Metal Toxicity Reduction on Seed Germination and Seedling Growth of *Raphanus* sp. and *Arabidopsis* sp. Using Date Seed Biochar

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ABSTRACT

The date seeds (DS) biochar produced at 550°C was found to be appropriate for remediation of metal-contaminated water. This was evident in the data, which showed that applying the biochar to *Raphanus* sp. and *Arabidopsis* sp. reduced metal stress and toxicity. The LC_{50} of all tested metals solutions on seed germination was increased significantly (P<0.05) in *Raphanus* sp. by 10.4, 2.3, 5, 1.8, and 3 folds, respectively, for Cu, Zn, Cd, Pb, and metals combination, and by 5, 3.4, 6, 5.5, and 2.5 folds in *Arabidopsis* sp. For seedling growth, the DS biochar enhanced the LC_{50} significantly (P<0.05) of the same metals in *Raphanus* sp. by 9.6, 9.2, 13.8, 12.1, and 1.6 folds, and in *Arabidopsis* sp. by 7, 3, 2.3, 2.9, and 2.7 folds, respectively. The LC of all metals was increased by 1.5 to 8 times and 1.5 to 12 times, respectively, for the seed germination and seedling growth of *Raphanus* sp. and *Arabidopsis* sp. Both plants were able to grow shoots at higher metal concentrations when the DS biochar was employed, as compared to the case when no DS biochar was utilized. In terms of shoot length, similar results were achieved, with the DS biochar application significantly enhancing shoot length (P<0.05), as compared to the case when no biochar was applied. Despite the fact that *Arabidopsis* sp. was more sensitive to metals than *Raphanus* sp., both plants raised their RGR and TI and reduced their phytotoxicity values in all metals following the DS biochar application, and sprouted at higher metal concentrations than before. These findings introduce a successful eco-friendly adsorbent for metal removal from aquatic environments, paving the way for more investigations.

Keywords: biochar, toxicity, heavy metals, *Raphanus* sp., *Arabidopsis* sp., seed germination, seedling growth.

INTRODUCTION

Heavy metals are naturally occurring compounds that are part of the Earth's crust. Their broad distribution in the environment as a result of their use in human activities (industrial, agricultural, technical applications, etc) has raised the concerns about their deleterious impact on ecosystems (Singh et al., 2011). Whether it was mining or irrigating with sewer wastewater, both contributed to metal poisoning (Stuckey et al., 2021; Prudnikova et al. 2020)

Metal toxicity refers to the hazardous effect of certain metals in specific forms and amounts on living organisms, which is dependent on multiple criteria, such as dose, exposure route, and chemical form (Tchounwou et al., 2012). Heavy metals (Fe, Co, Cu, Mn, Zn, Mo, etc.) can act as micronutrients and play a significant role in plant development. However, when threshold concentrations are exceeded, their effects are deemed harmful to plant development. Metal toxicity in plants, for example, is linked to the global increase in soil acidity, which affects 40 percent of the world's arable land, because its most poisonous forms are only available at acidic pH levels (Angulo-Bejarano et al., 2021). An increase in the Cu levels in soil due to human activity might cause phytotoxicity and lower plant yield (Trentin et al., 2022).

Cd and Pb, on the other hand, are not required nutrients for plants and can be phytotoxic at low quantities, albeit trace metal bioavailability is also influenced by other physicochemical parameters in the soil, such as pH (Stuckey et al., 2021; Kumpiene et al., 2017; Liu et al., 2009; McBride at al., 1997). Pb, Hg, Co, As, Cd, and Cr are only a few examples of heavy

metals, which are primarily hazardous and must be treated as well as removed from the environment (Rebello et al., 2021). As a result, one of the key challenges for scientists is to rid the environment of metals in order to lessen their toxicity. When utilized sequentially to produce the best results, combinatorial approaches employing physicochemical and biological methodologies are successful (Khalid et al., 2017).

One of the most important concerns facing the world is water scarcity, and wastewater reuse for irrigation purposes could be one of the most effective solutions (Ungureanu et al., 2020). Wastewater, on the other hand, may contain organic and inorganic pollutants (Chen et al., 2021). One of the most common sources of heavy metal contamination is industrial wastewater (Shrestha et al., 2021). As a result, extreme caution should be exercised when using such water in agriculture.

Several physical, chemical, and biological methods are utilized to remove heavy metals from contaminated environments. The approach for removing heavy metals varies based on the processed metal and sample type, whether soil or water (Rebello et al., 2021).

For eliminating heavy metals, a variety of processes were tried (ion exchange, membrane filtration, adsorption, electrochemical, and chemical precipitation), each with its own set of advantages as well as issues, obstacles, and limitations in application (Rajoria et al., 2022; Mishra et al., 2021; Yadav et al., 2021). Because of its high effectiveness and low cost, the adsorption method has advantages in treating wastewater to remove heavy metals (Singh et al., 2022; Cheng et al., 2021).

Pyrolysis was used to create a range of adsorbents, which were subsequently used to clean the metal-contaminated environment in a cost-effective manner (Chen et al., 2022). Due to its high porosity and presence of several functional groups, biochar has shown tremendous potential in the treatment of heavy metal-contaminated wastewater (Chai et al., 2021; Ngambia et al., 2019). In previous investigations, biochar was discovered to have a strong affinity for removing heavy metals from aquatic environments (Shan et al., 2020; Bandara et al., 2020). According to Li et al. (2022) and Gupta et al. (2020), the biochars made from organic waste can be utilized as green sorbents to remove heavy metals from contaminated water.

Plant models have been used to examine metal toxicity, according to the literature. The phytotoxicity of SPIONs was assessed using *Raphanus sativus* and *Lactuca sativa* as model plants (Hoffmann et al., 2022). *Arabidopsis thaliana* is a common model plant for studying metal toxicity, according to Angulo-Bejarano et al. (2021).

Using *Raphanus* sp. and *Arabidopsis* sp. as model plants to evaluate its phytotoxicity, this study aimed to show that using date seed biochar as a remediation agent can reduce the metal stress of contaminated irrigation water on seed germination and seedling growth, paving the way for further research and development.

MATERIAL AND METHODS

Plant materials, bacteria, biochar, and heavy metals

Radish (*Raphanus* sp.) and Cress (*Arabidopsis* sp.) seeds were purchased at the local market. The date seeds (DS) biochar was made in our lab at 550°C, as previously stated (Al-Tarawneh, 2022). The metal solutions of Cu, Zn, Cd, Pb, and metals combination (Cu + Zn + Cd + Pb) were prepared in deionized water from their salts of nitrate with concentrations of 0, 20, 50, 100, 200, 400, 600, 800, and 1000 ppm for the seed germination and seedling growth experiments. The 0 concentration was just deionized water and was used as a control. All metal solutions were set to a pH of 7.

Effects of metal toxicity on seed germination and seedling growth

Both plants (*Raphanus* sp. and *Arabidopsis* sp.) with and without biochar application were tested in triplicate for each metal concentration. To achieve this goal, 50 seeds were embedded on a double layer of filter papers in a 9 cm Petri dish with 10 mL of each metal concentration but no DS biochar. The experiment was repeated with the addition of 0.5 g of DS biochar with a grain size of < 250 μm between the filter papers. All Petri dishes were cultured at 25°C in an incubator with white light for 8 days and were examined daily. Seed germination, seedling growth, as well as shoot and root length were all measured at the end of the incubation period.

