# Phytoremediation of highly contaminated mining soils by *Jatropha curcas* L and production of catalytic carbons from the generated biomass

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## Abstract

This paper deals with the removal of heavy metals from marginal soil mixtures from the Cobre Las Cruces and Aznalcóllar mining areas containing high concentrations of metals (Cr, Fe, Ni, Cu, Zn, Cd, Hg, Pb and As) by means of phytoremediation using *Jatropha curcas* L., and the subsequent production of biocatalysts from the plant biomass. First, *J. curcas* L. was sowed in eight mixtures of these mining soils to study its adaption to these high-contaminated soils and its growth during 60 days in a greenhouse under conditions simulating the South of Spain's spring climate. Later, the most suitable soil mixtures for plant growth were used for 120-day phytoremediation under the same conditions. Heavy metal concentration in soils, roots, stems and leaves were measured by ICP-OES at the beginning, at the middle and at the end of the phytoremediation period, thus calculating the translocation and bioaccumulation factors. *J. curcas* L. was found to absorb great amounts of Fe (> 3000 mg $kg^{-1}$ plant) as well as notable amounts of Pb, Zn, Cu, Cr and Ni, and traces of As. Other metals with lower initial concentrations such as Cd, Hg and Sn were completely removed from soils. Finally, the plant biomass was subjected to

pyrolysis to obtain catalytic biocarbons, assessing the optimal temperature for the pyrolytic process by means of thermogravimetric analysis and Raman spectroscopy.

*Keywords*: bioaccumulation; *Jatropha curcas*; mining soils; phytoremediation; translocation factor.

## Introduction

Soil contamination by heavy metals (As, Cd, Cr, Cu, Pb and Zn) is one of the major environmental problems raising critical concerns for both human health and ecosystems (Singh et al., 2011) due to their carcinogenic and mutagenic effects on animals and humans (Sánchez-Chardi et al., 2009). Small quantities of these metals are required for human health, however in higher concentrations they become toxic or dangerous, affecting brain, kidney, lungs, liver and other important organs. Moreover, long-term exposure to them can cause physical, muscular and neurological degenerative processes, and even cancer (Järup, 2003). The clean-up of most of these soils is mandatory for the area to be reclaimed and to minimize the entry of potentially toxic elements into the food chain.

The main anthropogenic sources of heavy metals in soils are related to the mining industry. The mining sector produces a whole range of gaseous pollutants both solid and liquid. This can occur in different ways: by deposition from the atmosphere as sediment particles or brought by rainwater; by direct discharge of the liquid products of mining and metallurgical activity; or by infiltration of leachate from the mining environment. Specifically, mining and mineral processing of sulphide ore deposits produce large quantities of wastes, most of which are regarded as toxic or hazardous, due to the

formation of acid drainage, and to their heavy metal content (Jiménez-Moraza et al., 2006).

Phytoremediation basically refers to the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environment (Singh et al., 2003; Suresh and Ravishankar, 2004). It can be used for the removal of not only heavy metals but also organic pollutants such as polynuclear aromatic hydrocarbons, polychlorinated biphenyls and pesticides.

Phytoremediation employs on-site plants to absorb heavy metals and to prevent their further transport. Plants generally handle the contaminants without affecting topsoil, thus conserving its utility and fertility. They may improve soil fertility and increase organic matter content (Abhilash et al., 2012; Cobbett, 2003). Therefore, phytoremediation is an environmental ecotechnology that can be applied to remediate contaminated soils. It is a novel, cost-effective, efficient, environmental and eco-friendly remediation strategy that can be applied *in situ* and is solar-driven (LeDuc and Terry, 2005; Mukhopadhyay and Maiti, 2010). What is more, it has already been demonstrated that phytoremediation is a more ecological and economic technique than the conventional physical-chemical alternatives (González-Chávez and Carrillo-González, 2013).

Recently, it has been reported that *Jatropha curcas* L. has a high capacity for bioaccumulation, phytotranslocation and phytoremediation of heavy metals (Chang et al., 2014; González-Chávez et al., 2017; Marrugo-Negrete et al., 2015). *J. curcas* L. is a plant belonging to the Euphorbiaceae family. It is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas (Jamil et al., 2009; Pandey et al., 2012). This plant survives and grows on marginal, eroded and depleted lands. It requires little water to grow, although it does not tolerate heavy rains. The implementation of these fast growing plants in degraded or contaminated areas is important for improving soil

quality and preventing erosion. It also leads to organic matter enrichment and to greater diversity of microorganisms in the soil (Abhilash et al., 2013). This plant can also accumulate most metallic elements in quantities of up to 0.01% of its dry weight (1000 mg $kg^{-1}$) (Ahmadpour et al., 2014; Ghavri and Singh, 2010). Other studies have shown that Cr, Hg and Pb are not easily transferred to aerial plant biomass as they are mainly stored in root cells (Bernabé-Antonio et al., 2015; Marrugo-Negrete et al., 2015), while Zn is easily accumulated in green tissues (Yadav et al., 2009).

Although phytoremediation is a promising approach for the recovery of soils contaminated with heavy metals, its main drawback is the later use of the contaminated biomass generated over phytoremediation. As soon as the plants have absorbed the pollutants from the soil, it is necessary to withdraw them from the contaminated zone. The contaminated plants are regarded as residues and are kept in vegetable containers so the problem is not solved.

The implementation of these fast growing plants in degraded or contaminated areas is important for the soil quality, erosion prevention as well as organic matter enrichment and diversity of microorganisms in soil. In addition to the beneficial effects on the soil, the biomass obtained from *J. curcas* L. (stems, leaves and roots) could be used as a potential source of energy and other resources (Chavan et al., 2015; Teo et al., 2015, 2014). A potential alternative is to perform thermochemical processes such as pyrolysis to transform this biomass into high added-value products. Pyrolysis is a process for which a material is thermally decomposed in the absence of oxygen or any other oxidizing agent. The principal product obtained in the pyrolysis is pyrolytic carbon, which has numerous potential applications, such as its use as acid heterogeneous catalysts (Yee et al., 2011). Its properties depend mainly on the biomass composition, reactor type and the experimental conditions in which pyrolysis is performed.

In the present work, we have studied the growth and adaptation of *J. curcas* L. to highly contaminated soils by heavy metals under the climatic conditions of the South of Spain as well as the phytoremediation capacity (extraction of heavy metals from soil) of *J. curcas* L. The distribution of extracted heavy metals in roots, stems and leaves was analysed and quantified by ICP-OES. Bioaccumulation or bioconcentration factors (BAF) and translocation factors (TF) of heavy metals were also compared. Finally, we have assayed the formation of biocarbons, with catalytic properties, from *J. curcas* L. roots, determining the structure of the biocarbons by Raman spectroscopy.

## 2. Materials and methods

### 2.1. Soil samples from the mining land

Soil samples were collected in Cobre Las Cruces and Aznalcóllar mining areas in Andalusia, in the South of Spain.

In Cobre Las Cruces mine, 300 kg were taken from the storage zone for the original topsoil in the mining region (Fig. S1.A). This soil (NCS) is currently used for the subsequent restoration of the landscape and to minimize the visual impact of the mine.

In Aznalcóllar mine, samples were taken from the heap area with the highest concentration of leachate metals. Two zones of well-differentiated soils were found in this mine (Fig. S1.B and S1.C). One with yellow soil (YCS) and another one with grey soil (GCS), so both samples were taken. A surface area of 50 $m^2$ was selected placing an imaginary mesh with 5 m sides (i.e. two adjacent 25-$m^2$ grids for each soil). 5-kg samples were taken at the centre of each grid at three different depths (0 – 30 cm, 30 – 60 cm and 60 – 90 cm, respectively, from the surface), which were subsequently mixed and homogenized in a mixer for one hour.

Finally, the three soils were transported to a greenhouse. After their analysis, they were used as substrate for the trials.

### 2.2. Germination of *Jatropha curcas* L. seeds

Seventy *J. curcas* L. seeds from Argentina were sown in vermiculite in 8-cm diameter pots. They were germinated in a Fitoclima 18000EH climate chamber (Aralab, Portugal) under the following conditions: 25 ºC, 80% humidity and continuous lighting (Fig. S1.D). The plants were watered with Hoagland solution containing (in mmol $L^{-1}$) 6.0 $KNO_3$, 4.0 $Ca(NO_3)_2 \cdot 4H_2O$, 2.0 $NH_4H_2PO_4$, 1.0 $MgSO_4 \cdot 7H_2O$, 50.0 KCl, 25.0 $H_3BO_3$, 2.0 $MnSO_4 \cdot H_2O$, 2.0 $ZnSO_4 \cdot 7H_2O$, 0.5 $CuSO_4 \cdot 5H_2O$, 0.5 $H_2MoO_4$, and 20.0 Fe-EDTA, providing K, Ca, N and P, Mg, Cl, B, Mn, Cl, B, Mn, Zn, Cu, Mo, and Fe, respectively. Sixty seeds germinated, 85% of all those planted. The seedlings were kept in this climate chamber for about 6 weeks until they were 25 cm tall (Fig. S1.E). Immediately afterwards they were planted into 20-cm diameter plant pots containing peat moss (Universal Compo Sana Substrate, Germany) and placed in a greenhouse. The climate in the greenhouse simulated that of spring in the South of Spain; that is, a daytime temperature of 25 ± 3 ºC and 18 ± 3 ºC at night, 60% humidity and between 8 and 14 h of light. The plants were kept under these conditions for about six weeks to acclimate before the beginning of both adaptation and absorption trials in mine soils, the resulting plant tall being 70 – 90 cm (Fig. S1.F).

