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Growth And Heavy Metals Uptake By Date palm Grown in Mono-And Dual Culture In Heavy Metals contaminated Soil

Mohebi Abdul Hamid

Member of Scientific Staff at Date Palm and Tropical Fruit Research Institute of Iran

Abstract: Plants are able to influence the availability of heavy metals in the rhizosphere due to root exudates and other mechanisms resulting in a change in their phytoextraction capability. The modern technology of co-planting techniques has therefore been introduced to phytoextract heavy metals from polluted agricultural soil. Separate tested were carried out with (monoculture) and inter cropping (dual culture) cultivation systems of date palm (*Phoenix dactylifera* L.) and sole crops of maize, alfalfa and sunflower in soil polluted with heavy metals. Corn inter cropped with date palm recorded significantly higher fresh and dry weights in the pot. Fresh and dry weights of date palm intercropped with corn were significantly higher ($P<0.05$) than the date palm intercropped with alfalfa. Sole corn recorded the highest copper and manganese uptake index and sole sunflower recorded significantly higher amounts of cadmium and lead uptake than the other treatments. Intercropping did not significantly affect the accumulation and removal of heavy metals in contaminated soil.

Key words: Intercropping · Phytoremediation · Corn · Alfalfa · Sunflower

INTRODUCTION

Heavy metal pollution has become one of the most serious environmental problems today [1].

There are a great numbers of small industrial plants and other sources of heavy metals emitting source in Khuzestan provinces of Iran [2].

Phytoextraction, as a promising tool of phytoremediation technology, uses plants to extract heavy metals from contaminated soil and accumulates them in the harvestable above-ground biomass. Two main strategies are usually discussed in investigations in to the technology. The first strategy suggests the use of hyper accumulator plants, which can naturally extract large concentrations of potentially hazardous trace elements from the soil. Low biomass yield is generally considered as a disadvantage of hyper accumulator species [3, 4]. The second strategy proposes the use of non-hyper accumulator plants with high biomass production [5]. Phytoextraction is based on large scale removal of metals. The accumulation of heavy metals can be further enhanced with the addition of mobilizing agents [6-9].

The total concentration of metals extracted from soil by plants is affected by factors such as concentrations of trace elements in the soil, their bio-available fractions, plant biomass production, tolerance and accumulation ability of used plant species and others. Yields of harvestable biomass can be affected by soil fertility and a number of other factors, including agronomic practices such as irrigation, application of fertilizers and weed and pest control [10]. The uptake of elements depends on the root architecture of the plant and its activity [11]. Plants are able to minimize the toxicity of heavy metals in the close environment (rhizosphere) or enhance the mobility of a required element bound into stable fractions of the soil due to several mechanisms [12-14].

Most of the identified hyper accumulator species produce only small harvestable biomass, which causes problems with their crop management and harvest. Cropping of species with lower metal accumulation capacity and higher yield production can solve agronomic practices. Intercropping of plants is mainly applied as an agricultural technique and aims at interspecific below-ground interaction, which may result in improved nutrient availability and increased yields of crops. However, it has been demonstrated that intercropping of the hyper accumulator *Thlaspi caerulescens* can increase the Cd uptake of non-accumulating plants such as barley [15]. Al-shayeb *et al.* showed heavy metals such as Pb, Zn, Cu, Ni, Cr and Li can be deposited from the atmosphere and

Corresponding Author: Mohebi Abdul Hamid, Date palm and Tropical Fruits Research Institute of Iran, P.O. Box 61355-16 Ahwaz - Iran, Tel: +986112423395, Fax: +986112423410, Cell: +989166165985.

Table 1: Some of soil characteristics

Cu (mg/kg)	Mn (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Texture %	O.C %	EC (dS/m)	pH
0.58	8.16	4.32	137.43	CL	1.2	3.2	7.5

taken up from the soil to be retained by the leaflets of date palm, they showed that date palm can be used to monitor polluted sites [16].