Seeds were classified as germinated when the seed coat was broken and visible, and as inhibited if the seed coat was not broken throughout the 8-day incubation period. When the seed coat was broken and the embryo grew, the seedling was regarded grown; however, when the seed coat was broken but the embryo did not grow, it was recorded as inhibited (Li et al., 2005).

The lethal concentration 50 (LC_{50}) of the investigated metals on the seed germination and seedling growth of *Raphanus* sp. and *Arabidopsis* sp. were revealed from the results, where 50% killing or inhibition occurred.

According to (El Rasafi et al., 2016), the relative germination rate (RGR), germination percentage (GP), tolerance index (TI), and phytotoxicity percentage (PT) were calculated in equations 1, 2, 3, and 4.

$$RGR = \frac{\text{Germination percentage of metal treatment}}{\text{Germination percentage of control}} \quad (1)$$

$$GP = \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100 \quad (2)$$

$$TI = \frac{\text{Mean of root length in metal treatment}}{\text{Mean of root length in control}} \times 100 \quad (3)$$

$$PT = \frac{\text{Root length in control} - \text{Root length in metal treatment}}{\text{Root length in control}} \times 100 \quad (4)$$

Data analysis

The student's t-test was used to statistically assess all of the results, which were expressed as mean ± SD. Using the SPSS computer software, basic linear regression was applied to examine the correlation between the data. The lowest limit of significance was defined as a P value < 0.05.

RESULTS

As previously indicated, the DS biochar used in this study was produced in our laboratory at 550°C with 69% porosity, 7.8 pH, and 833 mg/kg of Cd absorption capacity (Al-Tarawneh, 2022). By embedding the seeds of *Arabidopsis* sp. and *Raphanus* sp. in artificially polluted water spiked

Table 1. Effect of DS biochar application on LC_{50} of Cu, Zn, Cd, Pb, and metal combination on seed germination and seedling growth of *Raphanus* sp. and *Arabidopsis* sp., the results were expressed by mean ± SD, were *n* = 3

Metal	Parameter	DS Biochar application	*Raphanus* sp.		*Arabidopsis* sp.	
			LC_{50} (ppm)	Fold increment	LC_{50} (ppm)	Fold increment
Cu	Seed germination	- *	50 ± 5	10.4	10 ± 2	5.0
		+ **	520 ± 48		50 ± 8	
	Seedling growth	-	35 ± 4	9.6	10 ± 1.5	7.0
		+	335 ± 37		70 ± 15	
Zn	Seed germination	-	220 ± 31	2.3	35 ± 4	3.4
		+	510 ± 55		120 ± 11	
	Seedling growth	-	50 ± 4	9.2	20 ± 3	3.0
		+	460 ± 41		60 ± 5	
Cd	Seed germination	-	100 ± 9	5.0	10 ± 2	6.0
		+	495 ± 51		60 ± 5	
	Seedling growth	-	25 ± 3	13.8	35 ± 3	2.3
		+	345 ± 25		80 ± 6	
Pb	Seed germination	-	250 ± 28	1.8	10 ± 2	5.5
		+	450 ± 48		55 ± 4	
	Seedling growth	-	33 ± 4	12.1	35 ± 3	2.9
		+	400 ± 39		100 ± 8	
Metal combination	Seed germination	-	38 ± 4	3.0	12 ± 1	2.5
		+	113 ± 8		30 ± 3	
	Seedling growth	-	28 ± 3	1.6	10 ± 2	2.7
		+	44 ± 3		27 ± 2.5	

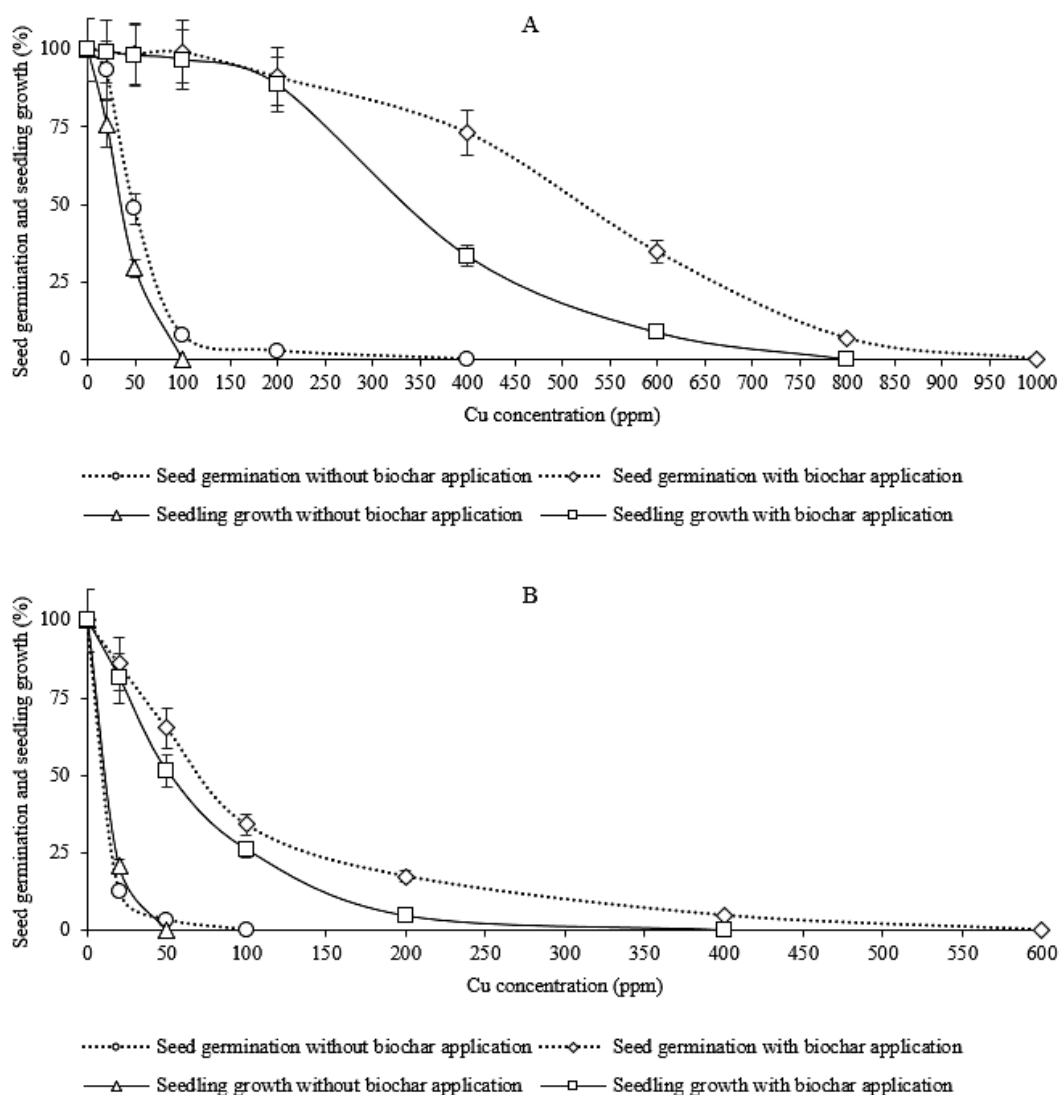**Note:** * – without biochar application; ** – with biochar application.

Figure 1. Effects of different Cu concentrations on seed germination and seedling growth of (A) *Raphanus* sp. and (B) *Arabidopsis* sp. with and without DS biochar application

with various metals concentrations, the ability of DS biochar to minimize metal stress on seed germination and seedling growth by treating contaminated water was tested in the current study.

