Usage of Jatropha Curcas Plant for Phytoremediation of Cd and Pb Contaminated Soil

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ABSTRACT
The heavy metals contaminated agricultural soil is a serious problem that causes negative impacts for various organisms. Jatropha plant may have considerable phytoremediation potential. Pot experiment was conducted at Antoniadis Garden, Horticulture Research Institute Alexandria branch Ministry of Agriculture, Egypt to study the effect of different combinations of Cd (14.4, 28.8 and 43.2 mg/kg soil) and Pb (247.7, 495.4 and 743.2 mg/kg soil) on growth and phytoextraction efficiency of jatropha plant. This plant can tolerate Cd and Pb up to high level of each, with survival 100%. The analysed data indicated significant reduction in vegetative traits (plant height, stem diameter, branches number, area/leaf, fresh and dry weights of leaves, stems and roots and length of the longest root). From application of such levels of Cd and Pb in relative to the control. A considerable increase was noticed in the content and uptake of Cd and Pb in the different plant organs, as well as total plant uptake in comparison to the control treatment. Bioconcentration factor (BCF) of shoots and roots was to be found < 1 under used levels of Cd and Pb. While translocation factor % (TF%) was > 100 except for Pb translocation factor of control which was less than 100. Also, total accumulation rat (TAR) of Cd and Pb was dependent on the levels of such elements in the combinations. Tolerance index (TI) of biomass and root was < 1, except for tolerance index of Pb in case of low levels of Cd and Pb, it was > 1. From the results of BCF, TF, TAR and TI, it can be recommended that Jatropha plant is a good candidate for research of phytoremediation and phytoextraction of Cd and Pb contaminated soil.

Keywords: Phytoremediation, heavy metals, Jatropha curcas, contaminated soil, cadmium, Lead.

1. Introduction
Growing environmental concern about multi-element contaminated soils highlights the importance of focusing more on ecologically friendly remediation strategies like phytoremediation (Razmi et al., 2021). Also, rapid industrialization, advanced agricultural habits and other anthropogenic activities add a significant quantity of toxic heavy metals into the environment, which encourages severe toxic effects on all shape of living organisms, alter the soil features and its biological activity (Manoj et al., 2020). Soil pollution by heavy metals and metalloids is a grave problem around the world (Sessitsch et al., 2013) which requirements to be directed and is still increasing along with the continuing industrialization and urbanization. Among other resources, the release of heavy metals via various applications in industry and agriculture is increasing environmental and human health risks related with exposure to these polluted soils and/or dust (Khan et al., 2010).

Metals like Cd and Pb are toxic and may pose a great threat to plants, animals, and humans by the food chain. Heavy metals can login the ecosystem by natural geological process or via anthropogenic processes as industrial and municipal wastes (Azez et al., 2015).
Lead (Pb) causes harmful effects on crop species like the physiological, biochemical, molecular and cytological levels. Preceding studies have appeared that Pb is also able of preventing shoot and root germination as explained by (Lamhamdi et al., 2011). Pb as well reasons chlorosis, decreased seed germination, growth, biomass, photosynthesis, nutrient and water uptake and transport, change membrane permeability, encourage abnormal morphology, oxidative stress (ROS generation) in plants, and prevent enzymatic activity at the cellular level by reacting with their sulfhydryl groups (Saxena et al., 2020). Cadmium (Cd) has been notified to upset the enzymatic antioxidant system of plants by causing imbalances in cellular redox status by inducing oxidative stresses (Li et al., 2015). Plants approve different biochemical and molecular strategies, hormonal regulation, antioxidative enzymes, vacuolar, transporters and metal chelators to handle with the hurtful effects of metal pollution during metal contamination (Galano et al., 2015).

Phytoremediation, the use of plants to decrease the threat related mostly with heavy metal contaminated soils, has been actively examined as a low-cost choice that causes no deterioration in soil quality (Shrestha et al., 2019). The cost analysis of phytoremediation was carried out, and it was found that this technique is most cost effective than other treatment techniques, like ion exchange, solvent extraction, adsorption, oxidation-reduction, and reverse osmosis. Furthermore, phytoremediation enlarges the soil quality and soil biological processes (Wan et al., 2016).

Jatropha curcas L. belongs to family Euphorbiaceae, researche showed that jatropha is native to Central and South America (Pecina-Quintero et al., 2014). J. curcas is simply propagated by seeds or stem cutting; moreover, it propagated via tissue culture technique, it has a brief period of growth until the first fruit harvest (Corte-Real at al., 2016).

Jatropha curcas is a precious multi-purpose crop, traditionally it was used as medicine for wounds and leaves used as drinks against malaria. Jatropha plants used to control soil degradation, alleviate erosion, desertification and enlarge soil fertility, on the other hand, in last decades there is extra attention to use jatropha oil for produce biodiesel, it is accepting drought for longtime, it is growing well with treated wastewater, as well, it can be grown on marginal land. Jatropha curcas seeds have about 32-40% valuable oil used to produce biofuel; consequently, it could be the source for biodiesel production particularly in arid and semiarid regions. (Abobatta, 2019).