### 2.3. Preliminary assessment of *Jatropha curcas* L. adaptation to contaminated soils

First, the study of the adaptation of *J.* curcas L. to the three soils was made. Eight mixtures of soils were assayed (Table 1), 5 replicas of each trial being performed. Plants were let to grow in these soils for 60 days, being watered with tap water once a week. For further comparison, 5 plants were sown in vermiculite (V100) and watered with Houlang solution. Another 5 plants were analysed at day 0 as reference. Visual observations and

pH measures were carried out at days 0, 7, 14 and 60. Chemical analysis of the soil mixtures as well as of the plant biomass (roots, stems and leaves) were performed at the beginning and at the end of this adaptation period.

**2.4. Heavy metal absorption trials**

Three of the soil mixtures assayed in the adaptation trials, namely NCS100, YCS20 and YCS50, were selected to assess the heavy metal absorption by *J. curcas* L. Plants were sowed in 50-cm diameter pots and were let to grow for 120 days, being watered with tap water once a week. Ten replicas of each trial were performed. Five replicas of soils and plants were analysed after 60 days (first cut-off) and another 5 replicas after 120 days (second cut-off).

**2.5. Catalytic carbons production**

The dried and sieved roots of the plants were subjected to pyrolysis in an inert nitrogen atmosphere at 300, 350, 400, 450, 500, 550, 600, 650 and 700 °C, respectively. To do this, 0.5 g sample were placed in a 25 x 300 quartz tube and introduced in a Carbolite Tube Furnace MTF 12/38/250 equipped with a Eurotherm 2416CG temperature controller. The outlet of the quartz tube was connected to two bubblers submerged in ice to condense the flue gases (bio-oils), an extractor hood eliminating the uncondensed gases. The flow of inert $N_2$ gas was set to 6 L $h^{-1}$, and the heating rate was set to 30 ºC $min^{-1}$. The working temperature was maintained for 2 h. The resulting catalytic carbons were let to cool to room temperature.

**2.6. Soil and plant analysis**

Before being analysed, soils were weighted and dried in a stove at 60 ºC for 72 h. Once dried, the samples were sieved using a 2 mm mesh, removing pebbles but not the clots of soil. A mortar was used to reduce the size of the soil particles and to press them through the sieve. After being sieved, samples were carefully homogenized.

As for plant analysis, they were first cut-up, separating roots, stems and leaves. Next, all the parts of the plants were dried in a stove at 60 ºC to constant weight. Finally, they were ground using a Culatti DFH48 mill with a 2 mm sieve.

**2.6.1. pH**

To measure the pH of the soils, 10 g soil were weighed in a beaker and distilled water was added until a thick, homogeneous paste was obtained with no excess water. This was let to stand for 30 min before introducing the pH meter electrode to measure the pH (Ali et al., 2011).

**2.6.2. Phosphates**

The Olsen method was used to determine the amount of phosphates ($PO_4^{3-}$ or $HPO_4^{2-}$) in the soil samples. Using this method, it is possible to determine the phosphates that a plant can assimilate. It is based on a colorimetric assay which measures the optical density of the blue solution produced when reducing the phosphomolybdic complex ($(NH_4)PO_4(MoO_3)_{12}$) formed by orthophosphoric acid ($H_3PO_4$) and ammonium molybdate $(NH_4)MoO_4$ (Jackson, 1958). Firstly, seven standard solutions from 0.2 to 1 mg $kg^{-1}$ $KH_2PO_4$ were prepared, to which 8 mL 0.03M $(NH_4)MoO_4$ solution were added. The absorbance at 880 nm of the resulting solutions was measured in a Varian Cary 100 UV-visible spectrophotometer, and a calibration curve was obtained. To analyse phosphates in soil samples, 2.5 g soil were weighed in a beaker, then 50 ml 0.5 N $NaHCO_3$ were added and the mixture was stirred for 30 min before filtering. Finally, 8 mL 0.03M $(NH_4)MoO_4$ solution were added to the filtered solution and the absorbance at 880 nm was measured. Results were expressed as mg available phosphorus $kg^{-1}$ soil.

**2.6.3. Organic matter**

The organic matter in soil samples was determined by oxidising it with potassium dichromate ($K_2Cr_2O_7$) and sulphuric acid ($H_2SO_4$), measuring the reduced chromium

($Cr^{3+}$) by UV-Vis absorption spectroscopy (Jackson, 1958). Before analysing the samples, a calibration curve was made. Six standard solutions of glucose were prepared from 5 to 50 mg $mL^{-1}$, reacting 2 mL of each with 10 mL 0.17 M $K_2Cr_2O_7$ and 10 mL $H_2SO_4$. As the rate and intensity of oxidation depend on the amount and kind of organic matter in the samples and the reaction temperature, all the reactions took place in a 100 ºC water bath for 30 min. The same procedure was followed for the reaction of 0.3 g of each of the soil samples. After preparing the standard reactions and the samples, the absorption spectra were recorded between 400 and 800 nm. Results were expressed in g organic matter $kg^{-1}$ soil.

**2.6.4. Carbonates**

The percentage of total carbonates ($CaCO_3$) in soil samples was determined using a gasometric method known as Bernard's calcimetry. This method involves the indirect measurement of the carbonates in the soil by the gasometric determination of the $CO_2$ released when the carbonates in soil samples react with HCl. To calibrate Bernard's calcimetry, 0.25 g pure $CaCO_3$ were weighed and were let to react with 5 ml 1:1 HCl solution. The same procedure was followed to determine the carbonates in soil samples.

**2.6.5. Nitrates**

UV-visible absorption spectrophotometry was used to determine the content in nitrates ($NO^{3-}$) in soil samples, recording the absorption spectra of the samples at 220 nm in a Varian Cary 100 UV-visible spectrophotometer (APHA et al., 2005). The calibration curve was obtained from 10 standard solutions from 0.2 to 5.0 mg $kg^{-1}$ $KNO_3$ to which 1 mL 1 M HCl was added. To determine the content in nitrates in soil samples, 10 g soil were weighed in a beaker and then 50 mL distilled water were added, the mixture being stirred for 1 h before filtering. Finally, 1 mL 1 M HCl was added to the filtered solution. Results were expressed as mg nitrates $kg^{-1}$ soil.

### 2.6.6. Trace elements

For the analysis of the trace elements, both soil and plant samples were digested with concentrated $HNO_3$ and $H_2O_2$ in a microwave digester (Milestone Ethos One). After digestion, the amount of trace elements in samples was determined using a Horiba Jobin Yvon ICP-OES with automatic Horiba Jobin Yvon AS 500 sampler, a hydride generator and a CETAC AT+ ultrasonic nebulizer.

### 2.6.7. Elemental analysis

A LECO CHNS 932 elemental analyser, with a Sartorius M2P microbalance, was used to determine the C, N and H percentages in plant samples. This method is based on the complete and instant oxidation of the sample by means of combustion with pure oxygen at temperature varying between 100 and 1000 ºC.

### 2.6.8. Translocation factor (TF) and bioaccumulation factor (BAF)

Heavy metals translocation in plants was calculated using the translocation factor (TF) defined as follows:

$$TF = C_{aerial}/C_{root} \quad (1)$$

where $C_{aerial}$ and $C_{root}$ are metal concentrations (mg $kg^{-1}$) in the aerial part of the plant (stem and leaves) and root, respectively. Wherein, TF higher than 1 indicates that the plant translocates metals effectively from root to the aerial parts.

Furthermore, the bioaccumulation factor (BAF) was also determined by calculating the ratio of metal concentration in the aerial parts to that in the soil:

$$BAF = C_{plant\_tissue}/C_{soil} \quad (2)$$

where $C_{plant\_tissue}$ is the average metal concentration in the whole plant tissue (mg $kg^{-1}$) and $C_{soil}$ is metal concentrations in soil (mg $kg^{-1}$). BAF is used to categorize plants as

hyperaccumulators, accumulator and excluder based on the concentration of accumulated metals (>5 mg $kg^{-1}$, >1 mg $kg^{-1}$ and <1 mg $kg^{-1}$, respectively).

### 2.7. Biocarbons characterization

Raman spectroscopy was used for the structural characterization of the biocarbons. The Raman spectra were recorded using an i-Raman Plus (Microbean, Spain), equipped with a diode laser emitting at 785 nm as illumination source. The spectrum of a commercial graphite sample (99% carbon), supplied by Schunk Iberica, was also acquired for comparison purpose.

### 2.8. Statistical analysis

Statistical analysis was carried out with SPSS Statistics 24 (United States) software. Tukey's HSD (honestly significant difference) test in conjunction with an ANOVA (post-hoc analysis) was used to find averages of pH values and contents in heavy metals, carbonates, phosphates, nitrates and organic matter of different contaminated soils, and of heavy metal concentrations of plants sowed in different soils during phytoremediation, that are significantly different from each other ($p \leq 0.05$).

## 3. Results and discussion

### 3.1. Analysis of soil samples from Aznalcóllar and Cobre las Cruces mines

The contents of trace elements, carbonates, phosphates, organic matter and nitrates as well as pH were determined in triplicate for GCS, YCS and NCS samples. Table 2 shows the concentration of trace elements in soils from the Cobre las Cruces and Aznalcóllar mines and the maximum values proposed by the Andalusia regional government for a soil to be regarded as contaminated (Simón et al., 1999). The content of Cd, Cu, Zn, Pb, As and Hg in soils from Aznalcóllar mine significantly exceeded the maximum values established for these metals in Andalusia, while Cobre las Cruces mine soil did not. For

this reason, the topsoil from Cobre las Cruces mine was labelled as NCS (non-contaminated soil) while soils from the area of exploitation of Aznalcóllar mine were labelled as GCS and YCS (grey and yellow contaminated soil from Aznalcóllar mine, respectively). As for pH, very acidic values were found in the contaminated soils (YSC: 1.58 ± 0.08; GCS: 0.74 ± 0.02) while the pH of the NCS was close to neutral (7.47 ± 0.03), which accounts for de degree of contamination of the Aznalcóllar soils.