Seed germination and seedling growth

The results showed that DS biochar raised the LC_{50} of all investigated metal solutions on seed germination in *Raphanus* sp. by 10.4, 2.3, 5, 1.8, and 3 folds, respectively, for Cu, Zn, Cd, Pb, and metals combination, as well as by 5, 3.4, 6, 5.5, and 2.5 fold in *Arabidopsis* sp. For seedling growth, the DS biochar application increased the LC_{50} of the same metals in *Raphanus* sp. by 9.6, 9.2, 13.8, 12.1, and 1.6 folds, and in *Arabidopsis* sp. by 7, 3, 2.3, 2.9, and 2.7 folds, respectively (Table 1, Figures 1–5).

When compared to non-biochar application, all of the LC_{50} values improved significantly (P<0.05), independent of the metal tested, with improvements ranging from 1.8 to 10.4 times for seed germination and from 1.6 to 13.8 time for seedling growth in *Raphanus* sp. The same pattern of results was obtained in *Arabidopsis* sp., where the DS biochar application increased the LC_{50} by 2.3–5 times for seed germination and 2.5–7 times for seedling growth. It was noticed that both the *Arabidopsis* sp. seed germination and seedling growth were more susceptible to metals than *Raphanus* sp. In terms of lethal concentration (LC), the LC of all metals increased with 1.5 to 8 times on *Raphanus* sp. (seed germination and seedling growth) and 1.5 to 12 times on *Arabidopsis* sp. after the DS biochar application (Figures 1–5).
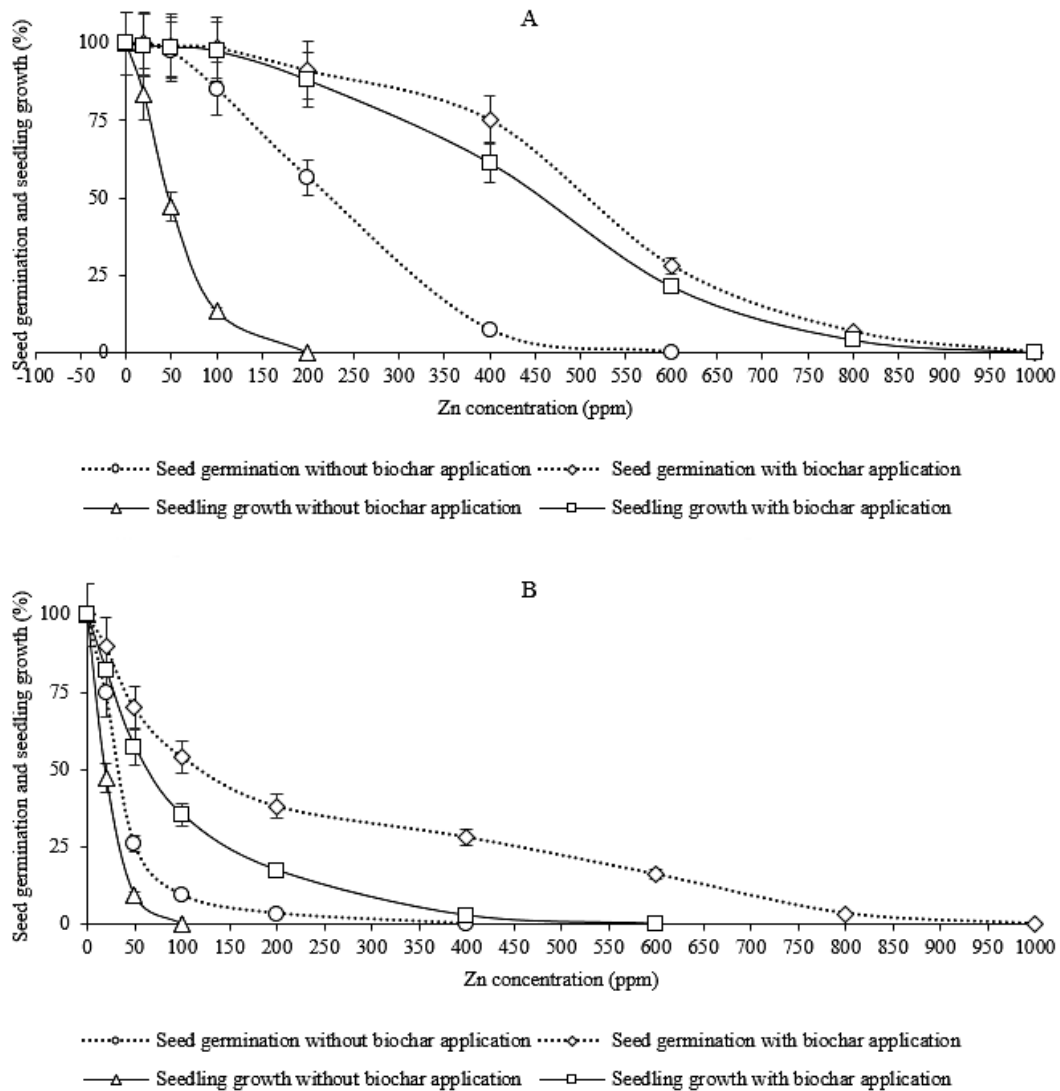
Figure 2. Effects of different Zn concentrations on seed germination and seedling growth of (A) *Raphanus* sp. and (B) *Arabidopsis* sp. with and without DS biochar application

Shoots production and length

When compared to the metals without the DS biochar application, the findings of shoot production demonstrated that the DS biochar application reduced the toxicity of all metals significantly (P < 0.05) by allowing both plants to develop shoots at higher metal concentrations. When the DS biochar was used, *Raphanus* sp. was able to produce shoots with 600 ppm Cu, compared to 50 ppm without the DS biochar. The results for the other metals followed a similar trend, with variations in survival concentrations for the development of shoots. The same pattern was also seen in *Arabidopsis* sp. (Figure 6). Similar findings were obtained in terms of shoot length, with DS biochar significantly increasing shoot length (P<0.05) when compared to non-biochar application (Table 2 and Figure 6).