Jatropha trees are used as hedges for it is unpalatable to animals (Corte-Real et al., 2016). The advantages of domesticating this plant is very good established and more specifically attention (i) its power to survive under diverse agroclimatic conditions, (ii) successful cultivation in degraded soils, (iii) production of high-quality oil and protein in seeds, (iv) broad adaptation, (v) short generation interval and (vi) big genetic variation. (Montes and Melchinger, 2016).

This study aims to evaluate the effect of different combination levels of cadmium and lead on growth, and Cd and Pb contents and uptake in different plant parts of Jatropha curcas. Estimate phytoextraction potential of Cd and Pb by plant organs of jatropha to use it as phytoremediator for Cd and Pb contaminated soil.

2. Materials and Methods

A pot experiment was carried out at Antoniadis Garden, Horticulture Research Institute, Alexandria branch, Ministry of Agriculture, Egypt to study the combinations effect of Cd and Pb at different concentrations during the period of 1st April 2020 to 1st August 2021 on vegetative growth of Jatropha curcas and the relation between the contents of these metals in plant organs and their concentrations in the soil.

2.1. Tree species.
6-month-old homogenous Jatropha curcas transplants of a uniform species height (10 ± 2 cm in height), the species was obtained from a local nursery.

2.2. Pollutant treatments
Cadmium nitrate Cd (NO$_3$)$_2$ at the rates of 40 [low concentration (L)], 80 [medium concentration (M)] and 120 [high concentration (H)] mg/kg soil, equal 14.4, 28.8, 43.2 mg Cd/kg soil. and lead nitrate Pb (NO$_3$)$_2$ at the rates of 400 [low concentration (L)], 800 [medium concentration (M)], and 1200 [high concentration (H)] mg/kg soil.
concentration (H) mg/kg soil, equal 247.7, 495.4, 743.2, mg Pb/kg soil. The used soil was placed in the plastic pots of 30 cm in diameter with 7.5 kg air-dried soil sprinkled with solutions of the aforementioned concentrations of metals and incubated for 60 d before being planted outdoors under a waterproof tarpaulin (from 29 January to 31 March). Soil without heavy metal contamination served as a negative control. Physical and chemical parameters of the used soil are presented in Table (1), According to Jackson (1973).

Table 1: Soil analysis before the plantation.

| Parameters                     | Value | Unit  |
|--------------------------------|-------|-------|
| Mechanical Analysis            |       |       |
| Sand %                         | 88.9  | %     |
| Silt %                         | 5     | %     |
| Clay %                         | 6.1   | %     |
| Soil texture                   | Sandy |       |
| pH (1:2)                       | 8.5   |       |
| EC (1:2, water extract)        | 0.368 | ds/m  |
| Organic matter (%)             | 0.53  | %     |
| CaCO₃                          | 2.1   | %     |
| Available nutrients            |       |       |
| Available nitrogen (N)         | 8.5   | mg/kg |
| Available phosphorus (P)       | 55    | mg/kg |
| Available potassium (K)        | 700   | mg/kg |
| Soluble cations                |       |       |
| Ca²⁺                           | 1.19  | meq/L |
| Mg²⁺                           | 1.95  | meq/L |
| Na⁺                            | 1.96  | meq/L |
| K⁺                             | 0.58  | meq/L |
| Soluble anions                 |       |       |
| Cl⁻                            | 2.16  | meq/L |
| CO₃²⁻                          | --    | --    |
| HCO₃⁻                          | 1.1   | meq/L |
| SO₄²⁻                          | 0.36  | meq/L |
| Heavy metals                   |       |       |
| Cd                              | 0.23  | mg/kg |
| Pb                              | 7.40  | mg/kg |

The treatments prepared from heavy metals were as following: Control; L Cd + L Pb; M Cd + M Pb; H Cd + L Pb; L Cd + H Pb; H Cd + L Pb; M Cd + H Pb; and H Cd + M Pb.

World soil average of Cd and Pb are 0.41 and 39.9 mg/kg soil and maximum allowable concentrations of such metals are 1 to 5 and 20 to 300 mg/kg soil, respectively (Kabata-Pendias, 2011).

2.3. Planting date
The identical growth face of *Jatropha curcas* transplants were transplanted into the pots, (one transplant/pot) on 1st April 2020. The transplants were placed in the natural field.

2.4. The experimental design
The experiment was designed using a complete randomized design as described by Snedecor and Cochran (1989). The experiment was consisted of 8 treatments replicated three times, three plants of each replicate, thus nine transplants for each treatment.

2.5. Agricultural practices
The agricultural practices were done during the experimental period, the plants were irrigated with tap water to reach the field capacity when needed.

