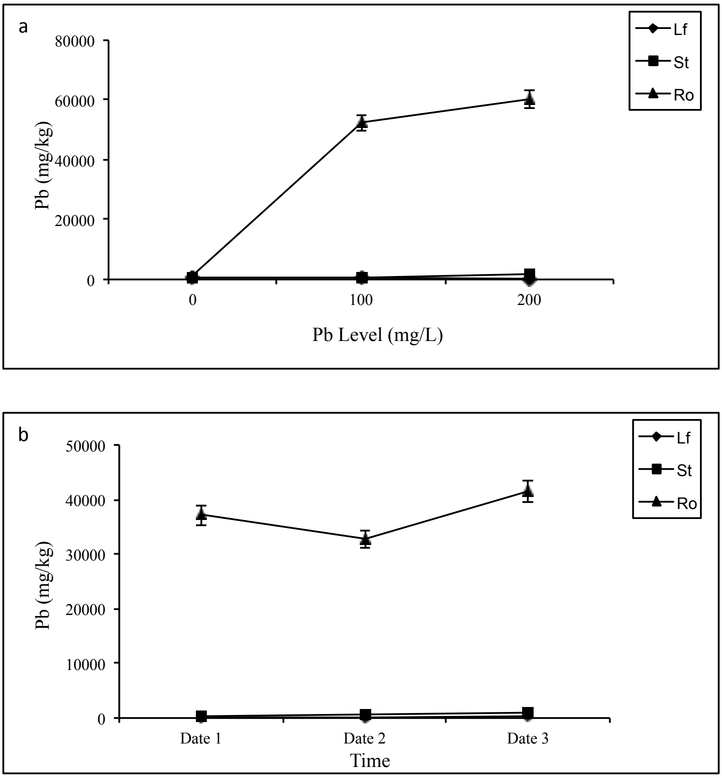

Figure 1 - Pb concentration (mg/L) in leaves (Lf), stems (St) and roots (Ro) of plants grown under different levels of Pb (a) and in relation to time after transplanting (b), 20th day (Date 1), 40th day (Date 2) and 60th day (Date 3). LSD bars are reported for each tissue for $P<0.05$.

Despite the high concentration of heavy metal in roots (Fig. 1a and 1b), the plants will survive. Similar to *J. curcas*, Romeiro *et al*. (2007) measured low Pb concentration in *Ricinus communis* aerial tissue, while founded high Pb concentration in roots. Another work (Luhach and Chaudhry, 2012) demonstrated the capability of roots of Jatropha to absorb and accumulate high levels of others heavy metal as compared to aerial tissues. On the opposite, *Brassica juncea* accumulate and translocate high quantity of Pb in aerial tissue (Kumar *et al*., 1995).

The low transition of heavy metal from root to areal tissues, could have two possible explanation: one explanation may be due to physiological and/or biochemical mechanism, activated by Pb presence, which required time before being activated. While the other one, the roots play an important role as a partial barrier to Pb translocation (Kumar *et al*., 1995; Sharma and Dubey, 2005).

Effect of Pb on mineral element accumulation

The results shows in Figure 2a, suggest that the quantity of Ca absorbed by *J. curcas,* decrease from level 0 to level 200 (mg/L). Lead induced a decrease of Ca concentration in areal tissues and this was more pronounced in stems at level 100 than in level 200. Nevertheless in proportion, the percentage of element translocate, despite Pb in nutrient solution, by plant to leaves and stems was the same in both Pb treatments. Ca has a detoxification action in the plant, so the maintenance of translocation percentage of element to areal tissue by *Jatropha curcas*, may be imputable to initial attempt of plant to contrast the presence of lead (Lotti, 1985). Next to this is to consider that one of the possible ways of lead entry into the plant was through the plasma membrane cation channels, situated on the cells of the root, similar to those of calcium (Sharma and Dubey, 2005). As a consequence, there were a probable competition between Pb and Ca transport, reducing the presence of nutrient and affecting the growth of the root and plant cells (Huang and Cunningham, 1996; Sharma and Dubey, 2005).

The amount of Mg (Fig 2b) decrease from Pb level 0 to level 100 in all tissues. However, the quantity of nutrient, as a percentage, translocated to areal tissue by the plant, was the same. The Zn amount in root progressively decreases with the increase of Pb levels in the solution and this reduction is more evident at Pb level 200. Nevertheless in the highest level of lead tested, the Zn translocated from roots to areal tissues, was higher than those detected in control and level 100 (Fig. 2c). As for Ca, there was probable a competition between Pb and both Zn and Mg, for the transport through cation channels of the root cells (Sharma and Dubey, 2005).