Relative germination rate

According to the data of RGR (relative germination rate), the beginning concentration of inhibitory impact of all metals on *Raphanus* sp. was considerably improved from 20 ppm without DS biochar to 100 ppm with DS biochar application. With the exception of Cu and Zn at 20 ppm, the stress generated by all metals on this plant was greatly decreased by raising the RGR with DS biochar application, regardless of the metal concentration. Despite the fact that *Arabidopsis* sp. was more sensitive to metals than *Raphanus* sp., *Arabidopsis* sp. enhanced its RGR values with DS biochar application in all metal concentrations except Pb at 20 ppm. Furthermore, after DS biochar application, both plants were able to sprout at higher metal concentrations than without it (Figure 7).
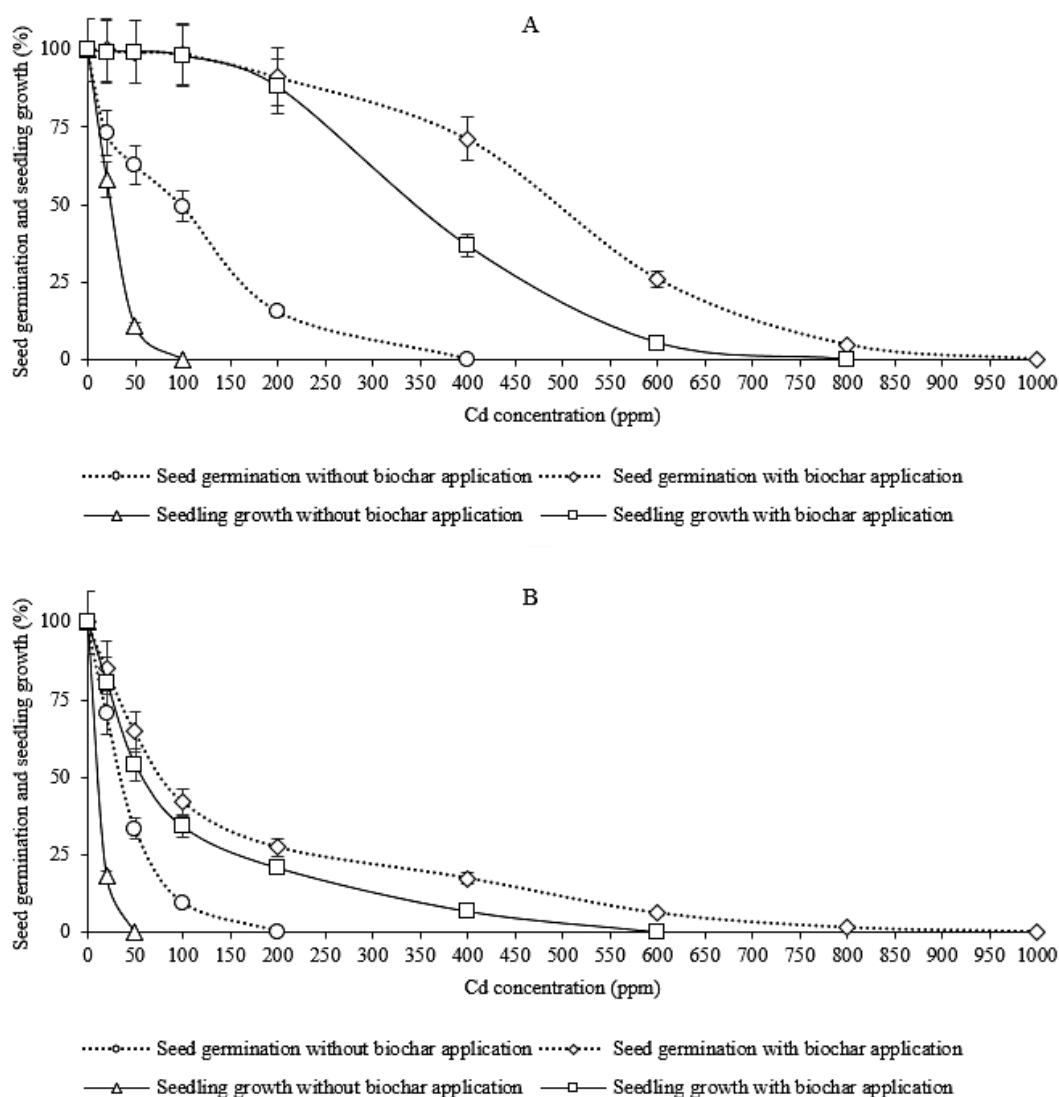
Figure 3. Effects of different Cd concentrations on seed germination and seedling growth of (A) *Raphanus* sp. and (B) *Arabidopsis* sp. with and without DS biochar application

Tolerance Index

When compared to non-biochar treatment, the results showed that DS biochar application to all metals had a significant (P<0.05) effect on the TI (Tolerance Index) of both plants. The significance of DS biochar application on Cu, Zn, Cd, Pb, and metals combination for *Raphanus* sp. was obvious at concentrations of 20–400, 200–600, 50–600, 50–600, and 20–200 ppm, respectively. In turn for *Arabidopsis* sp., 20–200, 50–200, 20–400, 20–200, and 50–100 ppm were obtained, indicating that *Raphanus* sp. was more tolerant of metal toxicity than *Arabidopsis* sp. There was no substantial influence on Tolerance Index outside of these concentration ranges (Figure 8).

Phytotoxicity

The root length was utilized to assess and evaluate the PT (phytotoxicity) of all metals on *Raphanus* sp. and *Arabidopsis* sp. The PT of metals on *Raphanus* sp. increased along with metal concentrations; however this toxicity was mitigated by using the DS biochar. By using the DS biochar, the metal concentrations that induce 100% toxicity were raised from 100 to 800 ppm for Cu and Cd, from 200 to 1000 ppm for Zn and Pb, and from 100 to 400 ppm for the metals combination. The metals concentrations that elicit 100% toxicity in *Arabidopsis* sp. were enhanced from 50 to 400 ppm for Cu and Pb, from 100 to 600 ppm for Zn and Cd, and from 100 to 200 ppm for the metals combination by employing the DS
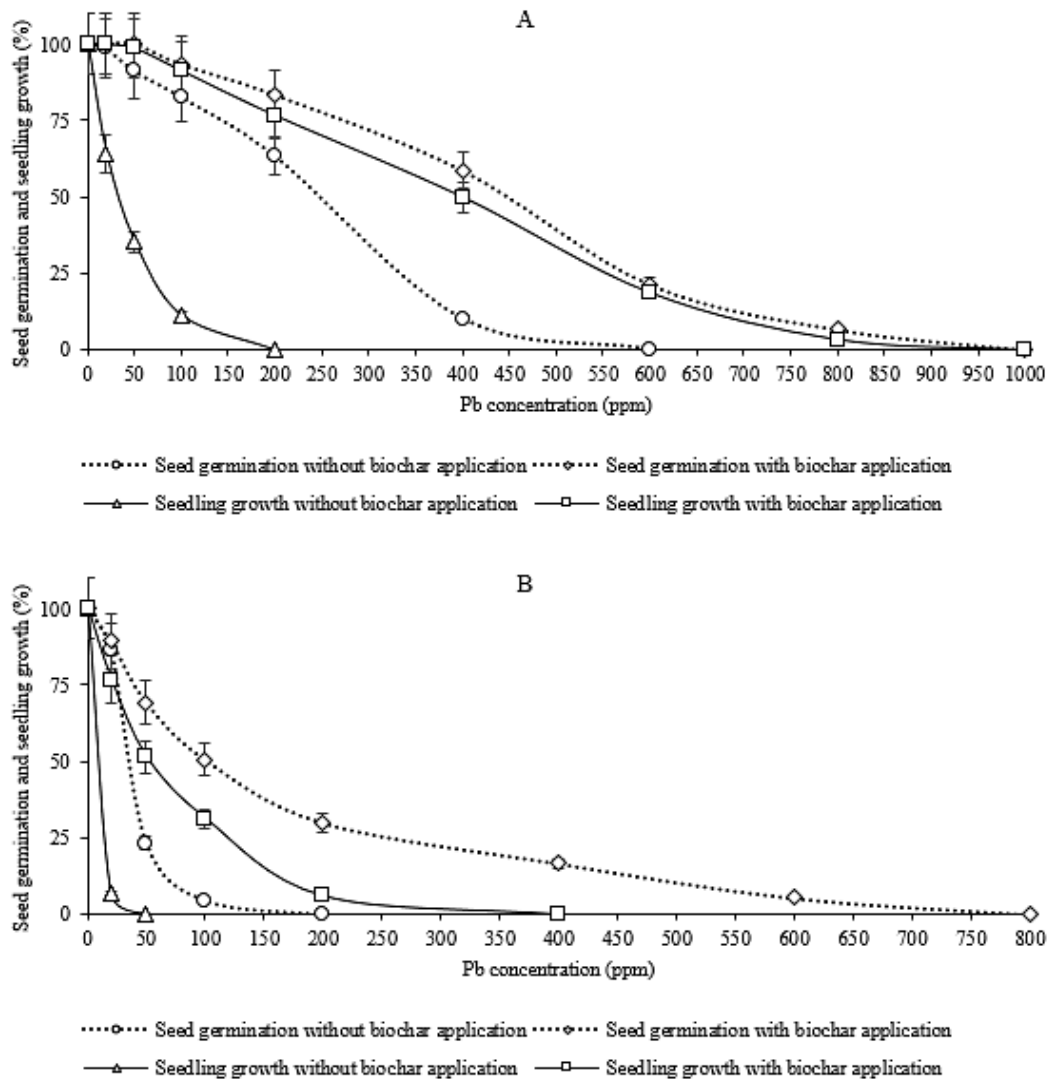
Figure 4. Effects of different Pb concentrations on seed germination and seedling growth of (A) *Raphanus* sp. and (B) *Arabidopsis* sp. with and without DS biochar application

