Influence of Heavy Metals on Seed Germination, Shoot Length, Root Length and the Profiling of Antioxidant Activity of *Linum Usitatissimum* L.

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Abstract: Being an important oilseed crop, flaxseed (*Linum usitatissimum* L.) grows mostly in temperate climates. The present study involved the seeds and seedlings of the flaxseed plant for analysis. The current experiment was carried out to determine the effect on seed germination, seedling growth and the antioxidant profiling of the plant when treated with heavy metal exposures. The heavy metals selected for the study were Zinc (Zn), Lead (Pb), and Copper (Cu). The inhibition caused by these metals varied from day to day as the experimental setup was upto 60 days and the observations started from the 15th day. The plant indicated inhibition on seed germination highest with Cu stress. The highest effect of heavy metal on reduced growth of shoot and root was exhibited by Pb and Cu stress. Also, when the antioxidant profiling was done, Zn had the most toxic effect on phytochemicals. The conclusions divulged that the metal toxicity was as follows: Pb > Cu > Zn.

Keywords: Flaxseed, Heavy metal, Seed germination, Phytochemical, Antioxidant Profiling

Introduction

The past few decades have witnessed an upsurge in prompt industrialization, anthropogenic activities along with the present agricultural approaches. This has led to augmented contamination rates of heavy metals in the environment, causing toxicity not only in flora but fauna as well as it disperses into the food chain as well (Kavamura and Esposito, 2010; Miransari, 2011). Prominent areas on earth have been adulterated with heavy metals via the extensive utilization of fertilizers, pesticides, insecticides, municipal wastewaters, compost waste, along with the releases from metalliferous mines or even manufacturing industries (Singh et al., 2016). Even though their presence in nature is as usual, but when it exceeds in limit through the extensive release it creates toxicity in the environment. Almost 53 elements that belong to d-block in the periodic table are labeled as 'heavy metals' depending upon their density of > 5g/cm^3 (Jarup, 2003).

Being demanding in very less quantity, heavy metals, for instance, cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), cadmium (Cd), lead (Pb), or zinc (Zn) can be detrimental to organisms when attaining extreme measures of these metals (Asati et al., 2016). It becomes very essential to study these heavy metals because they are tough to decay and accumulate in living organisms. These heavy metals readily dissolve in water because they have a higher density than water and then they generate positive ions in the solution. Despite the detrimental effects of these heavy metals, some of these including Copper (Cu), Zinc (Zn), and Lead (Pb) is elemental for the growth of the plant in lower concentrations and later may become toxic if turn upto extreme levels (Wintz et al., 2002). Comparable to other living beings, even plant exhibit sensitivity for deficiency and excessiveness too. What influence these metals bring in terms of inhibition of growth or even the death of the plant can be read in literature published (Carlson et al., 1991). As per literature, it is found that these metals have a visibly negative effect on seed germination and might also affect different plants or their processes in different ways. (Rasafi et al., 2016).

The current study aimed at the influence of heavy metals on the seeds of *Linum usitatissimum* (Linseed; Flax; Alsi) along with its seedlings to analyze the growth of shoot and root. This novel plant, *Linum usitatissimum*, belongs to the Linaceae family and has been reviewed in literature for its resourceful industrial applications such that of flax-fibre production and linseed oil extraction and also, presently it's been utilized extensively for the generation of the health-promoting phenolic metabolites (i.e., lignans & neolignans) which are naturally occurring in flaxseed (Abbasi et al., 2019). Being the two most important stages of phyto-lifespan, seed germination and seedling growth, guarantees a remarkable crop yield based on healthy seeds, therefore, higher productivity (Liu et al., 2011; Baruah et al., 2019). Several phytochemical analyses have divulged that the mean constituents of commercial seed were 41% fat, 28% dietary fibre, 20% protein content, 7.7% moisture and 3.4% of ash (Shim et al., 2014).