Taking into account the important role of the presence of iron oxides and hydroxides in the retention of heavy metals and their immobilization, the concentration of this element was calculated in the tree types of soils, obtaining 32213, 265357 and 104967 mg $kg^{-1}$ for NCS, GCS and YCS, respectively. The fact that the concentration of Fe in GCS was two and a half times higher than that in YCS and eight times greater than in NCS is coherent with the high concentrations of trace elements found in both soils from Aznalcóllar mine. The pH of the soil has also influence on the amount of carbonates ($CO_3^{2-}$), phosphates ($PO_4^{3-}$ or $HPO_4^{2-}$), nitrates ($NO^{3-}$) and organic matter that it contains (Table 3). At low pH, $CO_3^{2-}$ decomposes into $CO_2$, which accounts for the nil content in GCS and YCS. Besides, soil's microbiological activity is inhibited (Delwiche and Bryan, 1976; Einsle and Kroneck, 2004); this refers to the degradation of the soil's organic matter to obtain energy in the form of mineral nutrients, so the highest organic matter content was found in the soil with the lowest pH (GCS). Bacterial denitrification is also inhibited when pH is below 7, so that nitrates remained in contaminated soils. The optimum pH range for phosphorus absorption by plants is 6.0-7.0. In acidic soils, P tends to react with Fe and Mn, decreasing its availability. This can explain that GCS, the soil with the lowest pH and the highest Fe concentration, had the lowest phosphate content.

### 3.2. Preliminary trials

### 3.2.1. *Jatropha curcas* L. adaptation to highly contaminated soils

Once Cobre Las Cruces and Aznalcóllar mines soils were characterised, the first set of trials was carried out to assess whether *J. curcas* L. can adapt and grow in these soils. To do this, the plants that had been germinated in vermiculite were transplanted to 30-cm diameter pots in the soil mixtures defined in Table 1. Visual observations made during 60 days indicated that *J. curcas* L. tolerates up to 50% of YCS in the soil mixture. Plants sowed in GCS20 showed wilting of leaves at day 15, and most their leaves had fallen at day 60 (Fig. S2.A). Plants sowed in GCS50, GCS80, YCS80 and GYCS25 presented necrotic leaves at day 15, most of leaves fallen at day 60 (Fig. S2.B). Furthermore, severe necrotic streaks on the stem were observed in plants sowed in GSC80 and YCS80 (Fig. S2.C and S2.D). It is worth noting that *J. curcas* has been demonstrated to grow in mine residue soils with an markedly lower contamination (12 mg Cu $kg^{-1}$, 184 mg Zn $kg^{-1}$, 13 mg Pb $kg^{-1}$) with no phytotoxic symptoms (González-Chávez et al., 2017), but this plant has never been tested under the extreme conditions assayed in this work (Table 2). *J. curcas* has been found to survive on arsenic, chromium and zinc artificially contaminated soils up to 250, 100 and 3000 mg $kg^{-1}$, respectively (Yadav et al., 2009), the growth of the plant being inhibited at soil concentrations of 250 mg $kg^{-1}$ As, 100 mg $kg^{-1}$ Cr and 1000 mg $kg^{-1}$ Zn. These limits in As and Zn concentrations can be in agreement with our results, based on the plant survival on different soil mixtures and the composition of the original contaminated soils (Table 2). After 60 days, the plants were removed from the pots in order to carry out a complete chemical analysis.

### 3.2.2. Soil analysis

The pH of the soils was measured over time (Fig. 1). It was observed that the pH of the soils decreased between day 7 and day 15. This could indicate that *J. curcas* L. needs a

longer adaptation period to acclimate to the new substrates. From day 15 to the end of the trials the pH raised in the whole soils, but for NCS100. This pH increase was uneven, YCS20 being the soil reaching the maximum pH value (6.5) and with the highest slope. GCS20 and YCS20 were the solely contaminated soils that reached pH higher than 5 and 6, respectively, while the final pH values of GCS50, YCS50 and GYCS25 were between 3 and 4. On the contrary, the pH values of GCS80 and YCS80 were less than 2. These results are consistent with the anomalies observed in the evolution of the plants (Fig. S2) and it can be concluded that *J. curcas* L. cannot adapt to soils with percentages of contaminated soil of Aznalcóllar mine (either GCS or YCS) greater than 50%.

### 3.2.3. Analysis of *Jatropha curcas* L. biomass at the end of the adaptation period

The percentages of root, stem and leaf growth, with respect to reference plants analysed at day 0, for each soil after the 60-day adaptation period are illustrated in Fig. 2. The highest growth was found in stems, followed by roots and, finally, leaves. Obviously, the maximum biomass growth was attained in plants sowed in vermiculite and watered with Houlang solution (V100). As can been observed, the increase of GCS in soils resulted in a decrease in stem growth while the increase of YCS in soils led to the opposite effect (Fig. 2). High percentages of GCS or YCS in soils were detrimental to leaves growth. When comparing the growth of plants in soils containing GCS and YSC with that of plants sowed in non-contaminated soil (NSC100), it could be concluded that the presence of metals in contaminated soils is not only non detrimental to plant growth, but also biomass production is favoured to some extent, mainly in mixture soils containing YCS.

## 3.3. Absorption of heavy metals by *Jatropha curcas* L. in contaminated soils

As mentioned earlier, soils containing GCS or percentages of YCS higher than 50% led

to serious adaptation problems, resulting in partial or total necrosis of *J. curcas* L., thereby solely NCS100, YCS20 and YCS50 mixture soils were used in heavy metal absorption trials.

### 3.3.1. Analysis of NCS100, YCS20 and YCS50 soils after 60 and 120 days

The analyses carried out at days 0, 60 and 120 showed a pH increase in contaminated soils (YSC20 and YSC50) until reaching equilibrium (Fig. 3), which accounts for the good adaptation of *J. curcas* L. species to these contaminated soils.

Regarding macronutrients, the initial soil carbonate content drastically decreased over time (Fig. 4.A), because carbonates neutralize the acid excess caused by the presence of metals, leading to liberation of $CO_2$ and the increase in the pH (Fig. 3). The concentration of nitrates increased during phytoremediation in the three soils (Fig. 4.B). It could be due to the production of nitrates by nitrification bacteria and the potential inhibition of denitrification bacteria at pH lower than 7. Meanwhile, significant differences were not found for the amounts of phosphates and organic matter in the non-contaminated soil (Fig. 4.C and 4.D). By contrast, the concentration of phosphates increased at day 60 to subsequently decrease at day 120 in YCS20 and YCS50 soils (Fig. 4.C), the phosphates concentration at the end of the phytoremediation period being higher than the initial one. Furthermore, the organic matter content decreased in these contaminated soils (Fig. 4.D) due to low bacteria activity.

Table 4 illustrates the percentage of reduction of heavy metals in each soil at the end of the 120-day phytoremediation. Metals such as Cd, Hg and Sn decreased by 100% as a consequence of their low initial concentrations (<10 mg $kg^{-1}$), while concentrations of metals with initial concentrations between 10 and 1000 mg $kg^{-1}$ decreased by 30 – 70%, with the exception of As, which agrees with previous results (Tripathi et al., 2007; Yadav

et al., 2009). The concentration of Fe decreased by only 15-39% due to its high initial concentration (> 30000 mg $kg^{-1}$).

### 3.3.2. Analysis of the plants sowed in NCS100, YCS20 and YCS50 soils

While differences were hardly observed after 60 days, Fig. 5 clearly shows that the biomass production after 120 days drastically decreased when increasing the percentage of YCS in the soil. Thus, *J. curcas* L. barely grew in YCS50 from day 60 to day 120. The elemental analysis of roots, stems and leaves after 120 days (Fig. 6) showed that the absorption of these metals by the plants affects, above all, the content of N, which increased its percentage in leaves and decreased in roots and stems when increasing the percentage of YCS in the soil where the plant was sowed (Fig. 6.C). The higher percentage of N in YCS20 and YCS50 leaves can be related to the chelating power of metals such as Cu, Zn and Pb, which are able to fix nitrogen to form complexes (Wuana et al., 2010). Percentages of C and H were similar in each biomass section and soil.

The ICP-OES analysis showed that plants mainly absorbed and accumulated Fe (Table 5) because this was the predominant element in soils, although they also absorbed to a less extent Cr, Ni, Cu, Zn and Pb. The concentrations of these metals found can be considered as promising, since they were quite high. The concentration of Fe in plants was higher than the accumulation limit by *J. curcas* for most metallic elements (1000 mg $kg^{-1}$) reported by other authors (Ahmadpour et al., 2014; Ghavri and Singh, 2010).