2.6. The measurements
At the end of experiment on 1st August six plants for each treatment (two plants of each replicate) were randomly chosen to determine the following parameters.
2.6.1. Vegetative growth

Plant height (cm), stem diameter (cm) at 5 cm from the soil surface, branches number/plant, the leaf area (cm^2) using a C1-202 Laser area meter (Cid Bio- Science, USA), software, fresh and dry weights of leaves, stems and roots and the length of the longest root (cm). Whereas, harvested plants were separated into, roots, stems and leaves. They were twice washed, first with tap water to remove soil, and then with deionized water. Plant samples were over-dried at 80°C for 24 h (Rautio et al., 2010).

2.6.2. Cd and Pb content in the plant organs

Dry samples were ground to obtain a homogenous powder in a metal-free mill (IKa-Werke, M20 Germany).

Dried samples of 0.2 g were wet digestion produced was performed as follows:

Concentrated sulphuric acid (5 ml) was added to the sample and mixture was heated for 10 minutes, then 0.5 ml perchloric acid was added and heating was continued until a clear solution was obtained. The solution was left to cool then filtered and diluted to 50 ml with distilled water (Evenhuis et al., 1980). The digested samples were prepared for measuring.

Cd and Pb concentration (mg/kg dry weight) were determined in different plant parts using Perkin, 3300 Atomic Absorption Spectrophotometer (Page et al., 1982).

The uptake of Cd and Pb was calculated as follows: Cd and Pb concentration × dry weight (leaves, stems and roots/1000 and total uptake = uptake of leaves + stems + roots (mg/plant).

2.6.3. The indicators to determine the potential of Jatropha curcas for phytoextraction of Cd, Pb in contaminated soil.

Bioconcentration factor (BCF) = \frac{\text{Metal concentration in the plant organ (mg/kg d.w.)}}{\text{Metal concentration in the soil (mg/kg soil.)}}

Depending on BCF values, accumulation efficiency was estimated using one of four groups: BCF > 1 (intensive), 1-0.1 (medium), 0.1-0.01 (week) and 0.01-0.001 no accumulation (Kabata-Pendias and Pendias, 1999).

Translocation factor\% = \frac{\text{Metal content in the shoots (mg/kg d.w.)}}{\text{Metal content in the roots (mg/kg d.w.)}} \times 100

TF\% to estimate the metal efficiency ion transport from roots to aerial plant organs (Maiti and Jaiswal, 2008) whereas, shoots = leaves and stems.

Total accumulation rate (TAR) mg/kg d.w. = \frac{\text{Metal concentration in shoots × shoots d.w. + metal concentration in roots × roots d.w.}}{21(\text{shoots d.w. + roots d.w.})}

TAR was estimated according to Aksorn and Chitsomboon (2013).

Tolerance index biomass (T_{lb}) = \frac{\text{D.W. of treated plant (g/plant)}}{\text{D.W. of control plant (g/plant)}}

Where, plant d.w. was referred to leaves + stems + roots.

(T_{lb}) to estimate resistance of tested Jatropha curcas in Cd and Pb contaminated soil, to calculate (T_{lb}) according to Wilkins (1978), there are 3 values: (T_{lb}) < 1 (a net decrease in biomass and a stressed condition of plants), T_{lb} = 1 (no difference relative to control treatment) and T_{lb} > 1 (a net increase in biomass and correct plant development).

Due to the highest accumulation of Cd and Pb in Jatropha curcas roots, the tolerance index of root (T_{lr}) was also calculated according to eq:
2.7. Statistical analysis

Duncan’s Multiple Range Test method was used to compare the treatments means (Snedecor and Cochran, 1989). A significant difference was considered at the level of p < 0.05.

3. Results and Discussion

3.1. Aerial parts traits

Data presented in Table (2) showed the effect of different Cd and Pb levels in the soil on aerial traits of jatropha plant (plant height, stem diameter, branches number, area/leaf, fresh and dry weights of leaves and stem). Data cleared that all various combination of Cd and Pb added to the soil caused significant decreases in the vegetative traits in comparison to the control in the most cases, except for one case of stem fresh weight, where M Cd H Pb and H Cd M Pb treatments gave nonsignificant increment in related to the control plants. Also, some treatments resulted in nonsignificant decreases in comparison to the control such as stem diameter, area/leaf, leaves dry weight, stem fresh and dry weights. In spite of the adverse effect of used Cd and Pb concentration the survival of jatropha plants was 100%. It means that Jatropha plant has good tolerance against Cd and Pb up to the high levels used. Some phytotoxicity symptoms of HM$_S$ used were noticed such as leaf chlorosis and necrotic spots on adult leaves, yellowish and browning roots. These the visual phytotoxicity were decreased with increasing the plant age.