Phytoextraction experiments generally show the cropping of selected species as a monoculture. Based on knowledge of plant characteristics and their ability to mobilize heavy metals in the soil, newly designed technologies of intercropping with different plant species are being tested [17,18]. Recently, a co-planting system (i.e., the growth of a metal hyper accumulator plant associated with a low metal accumulating crop) was introduced simultaneously to phytoextract heavy metals as well as to develop an agricultural production based practice for contaminated sewage sludge [19]. Results show that this co-planting system can effectively remove heavy metals from the sewage sludge by the hyper accumulator plant while the harvested products from the agricultural crop meet the Chinese standards (Table 1) for animal feeds [20].

Hyper accumulator could change conditions and selected element bio-availability in the rhizosphere shared with another plant species. This intercropping could help to enhance the uptake of heavy metals using higher biomass species [21].

This study aimed to focus on differences between monoculture and intercropping systems of corn, alfalfa, sunflower and date palm in their growth and ability to remove metal from soil polluted with heavy metals.

MATERIALS AND METHODS

Ahwaz is the capital city of Khuzestan province in Iran, an industrial zone that lies between longitudes of 47° 40' E and 49° 20' E and latitudes 31° 5' N and 32° 20' N at an elevation of 480-550m (a.s.l). The area hosts an array of industrial units spread over 63,238 km² of land.

Plants growing in zones around and in close vicinity to the various industrial units exhibit increased concentrations of heavy metals. A sample of bulk soil from a field (0-20 cm) near an oil well in the industrial area around Ahwaz city was taken and analyzed for Ec, pH, %O.C, soil texture, Cu, Mn, Cd and Pb (Table 1). The soil was air-dried and passed through a <5 mm stainless steel sieve. The soil samples were then transferred to polyethylene pots (30 cm length 20 cm width and 15 cm depth), each pot containing 10 kg soil. At the beginning of the experiment 240, 120 and 150 mg kg^{-1} of N, P and K respectively were added to the pots as fertilizer. The following cropping plan in three replications was applied:

- control (without vegetation)
- date palm
- corn
- alfalfa
- sunflower
- date palm with corn
- date palm with alfalfa
- date palm with sunflower

The first step was to cultivate the date palm. Then four seeds were sown in the pots as necessary. This study was carried out in a greenhouse. Day and night temperatures in the greenhouse were 30°C and 28°C respectively and natural sunlight was supplemented. The pots were irrigated as per the field capacity condition with distilled water. Samples from each pot were taken four times: at the time of cultivation then 1, 2 and 3 months after planting. Then the soil samples were analyzed for Cu, Mn, Cd and Pb. Whole plants, one of each plant species were removed from the soil after one growth cycle and then samples were taken from each pot. Soil samples were air dried at room temperature, crushed and pulverized to pass through a 2-mm stainless steel sieve. The root and shoot from each plant was separated and collected. Plant samples, after washing were dried at 70°C. Acid digests of each soil sample were prepared using the USEPA 3051A (1998) method. Digestion of soil samples was applied with 1:1 nitric acid (69% purity) and hydrochloric acid (28% purity) solution. 2g soil and 0.5g plant samples were digested in a mixture of concentrated nitric acid and hydrochloric acid. Soil and plant sample digests were used to measure total concentration of Cu, Mn, Cd and Pb using an Atomic Absorption Spectrometer (Varian 220AA).

Uptake Index (UI) was calculated as metal concentration of shoots multiple yield dry matter given in Equation 1.

$$UI = Sh\textit{oot metals}*\textit{Dry matter} \quad (1)$$

This study was conducted as a factorial experiment in completely randomized design with 3 replications. The statistical analysis and means comparison using SPSS16 software. Data was analyzed with the software SPSS 16 and then Duncan's test was performed for comparisons.

RESULTS

Yield of Tested Plant Species

Fresh Weight of Plants: The effect of different treatments on the fresh weight of plants was observed (Table 2). Of the different treatments corn intercropped with date palm (T6) recorded a significantly higher fresh weight at (121.10 g) in pot than the other treatments. Sole alfalfa was recorded as having the lowest fresh weight of plant in pot at (18.180 g).