The concentrations of Cu and Zn after 120 days were in the same range than those found in *J. curcas* established for 105 days on a mine residue amended or not amended with biochar and inoculated or not inoculated with the mycorrhizal fungus *Acaulospora* sp (González-Chávez et al., 2017). By contrast, these authors found much lower concentrations of Pb (less than 25 mg $kg^{-1}$) in their trials. Higher Cu concentrations (665 ± 1 mg $kg^{-1}$), based on total plant dry biomass, were found in *J. curcas* planted for 5

395 months in soils spiked with 400 mg $kg^{-1}$ Cu (Ahmadpour et al., 2014). However, our study
396 deals with much more contaminated soils with numerous heavy metals at high
397 concentrations at the same time, and the metal concentrations depend on plant growth. In
398 spite of achieving higher Cu concentrations in plant tissue, the maximum total Cu removal
399 from soils these authors achieved was 1.2% (Ahmadpour et al., 2014), while we achieved
400 Cu removal from soils of up to 72% (Table 4). Furthermore, *J. curcas* sowed in soils
401 artificially contaminated up to 10 mg Hg $kg^{-1}$ soil (using mercury nitrate solution) was
402 able to solely absorb up to 7.3 mg Hg $kg^{-1}$ biomass after 4-month trial (Marrugo-Negrete
403 et al., 2015). The low initial concentrations of trace elements (Cd, Hg and Sn) found in
404 the different parts of the plants (roots, stems and leaves) did not allow determining with
405 great precision their concentrations at the end of the heavy metal absorption trials. Since
406 these elements were not found in soils, it could be assumed that most of them were totally
407 absorbed by plants. Taken into account the different parts of the plant, 800.5, 633.3 and
408 872.1 mg metals $kg^{-1}$ plant were found in roots, stems and leaves, respectively, of
409 NCS100 samples; 1262.4, 1192.9 and 607.6 mg metals $kg^{-1}$ plant were found in roots,
410 stems and leaves, respectively, of YCS20 samples; and 2132.5, 446.4 and 421.2 mg
411 metals $kg^{-1}$ plant were found in roots, stems and leaves, respectively, of YCS50 samples.
412 As it can be observed, plants sowed in the most initially contaminated soil (YCS50) were
413 the ones that more metals accumulated in roots, which agrees with other authors' findings
414 (Ahmadpour et al., 2014; Ghavri and Singh, 2010; González-Chávez et al., 2017;
415 Marrugo-Negrete et al., 2015; Yadav et al., 2009). The first authors (Ghavri and Singh,
416 2010) detected 49.3 – 64.9 mg Fe $kg^{-1}$ *J. curcas* roots grown in garden soil for 100 days
417 while the Fe concentration in *J. curcas* roots grown in iron rich wasteland soil for 100
418 days was 6 folds higher (301.24 – 319.53 mg $kg^{-1}$). In our case, *Jatropha curcas* L. roots
419 sowed in SCN100, YCS20 and YCS50 soils for 120 days absorbed 622.2, 1124.3 and

420 1929.1 mg $kg^{-1}$ Fe.

421 Regarding the translocation of these metals in the plants, the metals are seen to remain
422 mainly in leaves and stems in most cases (Fig. 7). All the metals increased their
423 translocation to aerial parts of the plant over time for NSC100 and YCS20, except Cr in
424 plants sowed in NSC100 and Fe in plants sowed in YCS20. The metals translocation
425 factors for YCS50 showed and uneven behaviour, being the global translocation factor
426 for plants sowed in this soil lower than those for NCS100 and YSC20. The global
427 translocation factors (considering all the analysed metals) for NCS100, YCS20 and
428 YCS50 were 0.81, 1.95 and 1.37 at day 60, and 1.88, 1.43 and 0.41 at day 120,
429 respectively. Most of individual TF were higher than those found for the same plant for
430 Hg in mining soils artificially contaminated after the same phytoremediation time
431 (Marrugo-Negrete et al., 2015), for Cu in soils spiked with Cu in amounts of 0, 50, 100,
432 200, 300, and 400 mg $kg^{-1}$ (Ahmadpour et al., 2014), and for Cu, Zn and Pb in mining
433 soils with biochar addition and under the effect of mycorrihizal inoculation (González-
434 Chávez et al., 2017). Accumulation and distribution of heavy metals in plant tissues are
435 of major importance in phytoremediation of contaminated soils. If the objective is to
436 stabilize metals in roots for them not to enter in stems and leaves and therefore to prevent
437 metals from entering the ecosystem, low TF are desirable. Therefore, the low global TF
438 (0.41) obtained for the most contaminated soil (YCS50) at day 120 is a promising result.
439 On the contrary, the high TF obtained for some metals such as Cr, Ni, Zn and Pb cannot
440 be regarded as good results if the purpose is the phytoremediation of soils contaminated
441 with these metals.

442 With regard to bioaccumulation factors, is spite of the great Fe concentration absorbed by
443 plants (Table 5), BAF of Fe was very low for the 3 soils in the 2 days of sampling because
444 of the huge initial concentration of Fe (between 32213 and 265357 mg $kg^{-1}$), which could

not be removed in 120 days, so a great amount of Fe remained in the soils at the end of this period of time (Table 4). This also led to low global bioaccumulation factors. The global BAF for NCS100, YCS20 and YCS50 were 0.142, 0.097 and 0.041 at day 60, and 0.117, 0.083 and 0.049 at day 120, respectively. As can be noted, BAF decreased over time. This was due to the huge plant growth from day 60 to day 120 (Fig. 5). BAF (except for Fe) were in the range of those found for Hg, Cu, Zn and Pb in mining soils with *J. curcas* for a similar cultivation period (González-Chávez et al., 2017; Marrugo-Negrete et al., 2015), these soils having initial metal concentrations much lower than those used in this study, which highlights the good results achieved.

### 3.4. Characterization of the catalytic carbons.

Two main phases can be distinguished in the thermogravimetric curves obtained in the pyrolysis of the contaminated root samples at different temperatures. Fig. 9 depicts the weight losses obtained for the roots of plants sowed in YCS50 soil, which had the highest concentration of heavy metals (2132.5 mg $kg^{-1}$). The first, between room temperature and 350 ºC, is the active zone and is related to the degradation of polymers of hemicellulose and cellulose. The second phase, the passive zone, showed a decrease in the slope until 600 ºC as a result of the decomposition of lignin. Beyond this phase, the slope steepened again due to the loss of mass caused by the total degradation of the biomass.

Fig. S3 shows the Raman spectra of the pyrolytic carbons for this sample obtained from 300 ºC to 650 ºC and the spectrum of the standard graphite. For C-based materials containing C-$sp^2$ sites, the most intense features can be observed at about 1582, 1350, 1620 and 2700 $cm^{-1}$, which are called G, D, D' and 2D (also G') (Reich and Thomsen, 2004; Tai et al., 2009; Veres et al., 2008; Viana and Marques, 2015). The G band appears from a double degenerated phonon mode with $E_{2g}$ symmetry, while the D and D′ bands

emerge from the phonon modes with $A_{1g}$ symmetry (Ferrari and Robertson, 2000; Pimenta et al., 2007; Reich and Thomsen, 2004). The G band appears in all graphite samples, while the D and D′ bands only appear in the spectra of disordered samples, and highly crystalline graphite does not show these bands. So, the D band is attributed to disorders and defects in the graphite structure. Comparing the G-band (~ 1575 $cm^{-1}$) and the D-band (~ 1355 $cm^{-1}$) obtained for pyrolytic carbons with that of commercial graphite (Fig. S5), it can be observed that the sample pyrolysed at 550 ºC is the one that presents the intensity ratio of G and D bands, as well as their positions and widths, more similar to those of graphite. Samples pyrolysed at temperature lower than 550 ºC showed wider bands that overlap each other while samples pyrolysed at temperatures higher than 550 ºC showed lower intensity bands, leading to the formation of amorphous carbonaceous materials.

## Conclusions

The high percentage of germination obtained from the seeds of *Jatropha curcas* L. (85%) and the rapid adaptation to the spring climate simulated in the greenhouse make this species a plant with wide possibilities for phytoremediation of marginal soils. Nevertheless, *J. curcas* L. did solely survive in soil mixtures containing up to 50% of the highly contaminated mining soils assayed in this work.

With regard to metal removal from soils, metals with low initial concentrations (<10 mg $kg^{-1}$) such as Hg, Sn and Cd were completely removed, while the concentrations of metals within the range 10 – 1000 mg $kg^{-1}$ (Cr, Ni, Cu, Zn and Pb) were reduced between 30 – 70%, except As. The concentration of Fe in soils was solely reduced 15% as consequence of its high initial concentration (> 30000 mg $kg^{-1}$). On the other hand, the translocation

493 factors of Cr, Ni, Zn and Pb were high, which can be a hindrance for the phytoremediation
494 of soils containing high concentrations of these metals.
495 Finally, the structural characterization by Raman spectroscopy of pyrolytic coal obtained
496 from the contaminated roots of the *J. curcas* L. showed that the most suitable pyrolysis
497 temperature to obtain a graphite structure was 550 ºC.
498
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503 **References**
504 Abhilash, P.C., Powell, J.R., Singh, H.B., Singh, B.K., 2012. Plant-microbe
505 interactions: Novel applications for exploitation in multipurpose remediation
506 technologies. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2012.04.004
507 Abhilash, P.C., Singh, B., Srivastava, P., Schaeffer, A., Singh, N., 2013. Remediation of
508 lindane by Jatropha curcas L: Utilization of multipurpose species for
509 rhizoremediation. Biomass and Bioenergy 51, 189–193.
510 https://doi.org/10.1016/j.biombioe.2013.01.028
511 Ahmadpour, P., Soleimani, M., Ahmadpour, F., Abdu, A., 2014. Evaluation of Copper
512 Bioaccumulation and Translocation in Jatropha curcas Grown in a Contaminated
513 Soil. Int. J. Phytoremediation 16, 454–468.
514 https://doi.org/10.1080/15226514.2013.798614
515 Ali, Q., Ahsan, M., Khaliq, I., Elahi, M., Ali, S., Ali, F., Naees, M., 2011. Role of
516 Rhizobacteria in phytoremediation of heavy metals: An overview. Int. Res. J. Plant
517 Sci. 2, 220–232.