Table 2: Growth traits of jatropha as affected by Cd and Pb levels in the soil during the experimental period

| HM$_S$ Treatments (mg/kg dry soil) | Plant height (cm) | Stem diameter (cm) | Branches number/plant | Area per leaf (cm$^2$) | Leaves fresh weight/plant (g) | Leaves dry weight/plant (g) | Stem fresh weight/Plant (g) | Stem dry weight/ plant (g) |
|-----------------------------------|-----------------|-------------------|----------------------|----------------------|-----------------------------|----------------------------|--------------------------|--------------------------|
| Cont.                             | 174.67 a        | 2.96 a            | 36.33 a              | 189.52 a             | 140.61 a                    | 34.29 a                    | 300.57 a                 | 69.45 a                  |
| L Cd L Pb                         | 170.00 a        | 2.72 bc           | 17.67 d              | 127.37 c             | 130.86 b                    | 32.36 ab                   | 240.57 b                 | 57.17 b                  |
| M Cd M Pb                         | 109.00 c        | 2.95 a            | 30.33 b              | 128.37 c             | 107.20 c                    | 29.61 b                    | 244.47 b                 | 55.05 b                  |
| H Cd H Pb                         | 122.33 b        | 2.92 ab           | 26.00 bc             | 135.03 c             | 128.58 b                    | 28.92 b                    | 292.97 a                 | 58.09 b                  |
| L Cd H Pb                         | 108.00 c        | 2.75 abc          | 30.00 b              | 117.87 c             | 78.46 c                     | 19.27 d                    | 189.97 c                 | 38.10 c                  |
| H Cd L Pb                         | 126.00 b        | 2.85 ab           | 24.33 c              | 132.87 c             | 99.89 d                     | 24.20 c                    | 304.06 b                 | 61.65 ab                 |
| M Cd M Pb                         | 118.33 bc       | 2.60 c            | 28.00 bc             | 127.39 c             | 99.37 d                     | 30.07 ab                   | 260.196 b                | 64.45 ab                 |
| H Cd M Pb                         | 116.00 bc       | 2.70 bc           | 23.67 c              | 161.09 b             | 84.19 e                     | 21.33 cd                   | 304.25 a                 | 60.71 ab                 |

Means have the same letters are not significant according to Duncan’s Multiple Range at 0.05 probability.

At the end of the experimental period a decrease was found in aerial parts of jatropha plants with one exception. The other researches have also noticed growing inhibition, in spite of the harmful of stress symptoms that depend on plant capacity to tolerate HM$_S$ (Redovnikovic et al., 2017). Jatropha had grown during the experimental period with survival 100%. It means that Jatropha could grow in HM$_S$ environment (Kubatova et al., 2016). The pH of the used soil (8.50) and temperature in summer reached 40°C. These can partitionaly affect the uptake of essential elements, in addition to the adverse effect of HM$_S$ on essential nutrients in the soil (Dalcorno et al., 2013).

As well as the harmful effect of Cd and Pb on the growth may be due to that Cd accumulation interferes with the enzymes of Calvin cycle, carbohydrate metabolism, photosynthesis (Shi et al., 2010) and changes the antioxidant metabolism (Khan et al., 2009). Cd is toxic to plant cell even at low concentration (Dai et al., 2012). Also, Maqboal et al., (2019) revealed that Cd is a very toxic HM and it causes oxidative stress in plants, therefore, results in over production of ROS, electrolyte leakage and lipid peroxidation.

Plants faced with Pb toxicity have their photosynthetic tracks harmfully affected as it disturbs ultrastructure of chloroplast and blocks synthesis pigments including chlorophyll and carotenoids furthermore to plastoquinone. Pb blocks the Calvin cycle and electron transport chain and similarly results shortage of carbon dioxide by closedown stomatal pares (Sharma and Dubey, 2005). Pb lead to...
the reduction in photosynthesis, oxidative stress, DNA damage and harmful in mitosis (Pourrut et al., 2011).

These results are in accordance to those of Zhu et al., (2019), Farias et al., (2020) and Martin et al., (2020) on Jatropha curcas, and El-Mahrouk et al., (2020) on Populus nigra, who found that HMs had negative impacts on vegetative traits.

3.2. Root parameters

Data tabulated in Table (3) cleared that the fresh and dry weights of roots and the longest root length were differently significantly affected by application the different combination of Cd and Pb. Where all used HMs used had inhibition effect on the root traits in relative to the control with one exception in case of length of the longest root. The highest significant values of root fresh and dry weights resulted from control treatment as gave 191.21 and 82.16 g/plant. The longest root was recorded for L Cd L Pb and control with nonsignificant between them as resulted in 72.83 and 72.67 cm, respectively. On the opposite the significantly least values of such traits were 101.95 g, 35.72 g and 30.00 cm for M Cd M Pb, M Cd M Pb and H Cd M Pb treatments, each in turn. It is observed from data that root traits values differed according to the level of Cd and Pb in the combination.

The harmful impacts on the root characters are explained by a numerous study that Cd and Pb at any concentration have a reduction effect on the physiological and metabolic processes, soluble carbohydrate transport and root biomass and elongation (Gasecka et al., 2012, and Basa et al., 2014). Pourrut et al., (2011) mentioned that Pb strongly inhibits root elongation. Many physiological processes of plants as N-metabolism and oxidative reactions were prevented by Cd (Phalakornkule et al., 2010). Also, a significant reduction in root biomass and total length at 50 µm Cd, as well as, change in root architecture were found in Salix viminalis (Cosio et al., 2006).