In the roots of *Jatropha curcas*, exposed to Pb treatment, Fe content in tissues of plants, increased with the increase of heavy metal level in nutrient solution. No statistical difference in Fe content was observed between leaves at level 100 and 200.

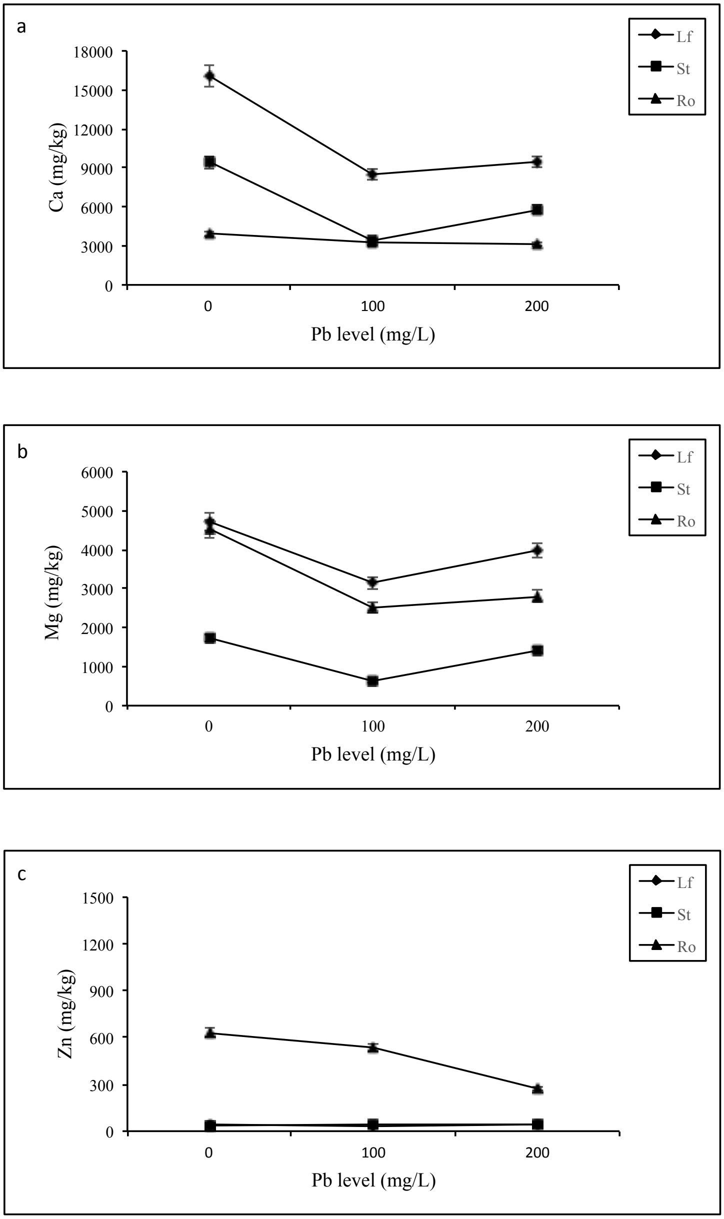

Figure 2 - Changes in minerals nutrients concentration (mg/kg) in leaves (Lf), stems (St) and roots (Ro) of Jatropha curcas. *grown in different levels of lead. a): Ca concentration; b): Mg concentration; c): Zn concentration; d): Fe concentration. LSD bars are reported for each tissue for P<0.05.*

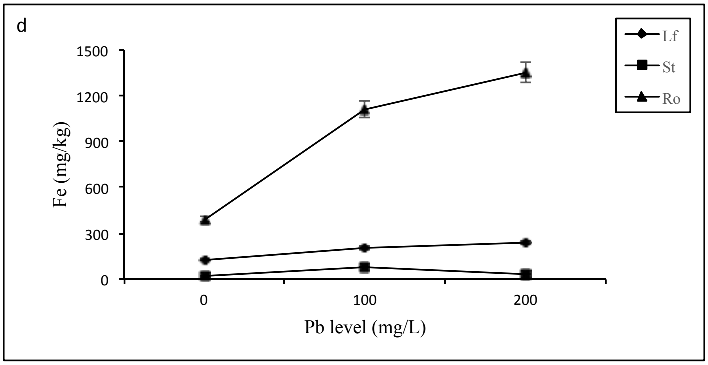

Figure 2 - continued

Only in stems of level 200 there was a decrease in Fe (Fig. 2d). The Pb toxicity affected processes such as the respiration of cells, the transport of electron, the mitochondrial and chloroplast activity and ATP synthesis (Sharma and Dubey, 2005 and Lotti, 1985). The high quantities of Fe in roots may be useful to contrast the toxicity Pb effects on the activities described above. In contrast, Huang and Cunningham (1996) and Sharma and Dubey (2005) found that Fe content decreases with increased exposure to Pb.