biochar. However, in *Raphanus* sp., the significance of enhancement (P<0.05) by reducing metal toxicity caused by the DS biochar was between 20 and 200 ppm Cu and metals combination, 100 to 400 ppm Zn, and 20 to 600 ppm Cd and Pb, and in *Arabidopsis* sp., it was between 20 and 200 ppm Cu and Pb, 50 to 200 ppm Zn, 20 to 400 ppm Pb, and 50 to 100 ppm metals combination. Thus, independent of the plant type or metal applied, the highest reduction in PT% as a result of the DS biochar application was obtained between 50 and 100 ppm metals concentration (Figure 9). These findings support the earlier observations that *Arabidopsis* sp. is more metal-sensitive than *Raphanus* sp.

DISCUSSION

In the current study, the influence of metal stress on two plants (*Raphanus* sp. and *Arabidopsis* sp.) growth indices was investigated with and without the use of DS biochar.

According to the findings of current study; Cu, Zn, Cd, Pb, and combination of metals had varied effects on seed germination and early seedling growth in *Raphanus* sp. and *Arabidopsis* sp. These findings aligned with those of El Rasafi et al., (2016), who discovered that metal toxicity on seed germination, root and shoot elongation differed depending on metal type and plant species. Many authors have described the influence of metals on seed germination as a
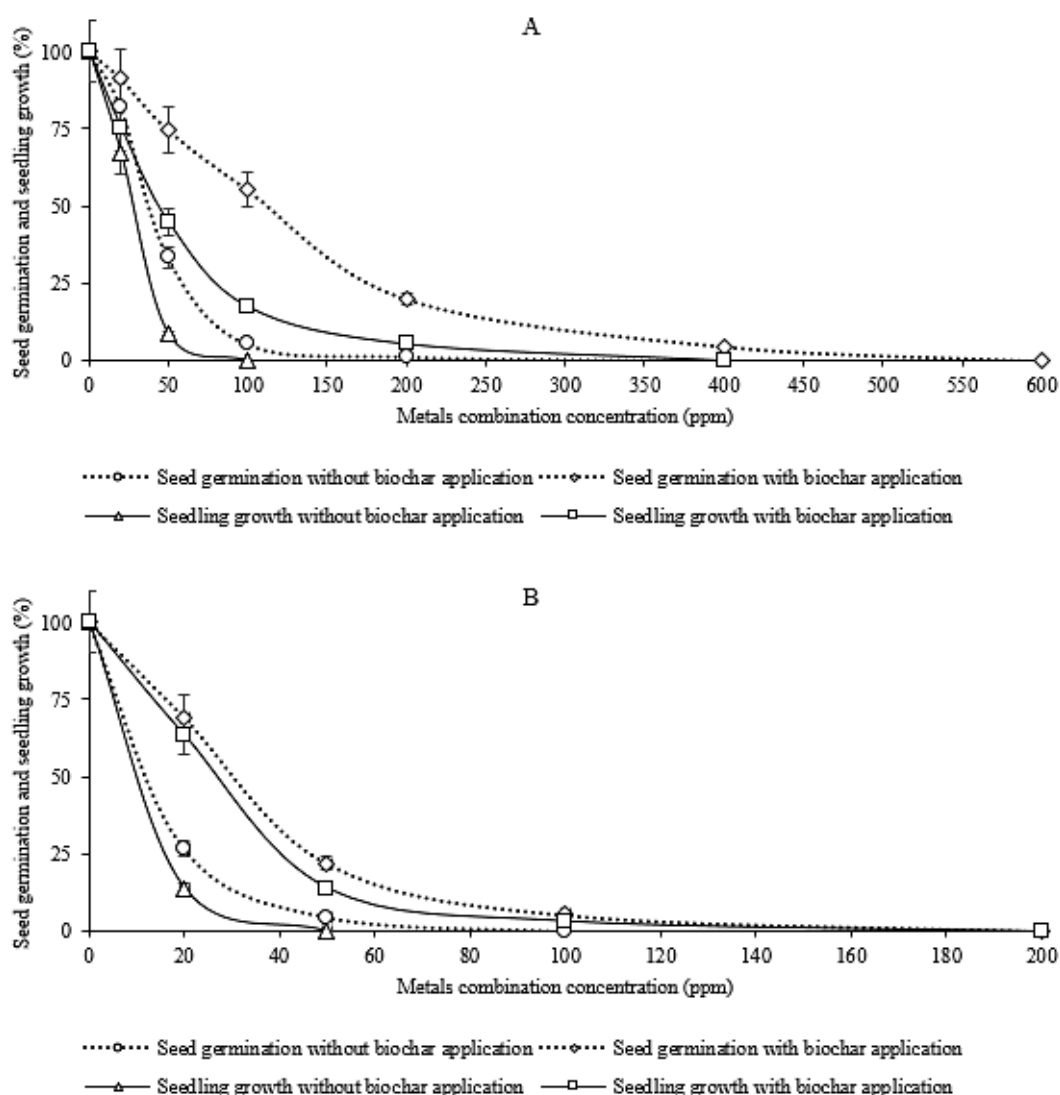
Figure 5. Effects of different concetrations of metals combination on seed germination and seedling growth of (A) Raphanus sp. and (B) Arabidopis sp. with and without DS biochar application

reduction in water intake and transport, as well as death or embryonic harm (El Rasafi et al., 2016; Wierzbicka and Obidzinska, 1998; Becerril et al., 1989). The degree of metal poisoning that affects germination varies by plant species and metal kind. In the presence of increased metal concentrations, some plants can sprout, but this is extremely detrimental to other species (Kranner and Colville, 2011).

The results also demonstrated that utilizing the DS biochar reduced metal stress by increasing the LC_{50} and LC values, which promoted seed germination and seedling growth. These findings could be construed as evidence that toxicity is related to the accessible percentage of metals due to increased water solubility of contaminants (Ahmad et al., 2012). According to the same author, biochar reduces the bioavailability

and bio accessibility of Pb in polluted soil by 75.8% and 12.5%, resulting in significant increases in *Lactuca sativa* seed germination and root length when compared to unamended soil by 360 and 189%, respectively.