These results are in harmony with those of Drzewiecha et al., (2017) on Salix purpurea and S. viminalis, El-Mahrouk et al., (2020) on Populus nigra and Sager et al., (2020) on Pisum sativum. They found that HMS had negative effects on the roots fresh and dry weights and total root length.

| HMs Treatments (mg/kg dry soil) | Root fresh weight /P (g) | Root dry weight /P (g) | Length of the longest root (cm) |
|--------------------------------|--------------------------|------------------------|-------------------------------|
| Cont.                          | 191.21 a                 | 82.16 a                | 72.67 a                       |
| L Cd L Pb                      | 169.12 b                 | 62.32 b                | 72.83 a                       |
| M Cd M Pb                      | 101.95 c                 | 35.72 d                | 40.73 b                       |
| H Cd H Pb                      | 118.22 de                | 50.42 bc               | 40.33 b                       |
| L Cd H Pb                      | 111.84 e                 | 45.62 cd               | 31.833 cd                     |
| H Cd L Pb                      | 139.85 cd                | 50.62 bc               | 39.00 bc                      |
| M Cd H Pb                      | 136.60 cd                | 48.86 bc               | 32.50 cd                      |
| H Cd M Pb                      | 143.01 c                 | 56.91 bc               | 30.00 d                       |

Means have the same letters are not significant according to Duncan’s Multiple Range at 0.05 probability

3.3. Cd and Pb contents and uptake in plant parts and total plant uptake

Data of Cd and Pb contents in plant parts and those of their uptake in plant parts and plant total uptake are presented in Tables (4 and 5), respectively. It is evident from data that all Cd and Pb combinations significantly increased Cd and Pb contents and uptake in green leaves, stems and roots, as well as, plant total uptake and Cd and Pb contents in the fallen leaves more than untreated plants. Also, Cd and Pb contents and uptake differed among the treatments according to HMs concentration in the treatments. Where Cd and Pb content increased in the plant organs with increase its levels in the soil. Also, increasing Cd in the treatment affecting Pb content and uptake when Pb at low or medium levels. The opposite is right.

In general Cd content in green leaves and roots was more than stems. While Cd uptake was in order of stems > roots > leaves for the treatments of cont., L Cd L Pb, M Cd M Pb, H Cd H Pb, but for H Cd L Pb, M Cd H Pb and H Cd M Pb treatments the uptake was in order of roots > stems > green leaves. On the other hand, Pb content and uptake were in order of roots > green leaves > stems, with some exception in case of Pb content. Also, it is obvious that Cd and Pb contents in the fallen leaves were negligible in comparison to its contents in the green leaves.
In regard to the relationship between the Cd and Pb levels in the soil and their contents and uptake in the different plant parts, where some factors affecting the uptake mechanism of HM$_S$(1) plant species and clones differ for uptake and accumulate HM$_S$ in their organs (Fahr et al., 2013), this confirms with our results, (2) medium pH because of increasing mobility of metals under low pH (soil acidity) (Dimitriou et al., 2012 and Mosseler and Major, 2017), while, in alkaline soil the geochemical mobility of metals be reduced (Smith and Giller, 1992) who mentioned that at soil pH 8 and above HM$_S$ mobility decreases. This is in agreement with this study because pH of the used soil was 8.5 and it raised during the experimental period, which might rather be partially reduced the geochemical mobility of HM$_S$ used, consequently the uptake by plant roots. Adsorption and sedimentation are been controlled by metal dynamics which it is affected by soil pH (Sharma and Raju, 2013). Moreover, the chelators of elements through plant all that were found in the rhizosphere by plant root release (Kinnersley, 1993), they have a more affinity for metals can help in element sequestering (Fulekar et al., 2009). That lead to most of Cd uptake still in the epidermis of root tips (Landberg and Greger, 1996). Pourrut et al., (2011) revealed that plants have severe defense strategies under Pb stress that reduced uptake it into the cell, sequestration of Pb into vacuoles by the formation of complex and binding of Pb and (3) the root zone can absorb HM$_S$ and store and metabolism them inside the plant tissue. Also, plant enzymes exuded from the roots are a one of phytoremediation mechanisms (Merkl et al., 2005).

The results showed that roots uptake and accumulated Cd and Pb more than leaves and stems, this was supported by Samuilov et al., (2016) on $P.$ tremula × $P.$ alba who found that the plant roots can be able to accumulate Cd and Pb more than the aerial parts. Likewise, Redovnikovic et al., (2017) mentioned that $P.$ negra received Cd at 10, 25 and 50 mg/kg soil and Pb at 400, 800 and 1200 mg/kg soil accumulated Cd and Pb in the plant parts in the majority of cases in order of roots > stems > leaves. Similar results were found by El-Mahrouk et al., (2019) on $S.$ safsaf, Xu et al., (2019) on $S.$ species and El-Mahrouk et al., (2020) on $P.$ nigra.

Data cleared that Cd and Pb contents in the fallen leaves were negligible in relative to their contents in green leaves. It means that during leaf senescence stage Cd and Pb move back might be into stems and roots. This keeps the soil from the risk of the fallen leaves during fallen stage.