Dry Weight of Plant: The effect of different treatments on the dry weight of plant was observed (Table 2). The records for the different treatments on the dry weight of plants were that corn intercropped with date palm (T6) and sole corn (T3) had the highest weights at 26.8g and 25.5g respectively and the dry weights did not differ significantly between corn intercropped with date palm (T6) and sole corn (T3). Next in order of the highest weight were sole sunflower (T5) and sunflower intercropped with date palm (T8) with of 19.16g and 18.36g, respectively and dry weight did not differ significantly between sole sunflower (T5) and sunflower intercropped with date palm (T8). This was followed by sole alfalfa (T4) and alfalfa intercropped with date palm which had a lesser amount of dry weight at 6.73g and 6.56g per pot, respectively and dry weight did not differ significantly between sole alfalfa and alfalfa intercropped date palm ($P<0.05$).

Date Palm

Fresh Weight of Date Palm: The effect of different treatments on the fresh weight of date palm was observed to be significant (Table 3). Date palm intercropped with corn resulted in fresh weight of 157.36 g and was significantly higher ($P<0.05$) than date palm intercropped with alfalfa at 62.42g. The fresh weight of date palm did not differ significantly between sole date palm and date palm intercropped with alfalfa or sunflower ($P<0.05$).

Dry Weight of Date Palm: The effect of different treatments on the dry weight of date palm was observed to be significant (Table 3). The dry weight of date palm intercropped with corn was recorded at 70.30g and was significantly higher ($P<0.01$) than date palm intercropped with sunflower at 43.09g, sole date palm at 42.11g or alfalfa at 36.67g. The dry weight of date palm did not differ significantly between sole date palm and date palm intercropped with sunflower or alfalfa.

Table 2: weight of plants (g) in the applied treatments

treatments	Fresh weight	dry weight
Corn	105.78^{A}	25.51 ^{A}
Alfalfa	*18.82* ^{B}	*6.73* ^{C}
Sunflower	*101.31* ^{A}	*19.16* ^{B}
date palm with corn	*121.11* ^{A}	*26.80* ^{A}
date palm with alfalfa	*19.39* ^{B}	*6.56* ^{C}
date palm with sunflower	*92.07* ^{A}	*18.37* ^{B}

*mean in each column with same letter are not significantly different ($P<0.05$)

Table 3: weight of date palm seedling plantlets (g) in the applied treatments

TREATMENTS	wet date palm	dry date palm
Date palm	157.36^{A}	70.50^{AB}
date palm with corn	108.89^{AB}	42.11^{AB}
date palm with alfalfa	62.42^{B}	35.67^{B}
date palm with sunflower	104.42^{AB}	43.09^{AB}

*mean in each column with same letter are not significantly different ($P<0.05$)

Copper Uptake Index: Significant differences ($P<0.05$) between the treatments were observed in the copper uptake indexes. Sole corn (T3) recorded the highest copper uptake index by an amount of 0.414 and did not differ significantly from sunflower intercropped with date palm (T8), corn intercropped date palm (T6) and sole sunflower (T5) with copper uptake amounts that ranged from 0.360 to 0. 374. Date palm (T7) had the lowest copper uptake index with a record of 0.132 and did not differ significantly with sole alfalfa (T4) with an amount of copper recorded at 0.145 (Fig. 1).

There were no significant differences shown on the copper uptake index between each sole plant and plants intercropped with date palm. Sole corn, corn intercropped with date palm, sole sunflower and sunflower intercropped with date palm were the same and replaced in the first class, sole alfalfa and alfalfa intercropped with date palm replaced in the second group (Fig. 1).

Manganese Uptake Index: The effect of different treatments on the manganese uptake index were observed to be significant ($P<0.01$). Corn intercropped with date palm (T6) by the amount of 3.024 was recorded as the highest manganese uptake and the sole alfalfa (T4) recorded the lowest manganese uptake with an amount of 0.609. (Fig. 2).

There were no significant differences made apparent by the manganese uptake index between each of the sole plants or plants intercropped with date palm.