APHA, AWWA, WEF, 2005. Standard methods for the examination of water and wastewater. Am. Public Heal. Assoc. Washington, DC, USA 1–2671.

Bernabé-Antonio, A., Álvarez, L., Buendía-González, L., Maldonado-Magaña, A., Cruz-Sosa, F., 2015. Accumulation and tolerance of Cr and Pb using a cell suspension culture system of Jatropha curcas. Plant Cell. Tissue Organ Cult. 120, 221–228. https://doi.org/10.1007/s11240-014-0597-y

Chang, F.-C., Ko, C.-H., Tsai, M.-J., Wang, Y.-N., Chung, C.-Y., 2014. Phytoremediation of heavy metal contaminated soil by Jatropha curcas. Ecotoxicology 23, 1969–1978. https://doi.org/10.1007/s10646-014-1343-2

Chavan, S.B., Kumbhar, R.R., Madhu, D., Singh, B., Sharma, Y.C., 2015. Synthesis of biodiesel from Jatropha curcas oil using waste eggshell and study of its fuel properties. RSC Adv. 5, 63596–63604. https://doi.org/10.1039/C5RA06937H

Cobbett, C., 2003. Heavy metals and plants - Model systems and hyperaccumulators. New Phytol. https://doi.org/10.1046/j.1469-8137.2003.00832.x

Delwiche, C.C., Bryan, B.A., 1976. Denitrification. Annu. Rev. Microbiol. 30, 241–262.

Einsle, O., Kroneck, P.M.H., 2004. Structural basis of denitrification. Biol. Chem. 385, 875–883. https://doi.org/10.1515/BC.2004.115

Ferrari, A., Robertson, J., 2000. Interpretation of Raman spectra of disordered and amorphous carbon. Phys. Rev. B - Condens. Matter Mater. Phys. 61, 14095–14107. https://doi.org/10.1103/PhysRevB.61.14095

Ghavri, S.V., Singh, R.P., 2010. Phytotranslocation of fe by biodiesel plant Jatropha curcas L. grown on iron rich wasteland soil. Brazilian J. Plant Physiol. 22, 235–243. https://doi.org/10.1590/S1677-04202010000400003

González-Chávez, M. del C.A., Carrillo-González, R., 2013. Tolerance of

Chrysantemum maximum to heavy metals: The potential for its use in the revegetation of tailings heaps. J. Environ. Sci. (China) 25, 367–375. https://doi.org/10.1016/S1001-0742(12)60060-6

González-Chávez, M. del C.A., Carrillo-González, R., Hernández Godínez, M.I., Evangelista Lozano, S., 2017. Jatropha curcas and assisted phytoremediation of a mine tailing with biochar and a mycorrhizal fungus. Int. J. Phytoremediation 19, 174–182. https://doi.org/10.1080/15226514.2016.1207602

Jackson, M.L., 1958. Soil chemical analysis. New York Prentice Hall 498.

Jamil, S., Abhilash, P.C., Singh, N., Sharma, P.N., 2009. Jatropha curcas: A potential crop for phytoremediation of coal fly ash. J. Hazard. Mater. 172, 269–275. https://doi.org/10.1016/j.jhazmat.2009.07.004

Järup, L., 2003. Hazards of heavy metal contamination. Br. Med. Bull. 68, 167–182. https://doi.org/10.1093/bmb/ldg032

Jiménez-Moraza, C., Iglesias, N., Palencia, I., 2006. Application of sugar foam to a pyrite-contaminated soil. Miner. Eng. 19, 399–406. https://doi.org/10.1016/j.mineng.2005.10.011

LeDuc, D.L., Terry, N., 2005. Phytoremediation of toxic trace elements in soil and water, in: Journal of Industrial Microbiology and Biotechnology. pp. 514–520. https://doi.org/10.1007/s10295-005-0227-0

Marrugo-Negrete, J., Durango-Hernández, J., Pinedo-Hernández, J., Olivero-Verbel, J., Díez, S., 2015. Phytoremediation of mercury-contaminated soils by Jatropha curcas. Chemosphere 127, 58–63. https://doi.org/10.1016/j.chemosphere.2014.12.073

Mukhopadhyay, S., Maiti, S.K., 2010. Phytoremediation of metal mine waste. Appl. Ecol. Environ. Res. 8, 207–222.

568 Pandey, V.C., Singh, K., Singh, J.S., Kumar, A., Singh, B., Singh, R.P., 2012. Jatropha
569 curcas: A potential biofuel plant for sustainable environmental development.
570 Renew. Sustain. Energy Rev. https://doi.org/10.1016/j.rser.2012.02.004
571 Pimenta, M.A., Dresselhaus, G., Dresselhaus, M.S., Cançado, L.G., Jorio, A., Saito, R.,
572 2007. Studying disorder in graphite-based systems by Raman spectroscopy. Phys.
573 Chem. Chem. Phys. 9, 1276–1290. https://doi.org/10.1039/B613962K
574 Reich, S., Thomsen, C., 2004. Raman spectroscopy of graphite. Philos. Trans. R. Soc. A
575 Math. Phys. Eng. Sci. 362, 2271–2288. https://doi.org/10.1098/rsta.2004.1454
576 Sánchez-Chardi, A., Ribeiro, C.A.O., Nadal, J., 2009. Metals in liver and kidneys and
577 the effects of chronic exposure to pyrite mine pollution in the shrew Crocidura
578 russula inhabiting the protected wetland of Doñana. Chemosphere 76, 387–394.
579 https://doi.org/10.1016/j.chemosphere.2009.03.036
580 Simón, M., Ortiz, I., García, I., Fernández, E., Fernández, J., Dorronsoro, C., Aguilar, J.,
581 1999. Pollution of soils by the toxic spill of a pyrite mine (Aznalcollar, Spain). Sci.
582 Total Environ. 242, 105–115. https://doi.org/10.1016/S0048-9697(99)00378-2
583 Singh, R., Gautam, N., Mishra, A., Gupta, R., 2011. Heavy metals and living systems:
584 An overview. Indian J. Pharmacol. 43, 246. https://doi.org/10.4103/0253-
585 7613.81505
586 Singh, O. V., Labana, S., Pandey, G., Budhiraja, R., Jain, R.K., 2003.
587 Phytoremediation: an overview of metallic ion decontamination from soil. Appl.
588 Microbiol. Biotechnol. 61, 405–412. https://doi.org/10.1007/s00253-003-1244-4
589 Suresh, B., Ravishankar, G.A., 2004. Phytoremediation—A Novel and Promising
590 Approach for Environmental Clean-up. Crit. Rev. Biotechnol. 24, 97–124.
591 https://doi.org/10.1080/07388550490493627
592 Tai, F.C., Lee, S.C., Chen, J., Wei, C., Chang, S.H., 2009. Multipeak fitting analysis of

593 Raman spectra on DLCH film. J. Raman Spectrosc. 40, 1055–1059.
594 https://doi.org/10.1002/jrs.2234
595 Teo, S.H., Rashid, U., Taufiq-Yap, Y.H., 2014. Heterogeneous catalysis of
596 transesterification of jatropha curcas oil over calcium–cerium bimetallic oxide
597 catalyst. RSC Adv. 4, 48836–48847. https://doi.org/10.1039/C4RA08471C
598 Teo, S.H., Taufiq-Yap, Y.H., Rashid, U., Islam, A., 2015. Hydrothermal effect on
599 synthesis, characterization and catalytic properties of calcium methoxide for
600 biodiesel production from crude Jatropha curcas. RSC Adv. 5, 4266–4276.
601 https://doi.org/10.1039/C4RA11936C
602 Tripathi, R.D., Srivastava, S., Mishra, S., Singh, N., Tuli, R., Gupta, D.K., Maathuis,
603 F.J.M., 2007. Arsenic hazards: strategies for tolerance and remediation by plants.
604 Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2007.02.003
605 Veres, M., Tóth, S., Koós, M., 2008. New aspects of Raman scattering in carbon-based
606 amorphous materials. Diam. Relat. Mater. 17, 1692–1696.
607 https://doi.org/10.1016/j.diamond.2008.01.110
608 Viana, G.A., Marques, F.C., 2015. Raman and thermal desorption spectroscopy
609 analyses of amorphous graphite-like carbon films with incorporated xenon.
610 Vacuum 112, 17–24. https://doi.org/10.1016/j.vacuum.2014.10.019
611 Wuana, R.A., Okieimen, F.E., Imborvungu, J.A., 2010. Removal of heavy metals from
612 a contaminated soil using organic chelating acids. Int. J. Environ. Sci. Technol. 7,
613 485–496. https://doi.org/10.1007/BF03326158
614 Yadav, S.K., Juwarkar, A.A., Kumar, G.P., Thawale, P.R., Singh, S.K., Chakrabarti, T.,
615 2009. Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by
616 Jatropha curcas L.: Impact of dairy sludge and biofertilizer. Bioresour. Technol.
617 100, 4616–4622. https://doi.org/10.1016/j.biortech.2009.04.062

618 Yee, K.F., Wu, J.C.S., Lee, K.T., 2011. A green catalyst for biodiesel production from
619 jatropha oil: Optimization study. Biomass and Bioenergy 35, 1739–1746.
620 https://doi.org/10.1016/j.biombioe.2011.01.017
621

Table 1. Percentages of non-contaminated soil from Cobre las Cruces mine (NCS) and grey (GCS) and yellow (YCS) contaminated soils from Aznalcóllar mine and pH of the resulting soil mixtures used to assess adaptation of *Jathopa curcas* L. to these soils.

| **Sample** | **NCS (%)** | **GCS (%)** | **YCS (%)** | **pH** |
|---|---|---|---|---|
| **V100** | 0 | 0 | 0 | 7.00 ± 0.10 [a] |
| **NCS100** | 100 | 0 | 0 | 7.47 ± 0.08 [b] |
| **GCS20** | 80 | 0 | 20 | 4.54 ± 0.08 [c] |
| **GCS50** | 50 | 0 | 50 | 3.50 ± 0.05 [d] |
| **GCS80** | 20 | 0 | 80 | 1.56 ± 0.03 [e] |
| **YCS20** | 80 | 20 | 0 | 4.60 ± 0.07 [c] |
| **YCS50** | 50 | 50 | 0 | 2.58 ± 0.03 [f] |
| **YCS80** | 20 | 80 | 0 | 2.14 ± 0.05 [g] |
| **GYCS25** | 50 | 25 | 25 | 2.76 ± 0.02 [h] |

Different lowercase letters in the pH column indicate significant differences according to ANOVA ($p \leq 0.05$).