### Table 4: Cd and Pb contents in jatropha plant parts as affected by Cd and Pb levels in the soil during the experimental period

| HM$_S$ Treatments (mg/kg dry soil) | Green leaf | Fallen leaf | Stems | Roots |
|-----------------------------------|------------|-------------|-------|-------|
|                                   | Cd (mg/kg | Pb (mg/kg | Cd (mg/kg | Pb (mg/kg | Cd (mg/kg | Pb (mg/kg | Cd (mg/kg | Pb (mg/kg | Cd (mg/kg | Pb (mg/kg |
| Cont.                             | 0.06 f    | 2.05 e     | 0.02 c  | 0.19 e  | 0.09 c    | 1.73 f    | 0.07 e    | 4.32 c    |
| L Cd L Pb                         | 0.25 de   | 18.31 cd   | 0.03 c  | 0.27 c  | 0.27 c    | 4.25 c    | 0.15 d    | 21.06 a   |
| M Cd M Pb                         | 0.31 cd   | 18.98 bcd  | 0.07 ab | 0.29 bc | 0.29 b    | 6.80 c    | 0.26 c    | 22.28 a   |
| H Cd H Pb                         | 0.35 bc   | 20.16 bc   | 0.09 a  | 0.55 c  | 0.55 c    | 9.12 ab   | 0.39 b    | 21.38 a   |
| L Cd H Pb                         | 0.21 e    | 22.57 a    | 0.03 c  | 0.55 c  | 0.55 c    | 9.67 a    | 0.13 d    | 18.11 a   |
| H Cd L Pb                         | 0.43 ab   | 17.61 d    | 0.08 a  | 0.40 d  | 0.26 c    | 5.56 d    | 0.46 a    | 12.94 b   |
| M Cd H Pb                         | 0.38 abc  | 20.95 ab   | 0.06 b  | 0.68 b  | 0.20 d    | 8.34 c    | 0.29 b    | 20.23 a   |
| H Cd M Pb                         | 0.45 a    | 18.90 bcd  | 0.07 ab | 0.56 c  | 0.33 ab   | 6.26 cd   | 0.49 a    | 21.43 a   |

Means have the same letters are not significant according to Duncan’s Multiple Range at 0.05 probability.

### Table 5: Cd and Pb uptake of jatropha plant parts and total uptake/plant as affected by Cd and Pb levels in the soil during the experimental period

| HM$_S$ Treatments (mg/kg dry soil) | Leaves uptake | Stem uptake | Root uptake | Total uptake/plant (mg) |
|-----------------------------------|---------------|-------------|-------------|------------------------|
|                                   | Cd (mg) Pb (mg) | Cd (mg) Pb (mg) | Cd (mg) Pb (mg) | Cd (mg) Pb (mg) |
| Cont.                             | 0.002 b 0.07 d | 0.006 a 0.12 d | 0.005 d 0.35 f | 0.013 c 0.54 d |
| L Cd L Pb                         | 0.008 a 0.59 a | 0.016 a 0.24 c | 0.009 cd 1.31 a | 0.032 b 2.15 a |
| M Cd M Pb                         | 0.009 a 0.56 ab | 0.016 a 0.37 b | 0.009 cd 0.79 de | 0.034 b 1.72 bc |
| H Cd H Pb                         | 0.010 a 0.59 a | 0.021 a 0.52 a | 0.020 b 1.05 bc | 0.051 a 2.16 a |
| L Cd H Pb                         | 0.003 b 0.43 bc | 0.006 a 0.37 b | 0.006 d 0.83 de | 0.015 c 1.63 c |
| H Cd L Pb                         | 0.01 a 0.43 bc | 0.015 a 0.34 b | 0.023 ab 0.65 e | 0.048 a 1.42 c |
| M Cd H Pb                         | 0.011 a 0.64 a | 0.012 a 0.54 a | 0.014 c 0.99 ed | 0.037 b 2.16 a |
| H Cd M Pb                         | 0.009 a 0.40 c | 0.019 a 0.38 b | 0.028 a 1.22 ab | 0.056 a 2.00 ab |

Means have the same letters are not significant according to Duncan’s Multiple Range at 0.05 probability.
3.4. Cd and Pb phytoextraction potential of jatropha

In order to evaluate Cd and Pb phytoextraction ability in jatropha HM$_s$ accumulation, BCF of shoots and roots, TF $\%$, TAR, TI$_l$ and TI$_s$ were calculated (Tables 6). Data indicated that Cd BCF shoots (Cd BCF$_s$) is dependent on Cd levels in the soil. Where Cd concentration increased in the soil Cd BCF$_s$ decreased. Therefore, Cd BCF$_s$ of control is higher than all Cd BCF$_s$ of used treatments. It is noticed from data that no significant differences among the values of Cd BCF$_s$ of used treatments, except for the control. Cd BCF$_s$ ranged from 0.015 for H Cd L Pb to 0.66 for control. As for Pb BCF$_s$, it took similar trend of Cd BCF$_s$, whereas, with increasing Pb level in the soil Pb BCF$_s$ decreased. Except for the control, the treatments of L Cd L Pb and H Cd L Pb realized Pb BCF$_s$ significant values in comparison to the other treatments which gave nonsignificant values of this parameter among themselves. Pb BCF$_s$ values ranged from 0.039 of each H Cd H Pb and M Cd H Pb treatments to 0.511 for the control.