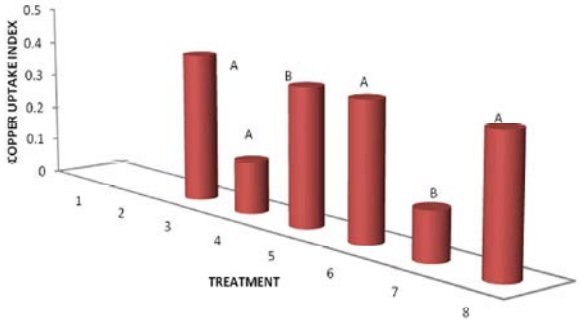

Fig. 1: Copper uptake index in the plants

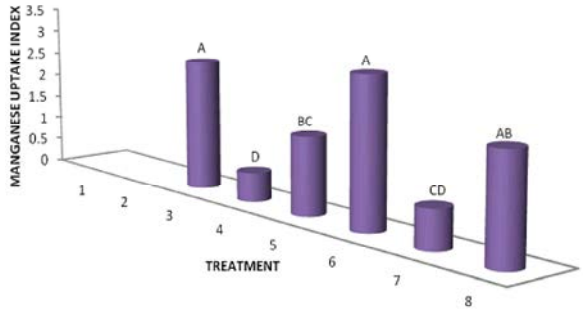

Fig. 2: Manganese uptake index in the plants

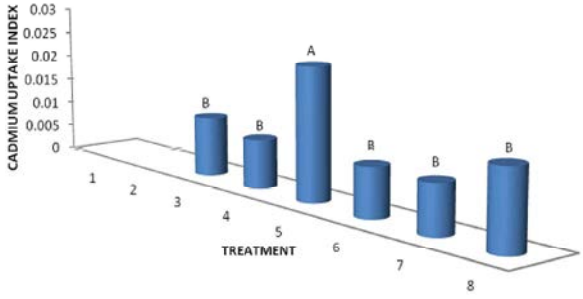

Fig. 3: Cadmium uptake index in the plants

Sole sunflower and sunflower intercropped with date palm were the same and replaced in the first class, sole sunflower and sunflower intercropped with date palm were the same and replaced in the second class, sole alfalfa and alfalfa intercropped with date palm were replaced in the third group (Fig. 2).

Cadmium Uptake Index: The effect of different treatments on cadmium uptake as shown by the index were observed to be significant ($P<0.01$). Among the different treatments sole sunflower (T5) recorded a significantly higher amount of cadmium uptake (0.025) than the other treatments (Fig. 3).

Lead Uptake Index: The effect of different treatments as per the lead uptake index were observed to be significant

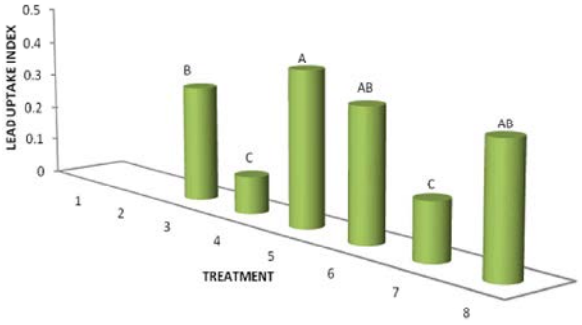

Fig. 4: Lead uptake index in the plants

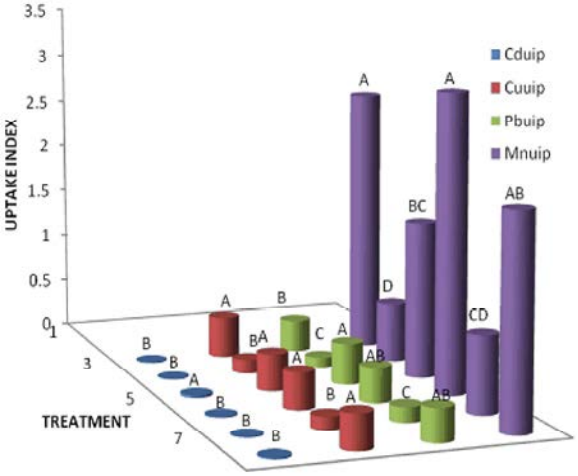

Fig. 5: Heavy metals uptake in the plants

in sole corn and corn intercropped with date palm ($P<0.05$). The maximum amount of lead uptake was evident on sole sunflower (T5) with an amount of 0.422. The minimum content of lead uptake index was for sole alfalfa (T4) with 0.103. It is remarkable that all the averages for treatments shown in the indexes are led by one group (Fig.4).