The aim of the present study performed was to enhance the comprehension of the proficiency of plants to raise in the presence of metallic stresses and to develop an understanding of plant responses when treated with distinctive elemental doses of metal contamination. The experiment covered the sequence of chosen parameters including seed germination, seedling growth (effect on the shoot and root length), along with its phytochemical screening (antioxidant profiling) from the view of metals which could have a negative impact on growth, influence the germination of seed, and growth of seedlings of our experimental plant *Linum usitatissimum*. Along with this, we also desired to distinguish the bioaccumulation potency of the experimental plant for these heavy metals; however, we hypothetically assumed that different amounts of the applied metallic stress will generate diverse effects on discrete stages of the plant life cycle.

Materials and Methodology

Sample Collection and Preparation

The Soil sample to be treated with heavy metals has been taken as a topsoil layer from Uttaranchal University Campus Dehradun, Uttarakhand and was air-dried, sieved to separate soil particles and then mixed thoroughly. For the treatment of soil with heavy metals; metals including Copper (Cu^{29}), Zinc (Zn^{30}), and Lead (Pb^{82}) were selected. The metal stock solutions were prepared in 200mg/100ml concentration. For the sterilization of seeds, the mature seeds of *Linum usitatissimum* L. were dipped in 70% ethyl alcohol solution (EtOH) for 1 minute. This was followed by surface sterilization by 2.5% sodium hypochlorite solution (NaOCl) for 8 mins. Hereafter, these seeds were taken out and rinsed with sterilized deionized water for 3 mins (Kavianifar et al., 2018).

Soil Treatment and Metal Exposure

The soil was taken from agricultural land, which was air-dried and filtered through an 8mm mesh-sized sieve. It was treated in autoclave twice at 121°C under 15Psi for 30-40 minutes in order to avoid contamination of any sort of microbes or fungi (Mukhtar et al., 2010). With this soil, pot culture experiments were performed. 4 pots were taken and labeled as Control, Copper Treated, Zinc Treated, and Lead Treated respectively and the autoclaved soil was added to each of the pots in adequate amount. This was followed by the addition of metal solutions of Cu, Zn and Pb to the respective pots (200mg/kg soil). These solutions were mixed with soil and kept for around 3-4 weeks to stabilize (Rasafi et al., 2016).

Experimental Set-up for Seed Germination

The seeds of *L. usitatissimum* were germinated and grown in laboratory conditions in the autoclaved petri-dish with filter paper. For the experiment 4 petri-dish were taken labeled as Control, Copper Treated, Zinc Treated, and Lead Treated respectively and to each of them, 10 seeds were placed using sterilized forceps. To this, 3ml of respective metal stock solution along with water is being added and has been observed for 10 days by the continuous water addition (10ml) to it at regular intervals.

Germination parameters

Seeds were observed each day (24hrs) and were recorded for 3 days, 7 days, and 10 days to evaluate the germination. By the end of the germination procedure, Percent germination (% Germination) was calculated using the formula to assess the performance under the applied treatment (Arnold et al., 1991; Tanveer et al., 2010; Sarma et al., 2014; Baruah et al., 2019) as below:

$$\%\ Germination = (Germinated\ seeds)/(Total\ seeds) \times 100$$

Experimental Set-up for Seedling Growth

For the evaluation of seedling growth under the given stress conditions of different metal solutions, a pot experiment was set up using the treated soil with metal exposure. 4 pots as above mentioned were taken and to each of them, 5 seedlings of the given plant sample were planted. Plants were kept in observation for 15 days, 20 days, 30 days, 45 days, and 60 days and watered twice per week with approximately 200ml of tap water. The shoot length and root length were observed in all 4 pots on the 15th and 20th days and the plants were left under further antioxidant development for its profiling as per the study.

Antioxidant Profiling

Methanol was used as the organic solvent for the preparation of extract of the selected plants and the methodology used was reciprocated from previous studies (Sundriyal et al., 2021; Sharma et al., 2016). The antioxidants including phenols, flavonoids, flavanols, and tannin were analyzed in the present study after 15, 20, 30, 45, and 60 days for each plant in the following experimental pots; Control, Copper Treated, Zinc Treated, and Lead Treated respectively.