Morphological characteristics

All morphological parameters (Table 1) were negatively affected by the presence of Pb in the nutrient solution, as compared to control plants. Fresh and dry weight of stems, leaves and roots were negatively influenced by Pb treatments. The water percentage in the tissues decreased from level 0 to level 200 and leaves are the most affected tissues by heavy metal. The plant height, diameter and number of leaves, decreased when exposed to lead treatment. However, no differences were observed between growth parameter of level 100 and 200 ($P<0.05$) and Level 200 of lead was the one that shows the highest phytoxicity. Symptoms of Pb toxicities to young plants were also observed in Liang *et al.* (2012) for what concern the decrease of height of stem and fresh and dry weight.

Low value of morphological parameters was probably due to the hydroponic conditions. In this type of culture there was not chemical and physical interaction between soil and plant that may affect the relations and mineral exchanges between the elements in the soil and the roots.

Tolerance indicators

Tolerance indicators are showed in Table 2. The translocation factor of Pb from roots to areal tissues. TF of treatment 200 mg/L of lead, increases with time. On the

Table 1 - Growths parameters of Jatropha curcas *plants, grown under different Pb levels. The parameter showed are significantly different ** = p<0.005 and ns= no significance to control plant; se=standard error.*

Morphological Parameters	Pb level (mg/L)			
	0	100	200	se
FW leaves (g)	7.8	2.1**	1.3 **	0.469
DW leaves (g)	1.4	0.3 **	0.2 **	0.085
FW stems (g)	15	4.6**	2.8 **	0.922
DW stems (g)	2.7	1.1 **	0.7 **	0.196
FW roots (g)	4.5	1.1**	0.6 **	0.391
DW roots (g)	0.6	0.1 **	0.09 **	0.062
% Total water	82	78 **	77 **	1.475
% Roots water	80	80 ns	85 ns	1.484
% Leaves water	81	58 **	16 **	5.768
% Stems water	82	75 **	73 **	1.453
Plant height (cm)	13	10.6**	9.1 **	0.482
Stem Diameter (mm)	12	7.3 **	6.2 **	0.323
Number leaves	7	3.2 **	3.3**	0.394

Table 2 - Values of Traslocation Factor (TF), Tolerance Index (TI) and Concentration Index (CI), of plants grown in presence of Pb in relation of time.

		Tolerance indicators						
Pb level	Date	TF value	TI diameter value	TI height value	CI-Ca	CI-Mg	CI-Fe	CI-Zn
Level 100	Date 1	0.3	73	82	0.63	0.62	2.1	0.8
	Date 2	1.4	55	64	0.48	0.58	3.8	0.7
	Date 3	1.1	57	83	0.44	0.52	1.7	0.8
Level 200	Date 1	1	65	74	0.63	0.68	3.4	0.4
	Date 2	3.1	49	56	0.62	0.8	3.1	0.6
	Date 3	4.8	43	67	0.63	0.79	2.7	0.5

contrary with 100 mg/L of Pb, TF increase until Date 2. The values of TI calculated for the height and the diameter were higher for the plant with the lower concentration of Pb. These results show that the plants better tolerate the lower level of Pb in the nutrient solution. Concentration index were always less than one unit, with the exception of Fe in which all value was more than one unit. No significant differences ($P<0.05$) were found among levels in relation to time.

Conclusions

The results suggest that *Jatropha curcas* can easily tolerate the presence of Pb in the juvenile stage, in the quantities imposed by the experimental trial, with an elevated accumulation of the heavy metal in the roots but a low translocation of the element to areal tissue. However, we could not consider *Jatropha curcas* as a plant suitable for the phytoremediation of contaminated soil but we could prospect a possible utilisation of Jatropha for the phyto-immobilization of these elements to reduce their availability in the soil of sub-tropical areas. Additional studies need to be performed over a longer duration period and also at field condition to investigate additional tolerance levels and derive more information about the processes involved in heavy metal uptake.

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