Table 2. Concentration of trace elements in Cobre las Cruces and Aznalcóllar mine soils and maximum values proposed for a soil to be declared contaminated in Andalucia (Simón et al., 1999).

| **Metal** | **Andalusia soils** | | **Cobre las Cruces** | **Aznalcóllar mine** | |
|---|---|---|---|---|---|
| **( mg kg$^{-1}$)** | | | **mine** | | |
| | **pH < 7** | **pH > 7** | **NCS** | **GCS** | **YCS** |
| **Cd** | < 2 | < 3 | 6 ± 1$^{a}$ | 27 ± 3$^{b}$ | 13 ± 2$^{c}$ |
| **Cr** | < 100 | | 59 ± 3$^{a}$ | 8 ± 1$^{b}$ | 11 ± 1$^{c}$ |
| **Cu** | < 40 | < 100 | 40 ± 2$^{a}$ | 270 ± 15$^{b}$ | 585 ± 29$^{c}$ |
| **Ni** | < 40 | < 50 | 23 ± 1$^{a}$ | 12 ± 1$^{b}$ | 14 ± 1$^{c}$ |
| **Zn** | < 200 | < 500 | 102 ± 5$^{a}$ | 2608 ± 124$^{b}$ | 2485 ± 124$^{b}$ |
| **Pb** | < 100 | < 200 | 94 ± 5$^{a}$ | 48686 ± 2450$^{b}$ | 4557 ± 228$^{c}$ |
| **As** | < 20 | | 18 ± 1$^{a}$ | 2366 ± 112$^{b}$ | 1121 ± 56$^{c}$ |
| **Hg** | < 1 | | 0 ± 0$^{a}$ | 58 ± 2$^{b}$ | 19 ± 1$^{c}$ |
| **Sn** | <20 | | 0 ± 0$^{a}$ | 14 ± 2$^{b}$ | 5 ± 1$^{c}$ |

Averages with different lowercase letters in the same row are significantly different ($p \leq 0.05$).

Table 3. Content in carbonates ($CaCO_3$), phosphates ($PO_4^{3-}$ or $HPO_4^{2-}$), nitrates ($NO^{3-}$) and organic matter of NCS, GCS and YCS samples.

| Soil | Carbonates (%) | Phosphates (mg $kg^{-1}$) | Nitrates (mg $kg^{-1}$) | Organic matter (g $kg^{-1}$) |
|---|---|---|---|---|
| **NCS** | $11.8 \pm 0.1^a$ | $16.8 \pm 0.4^a$ | $13.2 \pm 0.4^a$ | $12 \pm 2^a$ |
| **GCS** | $0 \pm 0.0^b$ | $0.12 \pm 0.1^b$ | $837 \pm 9.0^b$ | $72 \pm 17^b$ |
| **YCS** | $0 \pm 0.0^b$ | $9.1 \pm 0.3^c$ | $1093 \pm 63.0^c$ | $22 \pm 1^c$ |

Averages with different lowercase letters in the same column are significantly different ($p \leq 0.05$).

Table 4. Decrease in the concentration of heavy metals in NCS100, YCS20 and YCS50 soils after 120 days.

| | % Concentration decrease | | |
|---|---|---|---|
| **Metal** | **NCS100** | **YCS20** | **YCS50** |
| **Cr** | 28 | 29 | 27 |
| **Fe** | 39 | 23 | 15 |
| **Ni** | 32 | 52 | 67 |
| **Cu** | 72 | 39 | 23 |
| **Zn** | 54 | 45 | 37 |
| **Cd** | - | 100 | 100 |
| **Hg** | - | 100 | 100 |
| **Sn** | - | 100 | 100 |
| **Pb** | 69 | 58 | 51 |
| **As** | 9 | 5 | 5 |

Table 5. Concentration of main heavy metals in plants sowed in NCS100, YCS20 and YCS50 soils after 60 and 120 days.

| | **Cr (mg kg$^{-1}$)** | **Fe (mg kg$^{-1}$)** | **Ni (mg kg$^{-1}$)** | **Cu (mg kg$^{-1}$)** | **Zn (mg kg$^{-1}$)** | **Pb (mg kg$^{-1}$)** | **Total (mg kg$^{-1}$)** |
|---|---|---|---|---|---|---|---|
| 60 days | | | | | | | |
| **NCS100** | 104.0 ± 0.0$^{a}$ | 3308.1 ± 0.036$^{a}$ | 0.0 ± 0.0$^{a}$ | 97.9 ± 0.0$^{a}$ | 222.1 ± 0.0$^{a}$ | 12.0 ± 0.0$^{a}$ | 3744.2 |
| **YCS20** | 33.8 ± 0.0$^{b}$ | 3130.5 ± 0.050$^{b}$ | 35.8 ± 0.0$^{b}$ | 44.0 ± 0.1$^{b}$ | 160.8 ± 0.0$^{b}$ | 139.8 ± 0.0$^{b}$ | 3544.8 |
| **YCS50** | 74.5 ± 0.0$^{c}$ | 2114.4 ± 0.015$^{c}$ | 23.8 ± 0.0$^{c}$ | 54.4 ± 0.0$^{c}$ | 305.4 ± 0.0$^{c}$ | 31.4 ± 0.0$^{c}$ | 2603.9 |
| 120 days | | | | | | | |
| **NCS100** | 166.4 ± 0.0$^{d}$ | 1653.1 ± 0.014$^{d}$ | 76.0 ± 0.0$^{d}$ | 41.1 ± 0.0$^{d}$ | 203.1 ± 0.0$^{d}$ | 166.1 ± 0.0$^{d}$ | 2305.9 |
| **YCS20** | 119.6 ± 0.0$^{e}$ | 2503.7 ± 0.041$^{e}$ | 74.8 ± 0.0$^{e}$ | 40.4 ± 0.0$^{e}$ | 226.7 ± 0.0$^{e}$ | 97.8 ± 0.0$^{e}$ | 3062.9 |
| **YCS50** | 49.76 ± 0.0$^{f}$ | 2598.2 ± 0.015$^{f}$ | 19.5 ± 0.0$^{f}$ | 44.5 ± 0.0$^{f}$ | 242.0 ± 0.0$^{f}$ | 46.3 ± 0.0$^{f}$ | 3000.2 |

Averages with different lowercase letters in the same column are significantly different ($p \leq 0.05$).

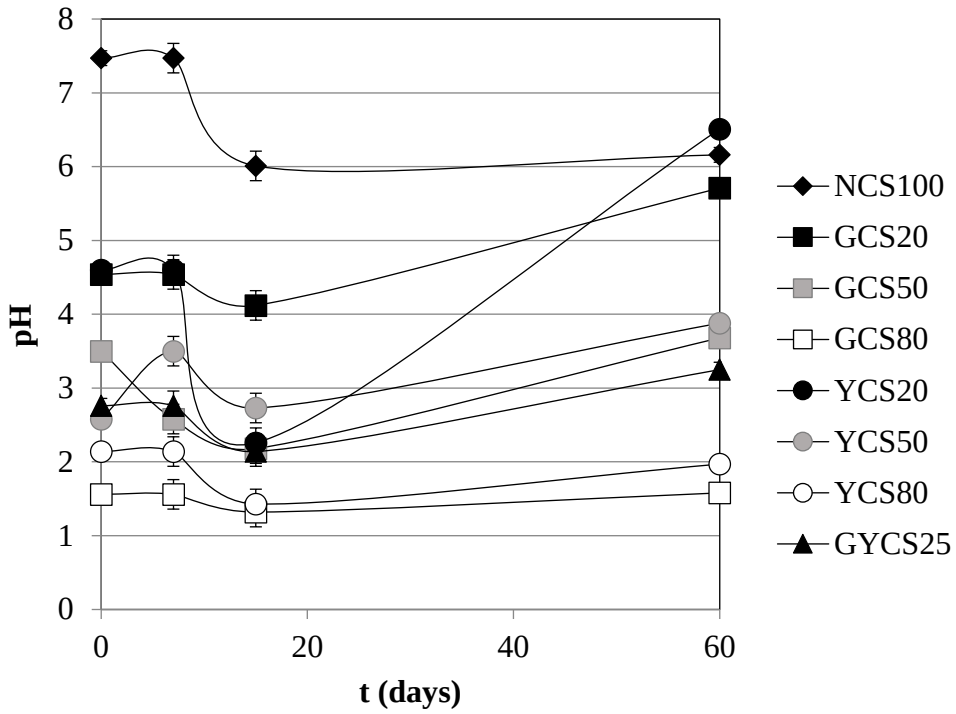


Figure 1: Evolution of pH over time for each soil sample assayed in the preliminary trials.