There were no significant effects on lead uptake index in sole alfalfa and intercropped date palm. Sole sunflower and sunflower intercropped with date palm had the same results that were replaced in the second class; sole alfalfa and alfalfa intercropped with date palm were replaced in the third group (Fig. 4).

Uptake of Elements by the Biomass: The total uptake of potentially toxic elements from the investigated soil and their total removal by harvested biomass of tested plant species are the most important factors for final evaluation of phytoextraction. The total removal by plant biomass was calculated from concentrations of elements in above-ground biomass with respect to the ratio of individual plant parts and their yields.

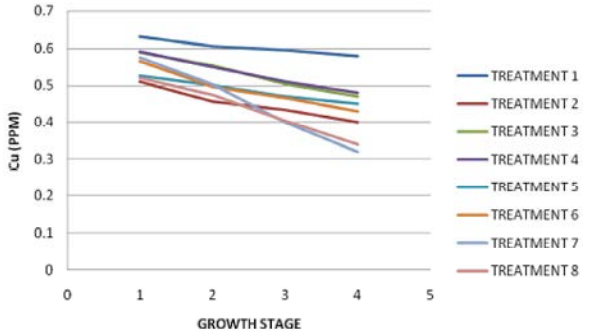

Fig. 6: Cu Concentration in the appllied treatments

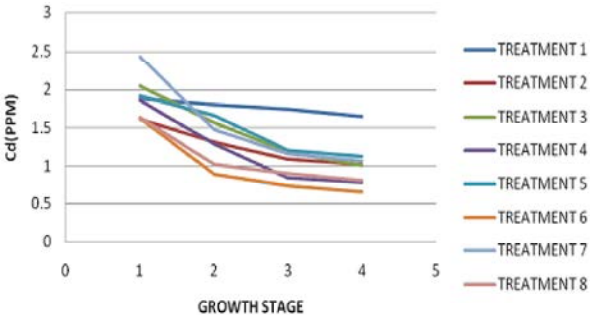

Fig. 8: Cd Concentration in the appllied treatments

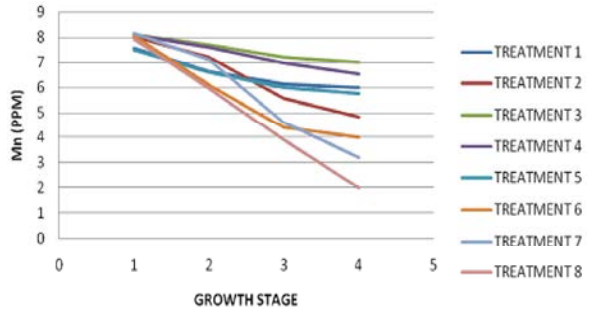

Fig. 7: Mn Concentration in the appllied treatments

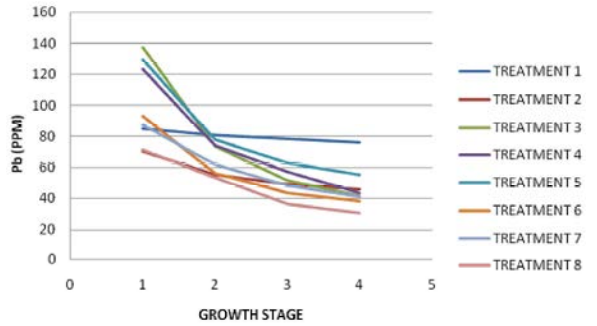

Fig. 9: Pb Concentration in the appllied treatments

All tested plant species showed that as well as concentrations of Cu, Mn, Cd and Pb accumulation in shoots from intercropping compared to separately planted crops intercropping did not affect concentrations of heavy metals in plants.