The addition of woody biochar reduced the Ag toxicity on *Hordeum vulgare* by increasing the LC_{50} and LC, indicating a strong potential for soil remediation, as the Ag toxicity was reduced in most endpoints studied in barley (Mocova et al., 2022). According to Soudek et al., (2017), different biochars exhibit adsorption capacities of 11.63–20.16, 7.83–20.08, and 70.92–200 mg/g for Cd, Cu, and Pb, respectively, thus, application of biochar reduce their toxicity on *Sorghum* seed germination. Increased pyrolysis temperature for biochar synthesis increases surface area and porosity, and consequently metal adsorption

Table 2. Shoot lengths (cm) of *Raphanus* sp. and *Arabidopsis* sp. after treatment with different metal concentrations and with and without DS biochar application, the results were expressed by mean ± SD, were *n* = 3

Metal concentration	Cu		Zn		Cd		Pb		Metal combination	
	-[a]	+[b]	-	+	-	+	-	+	-	+
Raphanus sp.										
0	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	1.7 ± 0.5	1.6 ± 0.5	1.5 ± 0.3	1.6 ± 0.4	1.7 ± 0.5	1.6 ± 0.4	1.6 ± 0.5
20	0.9 ± 0.2	1.6 ± 0.4	1.4 ± 0.3	1.6 ± 0.5	0.9 ± 0.2	1.4 ± 0.3	1.2 ± 0.2	1.5 ± 0.3	0.9 ± 0.3	1.2 ± 0.4
50	0.4 ± 0.06	1.5 ± 0.3	0.8 ± 0.04	1.2 ± 0.2	0.3 ± 0.03	1.3 ± 0.2	0.6 ± 0.02	1.1 ± 0.2	0.2 ± 0.01	1.0 ± 0.1
100	0.0[c]	1.3 ± 0.1	0.5 ± 0.03	1.1 ± 0.2	0.0	1.3 ± 0.2	0.3 ± 0.03	1.0 ± 0.2	0.0	0.7 ± 0.1
200	0.0	0.8 ± 0.2	0.0	0.9 ± 0.1	0.0	0.8 ± 0.2	0.0	0.8 ± 0.2	0.0	0.4 ± 0.1
400	0.0	0.5 ± 0.02	0.0	0.5 ± 0.03	0.0	0.4 ± 0.02	0.0	0.4 ± 0.02	0.0	0.0
600	0.0	0.2 ± 0.02	0.0	0.3 ± 0.02	0.0	0.1 ± 0.01	0.0	0.3 ± 0.	0.0	0.0
800	0.0	0.0	0.0	0.1 ± 0.01	0.0	0.0	0.0	0.1	0.0	0.0
1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Arabidopsis sp.										
0	1.2 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3
20	0.4 ± 0.1	0.9 ± 0.2	0.6 ± 0.2	1.1 ± 0.2	0.5 ± 0.2	1.0 ± 0.3	0.7 ± 0.2	1.1 ± 0.4	0.4 ± 0.1	0.9 ± 0.2
50	0.0	0.6 ± 0.2	0.2 ± 0.03	0.8 ± 0.1	0.0	0.7 ± 0.2	0.0	0.7 ± 0.3	0.0	0.4 ± 0.05
100	0.0	0.2 ± 0.05	0.0	0.7 ± 0.1	0.0	0.5 ± 0.1	0.0	0.6 ± 0.2	0.0	0.2 ± 0.03
200	0.0	0.1 ± 0.05	0.0	0.4 ± 0.04	0.0	0.3 ± 0.05	0.0	0.2 ± 0.03	0.0	0
400	0.0	0.0	0.0	0.1 ± 0.03	0.0	0.1 ± 0.04	0.0	0.0	0.0	0
600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
800	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0

Note: a – without DS biochar application; b – with DS biochar application; c – no growth.

capacity (Al-Tarawneh, 2022; Chatterjee et al., 2020; Guittonneau et al., 2010). The date seed biochars with the best Pb removal effectiveness were those with the highest pyrolysis temperature (Al-Tarawneh, 2022; Mahdi et al., 2018). According to Ahmad et al. (2012), date seeds biochars have a high affinity for binding heavy metals, making them capable of removing heavy metals from water. Usman et al. (2016) discovered that the date palm biochar produced at 700°C was successful in removing metals from aqueous solution with an adsorption capacity of 43.58 mg/g. Increased soil pH resulted in decreased metal bioavailability in the soil (Rehman et al., 2016).

Many authors concurred with these conclusions. It was discovered that using 1% biochar made from chicken manure boosted the biomass of *Brassica juncea* shoots by 353% (Park et al., 2011). By improving plant height, root length, shoot and root dry weight, the biochar generated from various sources was discovered to reduce Ni bioavailability and toxicicty on *Zea mays L.* growth (Rehman et al., 2016). Biochar addition reduced the bioavailable metal concentrations. The reductions in Cd, Zn, and Pb were 71, 87, and 92 %, respectively (Houben et al., 2013). According to Hernandez-Allica et al., (2008),

the concentrations of Cd, Zn, and Pb that induced 100% inhibition of rapeseed shoot growth were 92, 10916, and 328 mg/kg, respectively.

According to this study, as the Cu, Zn, Cd, and Pb concentrations increased; the tolerance index of all seedlings decreased considerably, with some differences across metals within and between plant species. In terms of metal concentrations and metal type, TI improved significantly (P<0.05) in both plants after the application of DS biochar. The metal effect, according to Mahmood et al., (2007), may be linked to the regulation of metal absorption induced by the changes in seed coat structure between species, resulting in differences in metal permeability. In general, the presence of DS biochar may reduce the impact of these metals on seeds due to its strong propensity to adsorb metals, lowering their accessible concentration in water for plants.

Phytotoxicity assays revealed that, depending on the metal type and plant species, PT increased along with metal concentrations until it reached 100% at a particular concentration, matching the findings of many prior investigations. Metal PT on plants increased proportionally with metal concentrations, according to numerous authors

(Ashagre et al., 2013; and Shaikh et al., 2013). The PT of both plants improved significantly (P<0.05) after the application of DS biochar, both in terms of metal concentrations and metal type.

Because of its significant proclivity to absorb metals, DS biochar may lessen their influence on seeds by lowering their accessible concentration in water for plants.
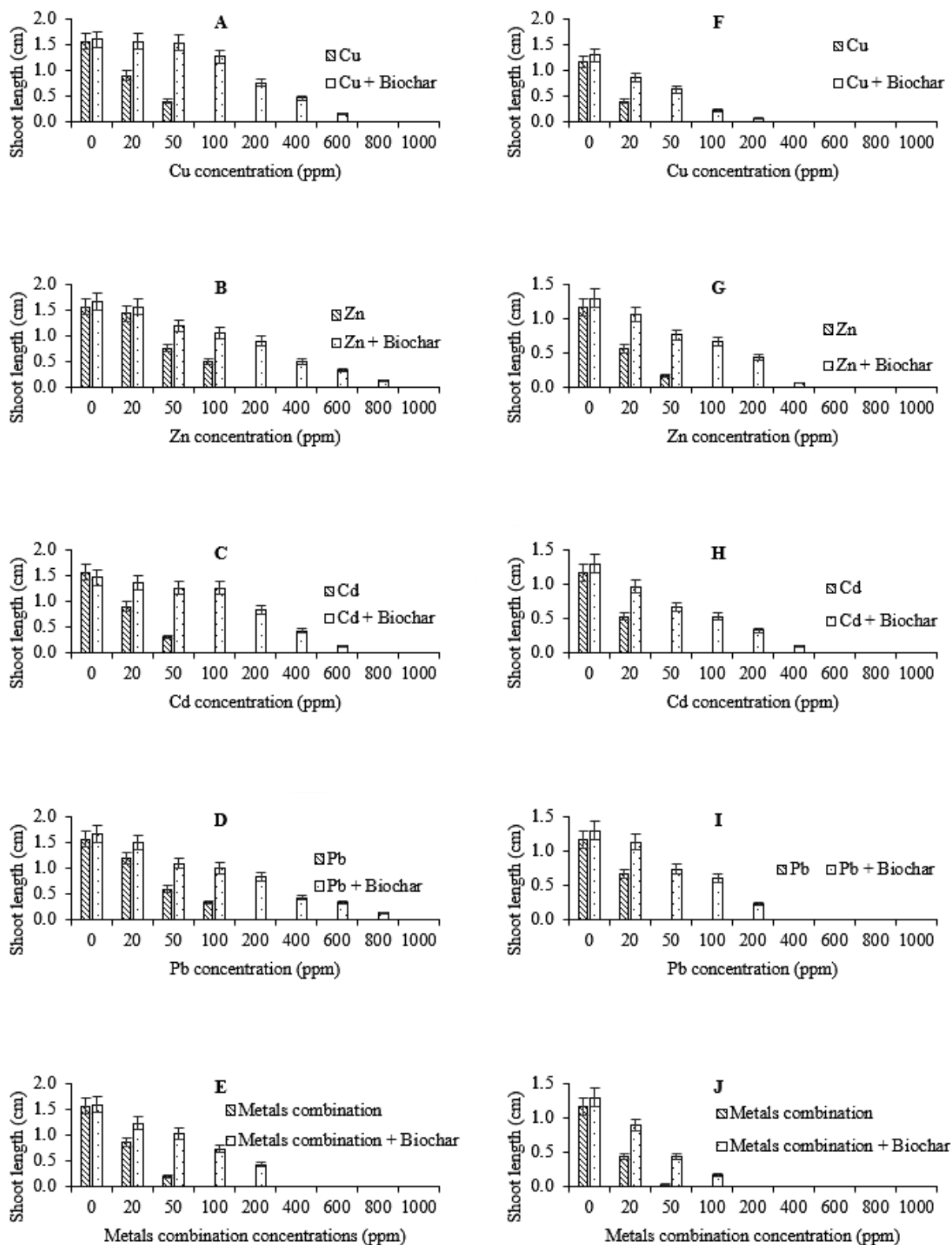