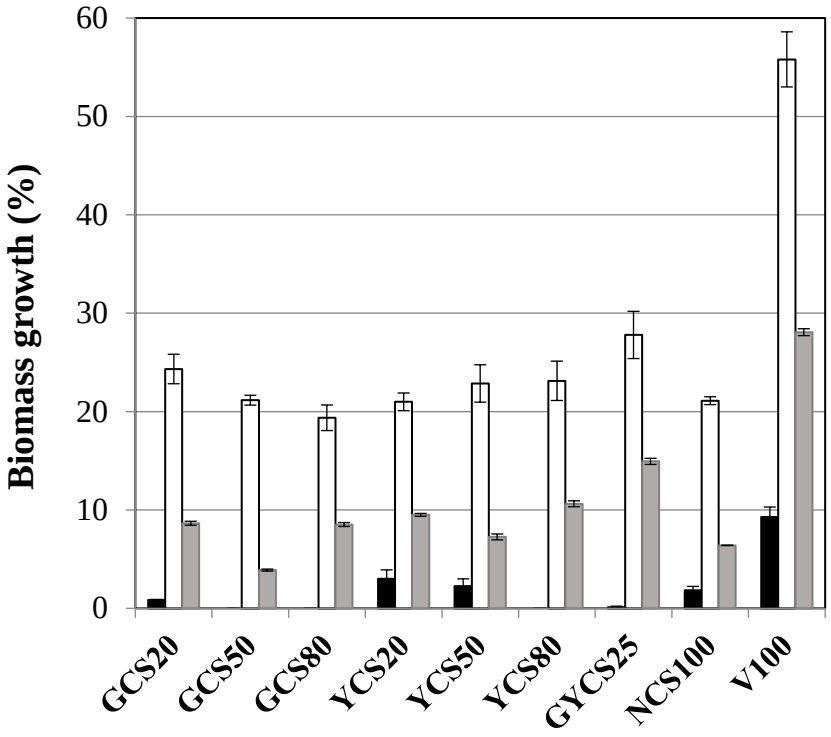


Figure 2. Percentage of growth of leaves (black bars), stems (open bars) and roots (grey bars) of *Jatropha curcas* L. in each soil at the end of the 60-day adaptation period.

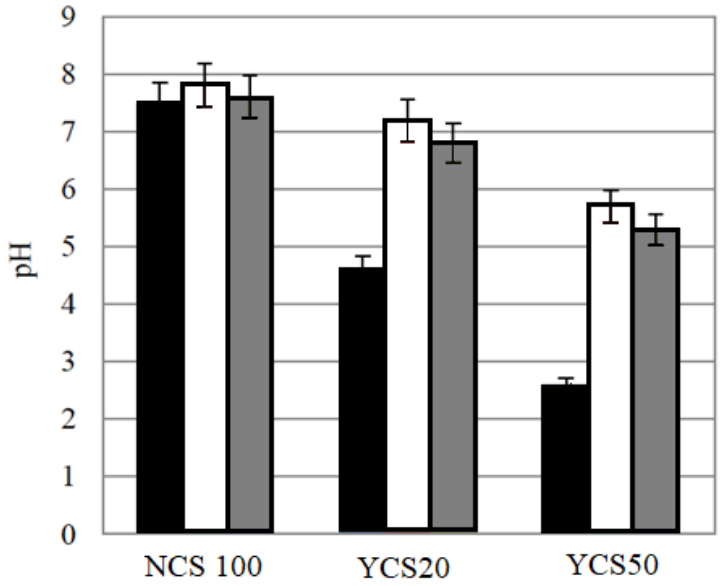


Figure 3: Changes in pH in soils at days 0 (black bars), 60 (open bars) and 120 (grey bars) during the heavy metal absorption trials.

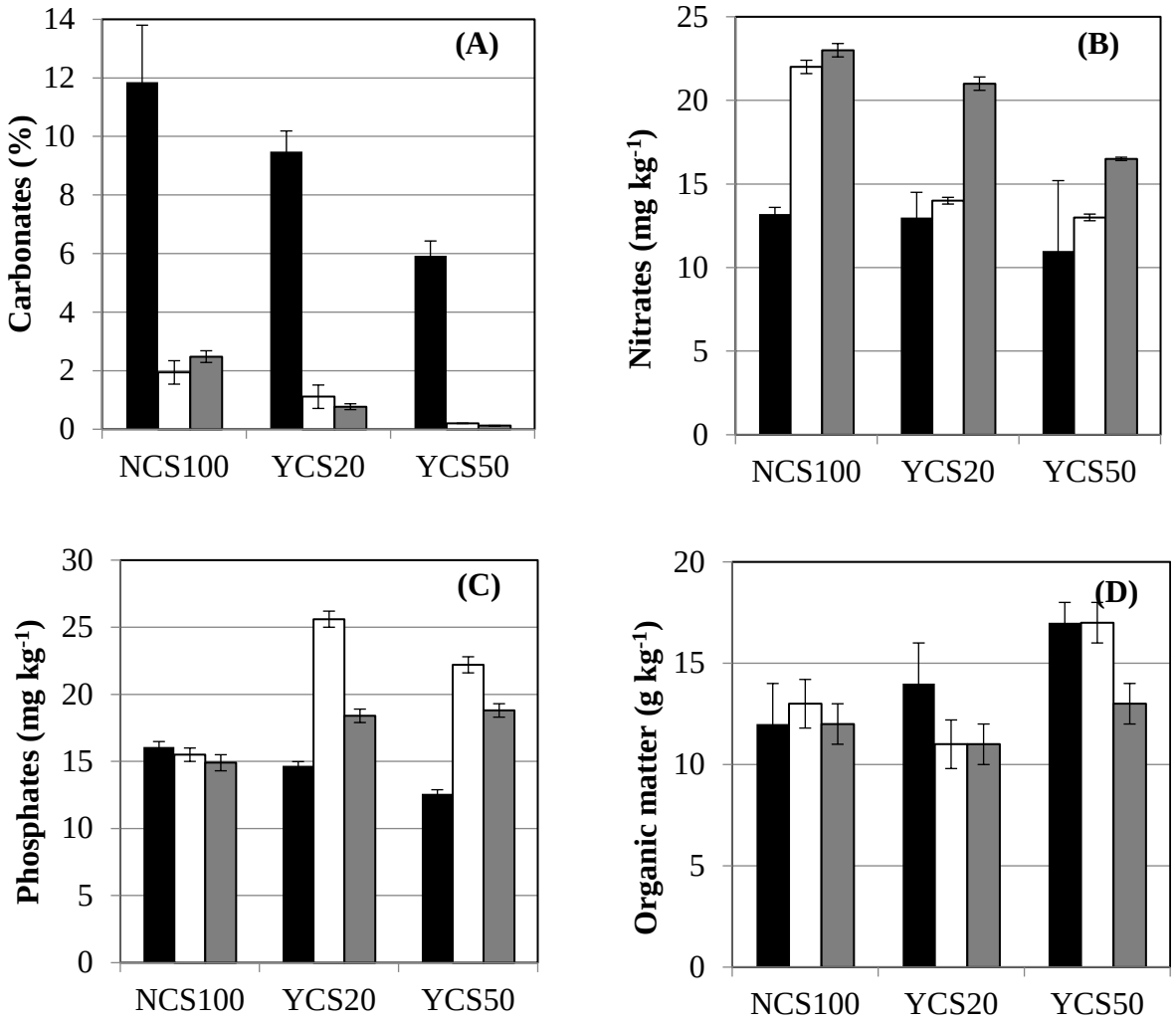


Figure 4: Evolution of the contents of (A) carbonates, (B) phosphates, (C) nitrates and (D) organic matter in NCS100, YCS20 and YCS50 soils at the beginning (black bars), after 60 days (open bars) and after 120 days (grey bars).

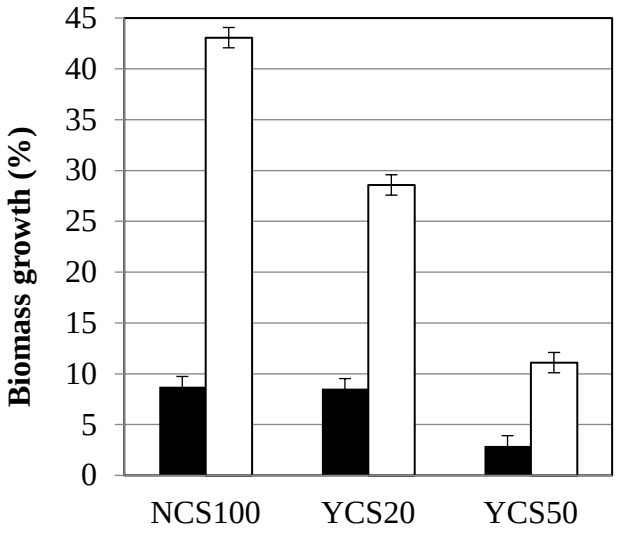


Figure 5: Percentage of biomass increased of plants sowed in NCS100, YCS20 and YCS50 soils after 60 (black bars) and 120 (open bars) days.