Regarding Cd BCF roots (Cd BCF$_r$), data showed that Cd BCF$_r$ for control was a significantly higher than the all other treatments that induced nonsignificant values among themselves. Cd BCF$_r$ ranged from 0.008 of each M Cd M Pb or L Cd H Pb treatments to 0.317 for control.

In respect to Pb BCF$_r$, which took similar trend of Pb BCF$_r$, but the differences among the used treatments were significant in same cases. Except for the control, which gave Pb BCF$_r$ (0.584) higher than the all used treatments, L Cd L Pb resulted in Pb BCF$_r$, significant value (0.082) in relative to the other treatments. Pb BCF$_r$ values ranged from 0.024 for L Cd H Pb to 0.584 for the control. It is noticed from data that BCF$_r$ of Cd or Pb was higher than BCF$_r$ of them for all treatments except for Pb BCF$_r$ of the control. Also, Pb BCF$_r$ shoots or roots $> $ Cd BCF$_r$ shoots or roots, except for BCF$_r$ of control where Cd BCF$_r$ $> $ Pb BCF$_r$.

The results focused on the potential of Cd and Pb phytoextraction by jatropha plant, whereas, BCF$_s$ and BCF$_r$ of Cd and Pb were calculated to evaluate the ion transport from soil to shoots and roots. Because plant biomass and ability of element translocated were dependent on the shoots phytoextraction efficiency (Shi et al., 2009). In our study BCF$_s$ or BCF$_r$ of Cd and Pb were week (BCF $= 0.1$-0.01) (Kabata-Pendias and Pendias, 1999). BCF$_s$ of Cd or Pb $< 1$ at the various used treatments. This is in accordance to Redovnıkovic et al., (2017) who found that poplar Italica BCF$_s$ of Cd or Pb $< 1$ at 10, 25 and 50 mg Cd/kg soil and Pb at 400, 800 and 1200 mg/kg soil.

Our data showed that BCF$_s$ at all used treatments of each Cd or Pb was $< 1$. This was supported by Rafati et al., (2011) who revealed that BCF$_r$ of P. alba is $< 1$ under 40, 80 and 160 mg Cd/kg soil. Additionally, BCF$_r$ $< $ BCF$_s$ of Cd or Pb in this study at all used treatments. It clears those roots of jatropha are the sinks of Cd and Pb and reflect this organ is lower effective to store them than other organs.

Concerning TF $\%$, it was calculated to determined ion translocation from roots to shoots. Data indicated that the values of TF of Cd or Pb was relative to their levels in the soil. The differences among the used treatments reached the significant level in the most cases. TF values of Cd was higher than TF of Pb under the all used treatments. Also, TF of such metals $> 1$($> 100$) except Pb TF of control. So, Cd TF $\%$ ranged from 151.50 to 353.71 for H Cd L Pb and L Cd L Pb treatments, while Pb TF $\%$ ranged from 87.57 to 188.37 for control and H Cd L Pb treatments, consecutively.

The movement of HM$_s$ from roots to shoots may occurs through the xylem. The free Cd and Pb concentrations can be impacted by cellular sequestration of such metals, and therefore, it can affect their movement within the plant (Fulekar et al., 2009 and Pourrut et al., 2011). TF $\%$ Cd or Pb in this study were $> 1$ (more than 100 $\%$). Our data are in harmony with those of Redovnıkovic et al., (2017) who found that TF Pb $> 1$ under 400, 800 and 1200 mg Pb/kg soil for P. nigra cv Italica, while TF Cd under 10 and 50 mg Cd/kg soil was $< 1$ (less than 100 $\%$), this was contrasted with our results. TF Cd $> 1$ of P. alba grown in 40-160 mg Cd/kg soil (Rafati et al., 2011). Thus, it is clear that BCF and TF values are dependent on species of plant and element ions kind and their levels. So, BCF shoots or roots of Cd or Pb $< 1$ and TF of them $> 1$ in this study. It means that jatropha plant can be used as a phytoextractor for Cd and Pb contamination. Also, these results are in accordance to those of El-Mahrouk et al., (2020) who found that Pb BCF$_r$ $< 1$ and Pb TF $> 1$ of P. nigra grown under 250, 450, 650 and 850 mg Pb Cl$_2$/kg soil. They added that P. nigra can be used as phytoextractor for Pb contaminated soil.