Figure 6. Shoots production and length of *Raphanus* sp. (A-E) and *Arabidopsis* sp. (F-J) when treated with different concentration of different meteal, with and without DS biochar application
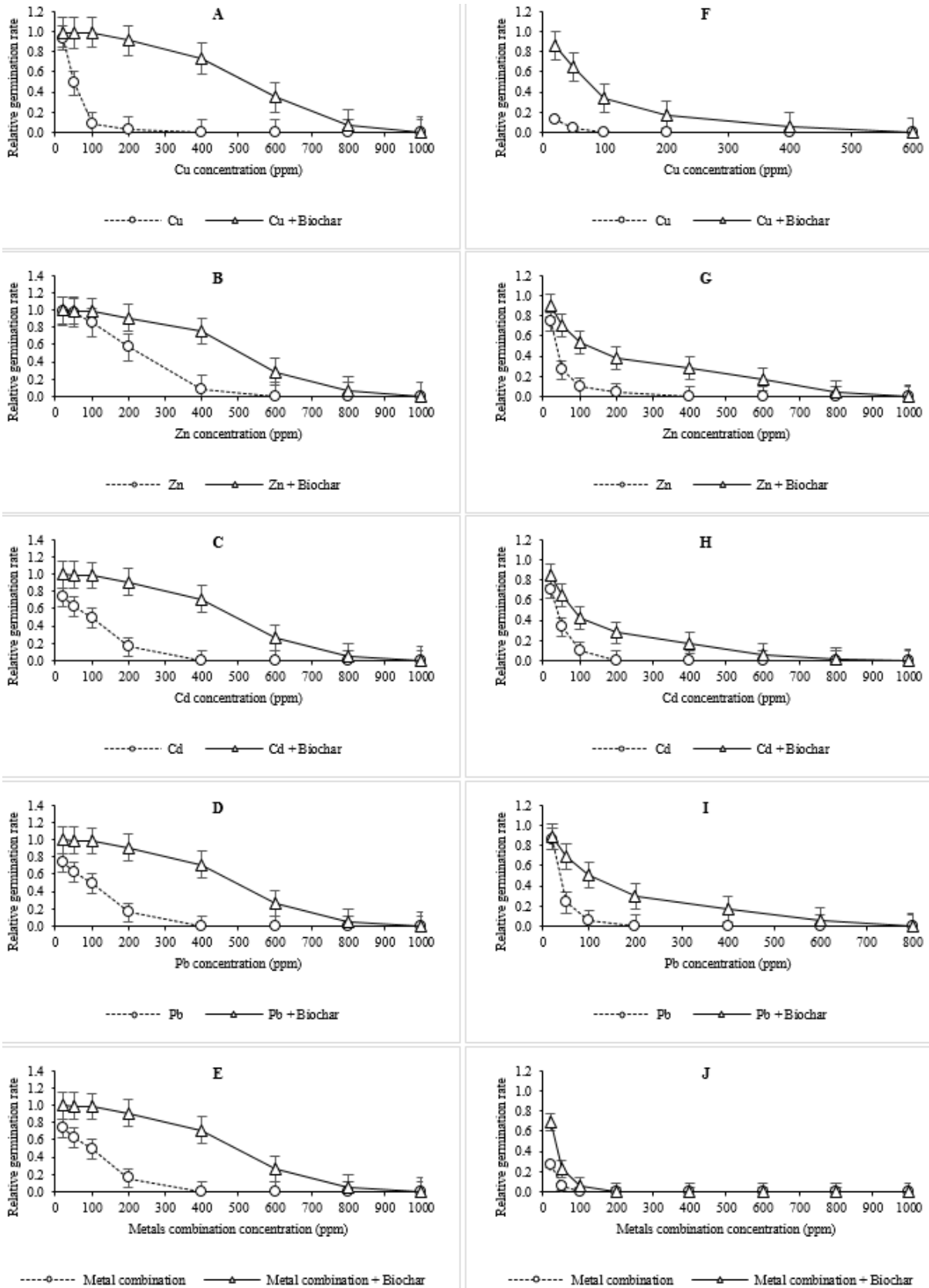
Figure 7. Relative germination rate of Raphanus sp. (A-E) and Arabidopsis sp. (F-J) when treated with different metals, with and without DS biochar application
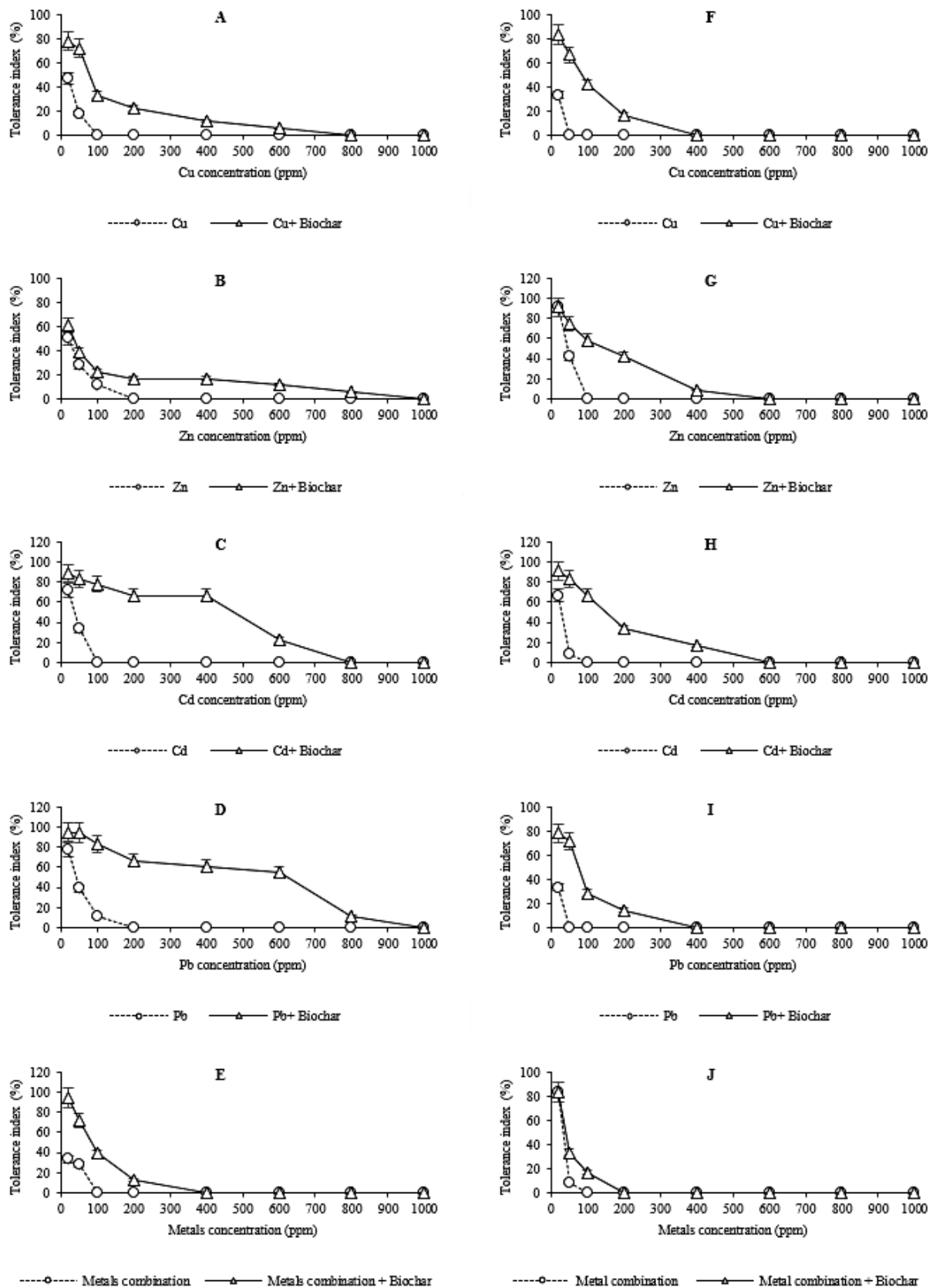
Figure 8. Effect of different metals concentrations on the Tolerance Index (TI) of Raphanus
sp. (A-E) and Arabidopsis sp. (F-J), with and without DS biochar application
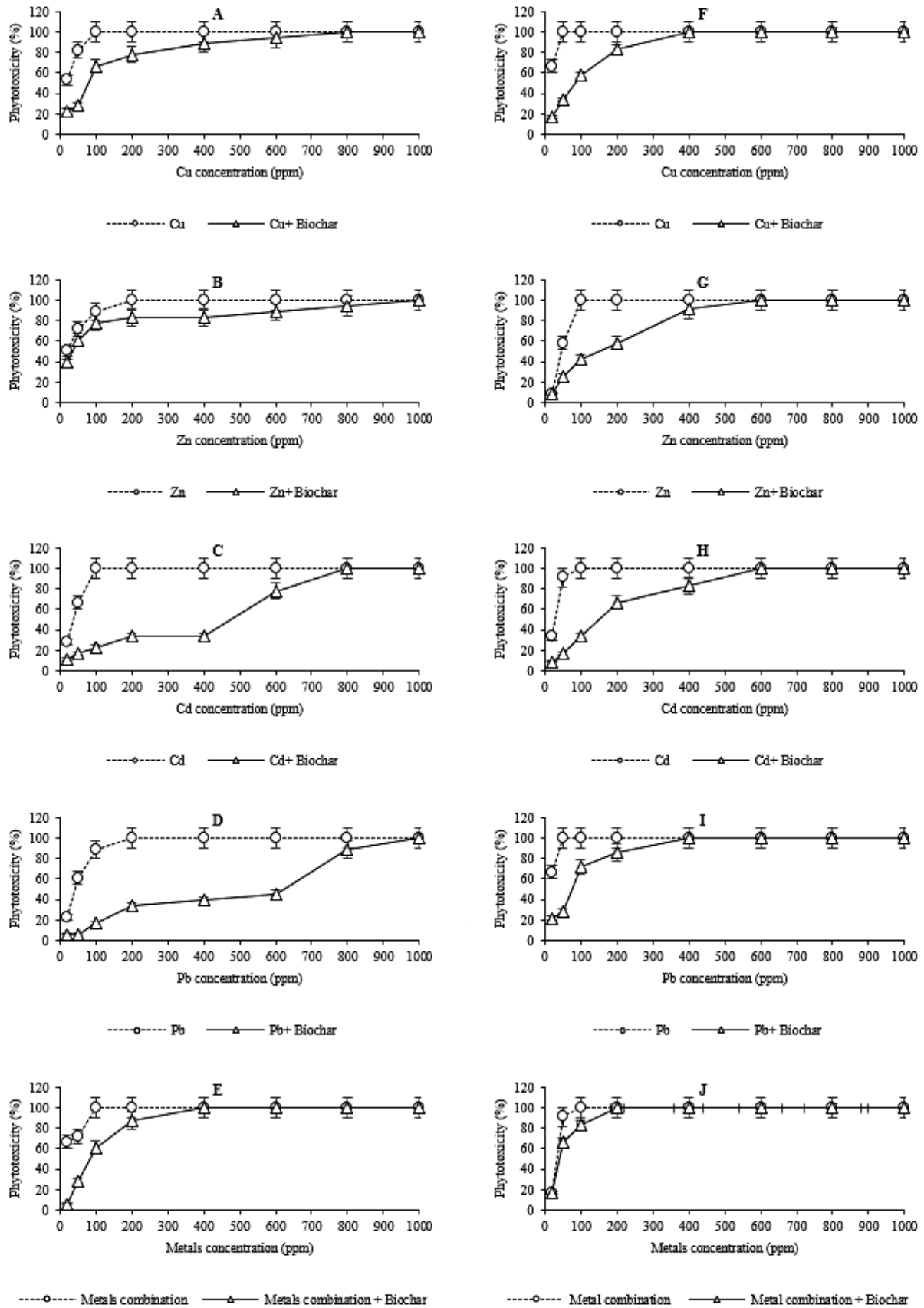
Figure 9. Effect of different metals concentrations on the phytotoxicity (PT) or *Raphanus* sp. (A-E) and *Arabidopsis* sp. (F-J), with and without DS biochar appliacation

CONCLUSIONS

On the basis of on the findings of the current study, DS biochar was shown to be a good provided raw material for remediation of metal contaminated water, hence lowering metal stress and toxicity on plants and seeds. This could be owing to the fact that metal toxicity is decreased. Evidence showed the biochar made from date seeds at 550°C changed the partitioning of Cu, Zn, Cd, and Pb from the easily exchangeable phase to the less accessible organic bound portion. In conclusion, independent of the metal employed or its concentration, biochar proved successful in immobilizing metals, reducing heavy metal bioavailability and phytotoxicity, as well as increasing the tolerance index of *Raphanus* sp. and *Arabidopsis* sp. More research into biochar production and optimization is therefore needed in order to improve its sorption capability for metals cleanup in aquatic environments.

Acknowledgements

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