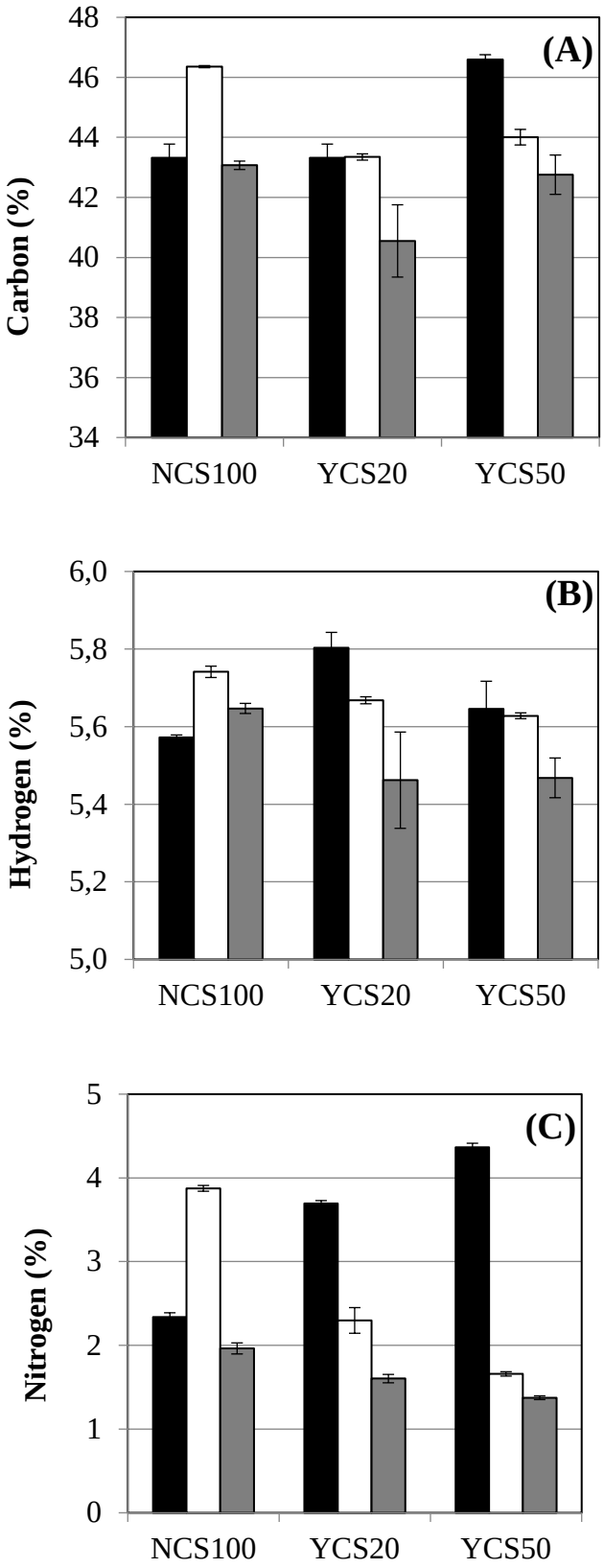


Figure 6. Elemental analysis of carbon (A), hydrogen (B) and nitrogen (C) in leaves (black bars), stems (open bars) and roots (grey bars) of plants sowed for 120 days in

NCS100, YCS20 and YCS50 soils.

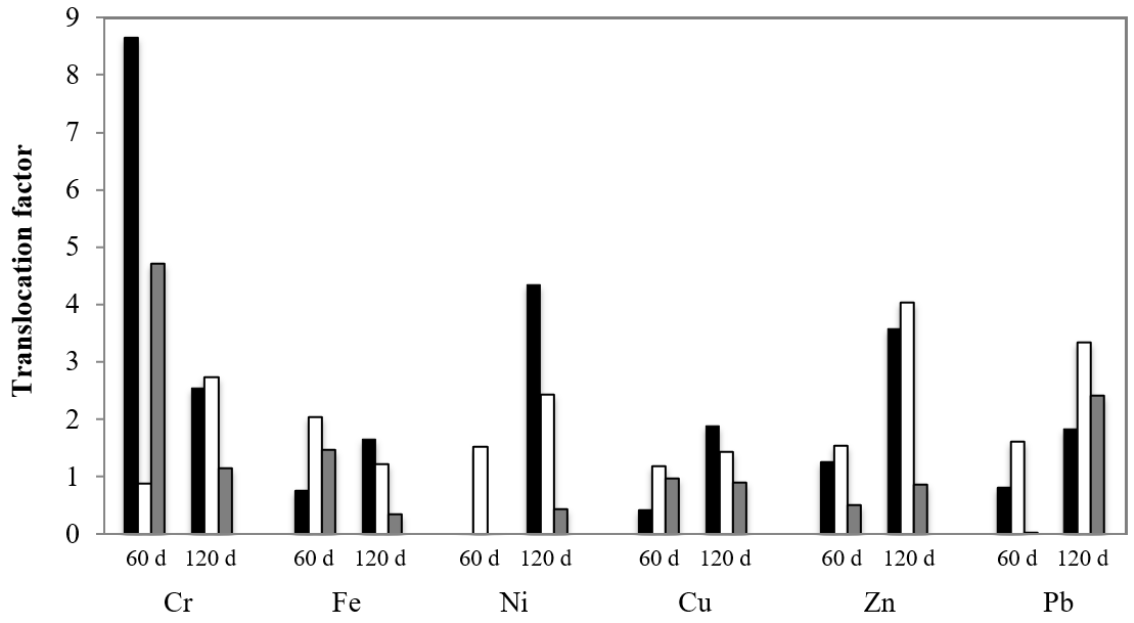


Fig. 7: Translocation factors of Cr, Fe, Ni, Cu, Zn and Pb of *Jatropha curcas* L. plants sowed in NCS100 (black bars), YSC20 (open bars) and YCS50 (grey bars) soils after 60 and 120 days.

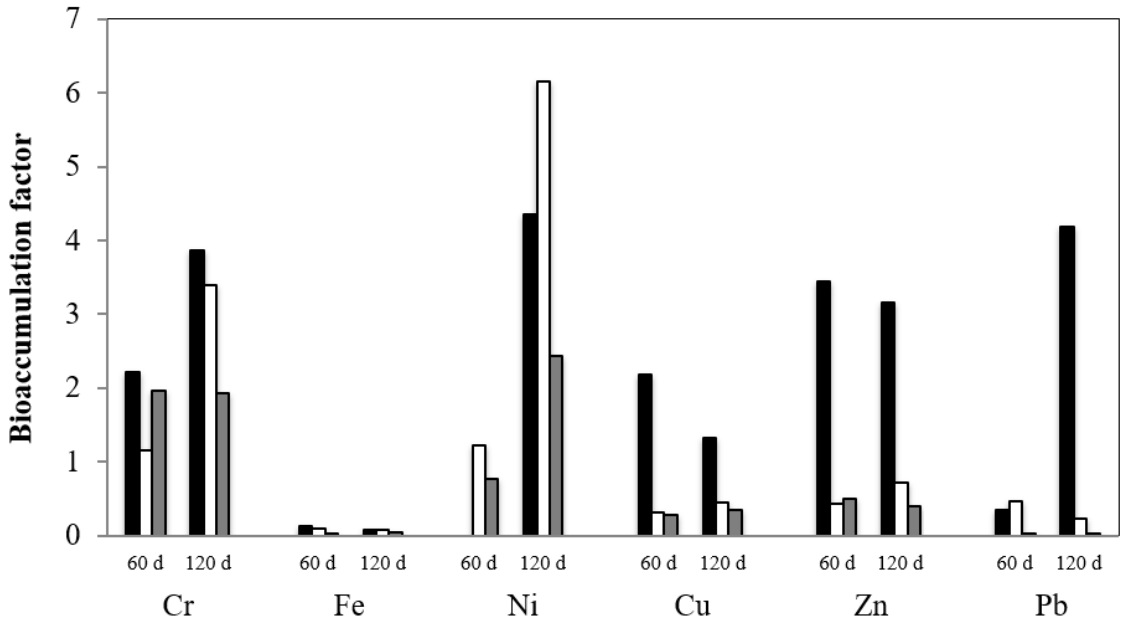


Fig. 8: Bioaccumulation factors of Cr, Fe, Ni, Cu, Zn and Pb of *Jatropha curcas* L. plants sowed in NCS100 (black bars), YSC20 (open bars) and YCS50 (grey bars) soils after 60 and 120 days.

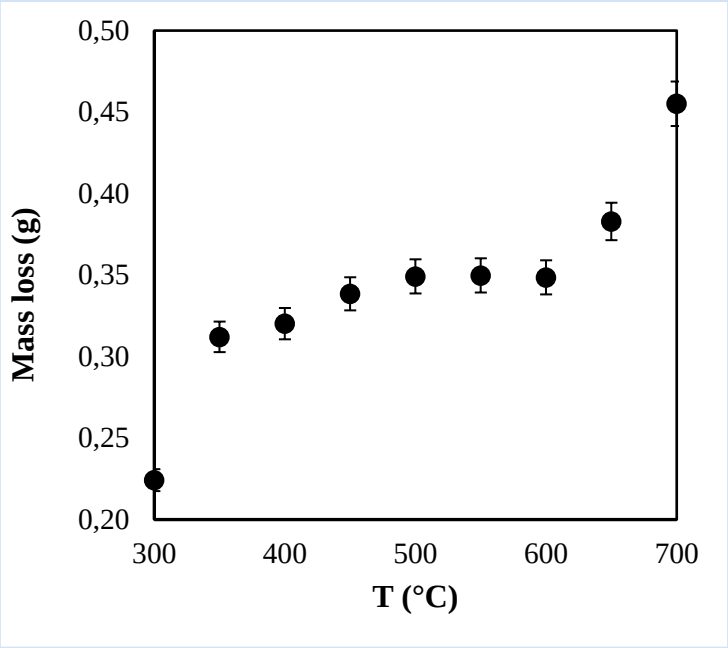


Figure 9. Thermogravimetric curve obtained in the pyrolysis of the roots of *Jatropha curcas* L. planted in the YCS50 soil.