As for TAR, it was calculated to determine the accumulation of Cd and Pb in the whole plant. Data in Table (6) cleared that TAR of either Cd or Pb was dependent on their levels on the soil. Whereas, TAR increased with increase the level of HM$_s$ used in the soil. Therefore, Cd TAR ranged from 0.005
Table 6: BCF shoots, BCF roots, TF, Cd and Pb total accumulation rate, TI_b and TI_r of jatropha as affected by Cd and Pb levels in the soil during the experimental period

| HM Treatments (mg/kg dry soil) | BCF shoots | BCF roots | TF (%) | TAR | TI biomass | TI roots |
|--------------------------------|------------|----------|--------|-----|------------|---------|
|                               | Cd  | Pb  | Cd  | Pb  | Cd     | Pb     | Cd (mg/plant) | Pb (mg/ plant) |       |           |
| Cont.                          | 0.66 a | 0.511 a | 0.317 a | 0.584 a | 214.28 bc | 87.57 e | 0.005 f | 0.19 d | 1.00a | 1.00 a |
| L Cd L Pb                      | 0.036 b | 0.088 b | 0.010 b | 0.082 b | 353.71 a | 107.50 de | 0.017 d | 1.04 bc | 0.82 b | 1.01 a |
| M Cd M Pb                      | 0.020 b | 0.051 c | 0.008 b | 0.044 c | 230.02 bc | 115.99 cde | 0.023 c | 1.18 ab | 0.65 cd | 0.56 b |
| H Cd H Pb                      | 0.016 b | 0.039 c | 0.009 b | 0.028 de | 187.56 c | 138.22 cd | 0.029 ab | 1.26 a | 0.74 bc | 0.56 b |
| L Cd H Pb                      | 0.025 b | 0.042 c | 0.008 b | 0.024 e | 294.60 ab | 178.10 ab | 0.012 e | 1.24 a | 0.55 d | 0.44 bc |
| H Cd L Pb                      | 0.015 b | 0.090 b | 0.010 b | 0.050 c | 151.50 c | 188.37 a | 0.028 b | 0.92 c | 0.75 bc | 0.53 bc |
| M Cd H Pb                      | 0.019 b | 0.039 c | 0.009 b | 0.025 e | 200.96 bc | 145.45 bc | 0.022 c | 1.25 a | 0.77 bc | 0.45 bc |
| H Cd M Pb                      | 0.017 b | 0.050 c | 0.011 b | 0.042 cd | 159.57 c | 117.94 cde | 0.031 a | 1.13 ab | 0.75 bc | 0.41 c |

Means have the same letters are not significant according to Duncan’s Multiple Range at 0.05 probability.
to 0.031 mg Cd/plant for control and H Cd M Pb treatments. While Pb TAR ranged from 0.19 to 1.26 mg Pb/plant for control and H Cd H Pb treatments, respectively. Also, the differences among treatments used reached the significant level in some cases. Thus, it may be the transport of element ion from roots to aerial parts affect the metal ion accumulation in the plant.

Concerning TI, to estimate the jatropha tolerance for Cd and Pb phytoextraction and to measure the plant ability to grow in HM5 contaminated soil, TIb and Tib were calculated (Table 6). Data showed that all used HM5 treatment decreased Tib and Tib of Jatropha plant in comparison to control, except for L Cd L Pb treatment which recorded nonsignificant increase (0.01) over than the control. In general, either Tib or Ti values were dependent on the concentration of each metal in the treatment. Tib ranged from 0.55 to 1.00 for L Cd H Pb and control treatments, while Ti ranged from 0.41 to 1.01 for H Cd M Pb and L Cd L Pb treatments, consecutively.

Tib values at all HM5 used treatments was lower than one, it means that the plants were under stress and the biomass production was limited or a net decrease was occurred in biomass and stressed condition of plants (Wilkins, 1978). Too, Ti < 1, there is a limited length of root. Likewise, El-Mahrouk et al., (2019) on Salex safsaf and El-Mahrouk et al., (2020) on P. nigra, they found that Tib and Ti of the two species were less than one under 20, 40, 60 and 80 mg CdCl2 /kg soil and 250, 450, 650 and 850 mg PbCl2 / kg soil. Thus, jatropha plant had a good tolerance against Cd and Pb up to 14.4 and 247.7 mg/kg soil, respectively, because of Tib and Ti > 0.8 (Wu et al., 2010).

Generally, it can mention that TAR may be related to Tib, Ti, and TF of metal kind and its level in the soil. Thus, it can be concluded that BCF, TF, TAR, and Tib or Ti are factors that could be used to classify Cd and Pb hyper accumulators. Thus, the results imply jatropha is a good candidate plant for phytoremediation application in Cd and Pb contaminated soil.

4. Conclusion

Jatropha curcas had high survival rate 100% under Pb and Cd treatments up to 743.2 and 43.2 mg/kg soil, respectively. Also, the used levels of Pb and Cd had an adverse effect on vegetative traits. Additionally, in compared to the control treatment, there was a significant increase in the content and uptake of Cd and Pb in the various plant organs, as well as total plant uptake. According to BCF, TF, Tib and Ti, Jatropha curcas can use as phytoextractor for Pb and Cd contaminated sandy soil at the used levels in this study.

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