Alfalfa with or without date palm (sole alfalfa or intercropping with date palm) treatments accumulated lower concentrations of all studied metals compared to corn and sunflower.

Heavy Metals in Soil: Concentrations of heavy metals in the soil used for this experiment are shown in (Figs. 6, 7, 8 and 9). We compared the total amounts of elements in investigated soil samples (pots) to determine an impact on planted species. Control pots without vegetation covers were used. Concentrations of copper were significantly higher in control pots without vegetation than the other treatments in all growth stages (Fig. 6).

Concentrations of Mn in control pots (mg kg-1) were similar to the results for dual culture cropping (Fig. 7). The contents of Pb (Fig. 8) and Cd (Fig. 9) in the soil of corn intercropped with date palm were significantly lower compared to control pots.

Calculations based on data in (Figs. 6-9) ascertained percentages of heavy metals at the different growth stages. Figures showed the removal of copper from the soil by corn, alfalfa and sunflower and the removal of manganese by corn was uniform in all the different growth stages. But more than 80% manganese by alfalfa and sunflower and 80% cadmium and lead by corn, alfalfa and sunflower was removed from the soil in the early growth stages (about two months after cultivation).

Uptake Index of Heavy Metals in the Different Treatments: Uptake indexes of heavy metals showed that manganese had the highest (0.61-3.024) and cadmium the lowest uptakes (0.009-0.025), respectively.

DISCUSSION

Growth of Plants According to Planting System: No symptom of plant toxicity in contaminated soil was found. Comparison of plant yield between mono culture cropping and intercropping systems, no significant differences between the treatments were found. When plants were not colonized by AM, there were no significant differences between the shoot dry weight of *Eucalyptus,* either when cultivated alone or cultivated with *P. vulgaris* at the same concentration of Pb [22]. No effect of intercropping on yield production compared to monoculture cropping system was determined by Whiting *et al.* [23].

Accumulation of Heavy Metals: Cd and Pb concentrations in shoots of tested species decreased and sunflower, in mono or dual culture had a Pb concentration higher than alfalfa. Also, sunflower in mono culture had a Cd concentration higher than corn. *P. vulgaris* enhanced the uptake of Pb by *Eucalytus* roots [22]. Concentrations of heavy metals in harvestable biomass of hyper accumulator *A. halleri* and willow *S. caprea* grown in combination decreased as indicated by Unterbrunner *et al.* [24]. The possible competition of experimental plants for available nutrients can be speculated and needs to be evaluated in further research. Moreover, the affectivity of intercropping to facilitate the uptake of heavy metals for accumulation in above-ground biomass is specific to plant species as documented by Whiting *et al.* [23]. In fact, intercropping sunflower and date palm caused a significant increase in Cd and Pb accumulation. Gove *et al.* demonstrated that intercropping *T. caerulescens* with *Hordeum vulgare* caused a significant increase in Cd and a decrease in Zn concentrations in barley biomass [21]. Ingwersen *et al.* demonstrated that intercropping *T. caerulescens* with *Brassica juncea* did not affect Cd accumulation in tested plants [25].The combination of *Sedum alfredii* with *Zea mays* induced a higher accumulation of Zn in hyper accumulator plants [26].

CONCLUSION

Intercropping of plants with date palm did not significantly affect accumulation of Cd, Pb and Zn compared to the monocultures. The phytoextraction rate of hyper accumulator plants in this soil was proportionally low in relation to soil contamination. Intensive uptake of heavy metals by corn roots decreased their concentration in the soil and improved date palm growth. The association of date palm with corn can further help to improve the resistance of date palm to contamination by heavy metals. Therefore, intercropping developed conditions for phytoextraction by date palm in heavy metal contaminated soil. However, the efficiency of this treatment to combat heavy metal contaminated soil is still limited and alternative remediation techniques can be considered at this particular site.

Sunflower is more suitable than corn and alfalfa for the removal of cadmium. Corn, with or without date palm is more suitable for the removal manganese than alfalfa and sunflower mono or dual cultures.

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