Determination of Total Phenol Content

The Folin–Ciocalteu assay was followed up for the detection of total phenol content in the methanolic extract of *Linum usitatissimum* with the slightest modifications (Lahmadi et al., 2019b). Aliquots of plant extract and standard gallic acid (20, 40, 60, 80 and 100 µg/ml) were added to volumetric flasks and then the volume was made upto 5ml and for reagent blank distilled water was used. Furthermore, 0.25ml of Folin-Ciocalteu's reagent along with 1ml of saturated Na_2CO_3 were added to plant extracts and gently mixed. As the observation of phenols, blue color was seen, which was developed due to the redox reaction with phosphomolybdic acid. This was followed by standing the mixture for a few minutes and then placed at 25°C for 1-hour incubation and later absorbance be read at 765nm.

Determination of Total Flavonoids

For the determination of total flavonoids contention in the plant extract, the aluminium chloride method (Lahmadi et al., 2019b) was employed. Different aliquots (20, 40, 60, 80 and 100 µg/ml) were prepared using plant extract and standard solution of quercetin in 10ml volumetric flask and volume was made upto 3ml with distilled water. To these aliquots, 5% $NaNO_2$ (300µl) and 10% $AlCl_3$ (300µl) were sequentially added. The volumetric flask was put on a stand for at least 5min and then 1N NaOH (2ml) was added and briskly shaken. At 510nm, absorbance was recorded against blank, and the concentrations were expressed as mg of quercetin equivalent (QE) per gram of sample (mg QE/g dw).

Determination of Total Flavanols

Estimation was performed by making aliquots (20, 40, 60, 80 and 100 µg/ml) of 2ml of plant extract and standard solution. To this, 2ml of 2% $AlCl_3$ Solution (in ethanol) was mixed and left to stand for a minute. After this, 3ml of 50g/I CH_3COONa (Sodium acetate) is added and mixed briskly. After the reaction to stand at 20°C for 2.5 hours, the absorbance was recorded at 440nm, and the concentrations were expressed as mg of quercetin equivalent (QE) per gram of sample (mg QE/g extract).

Determination of Total Tannin Content

Tannin content was estimated using the Folin-Ciocalteu's method (Sundriyal et al., 2021). It was done by making 1ml aliquots (20, 40, 60, 80 and 100 µg/ml), to which 500 µl of Folin-Ciocalteu's reagent was added, followed up by the addition of 1ml of 7.5 of Na_2CO_3 and final volume made upto 5ml. The reaction was shaken briskly and then placed at 20°C for 30mins for incubation. Finally, the absorbance was taken at 740nm and the tannin content in the extract was expressed as mg of tannic acid equivalent per gram of extract (TE mg/g extract) (Nithya et al., 2016).

Results and Discussion

Seed germination under heavy metallic stress

The obtained results as depicted in Figure 1; Table 1, demonstrate the correct percentage of seeds of *Linum usitatissimum* that had been germinated along with given metallic stress. The percentage germination was calculated using the formula mentioned above. For the seeds of experimental plant species, out of the 10 seeds taken for each metallic stress, 80% of them germinated in Zinc (Zn) stress, 70% in Lead (Pb) stress and 10 % in copper (Cu) stress. The most distinct inhibitory effect of heavy metals on seed germination of *L. usitatissimum* was recorded in copper stress (10% Germination). The copper stress when stimulated in plant growing conditions, it generates biomass mobilization via releasing the glucose-fructose which ultimately impedes the breakdown of starch or sucrose in reverse tissues and along with this it leads to reduced germination of seeds (Pena et al., 2011; Sfaxi Bousbih et al., 2010). Literature reviews related to proteomics have publicized that the toxicity of Cu does inhibit the germination of seed via the down-regulating activity of α-amylase or enolase (Sethy and Ghosh, 2013). Additionally, studies in 1982 have revealed that seed germination when observed in *Spartiana alterniflora* and *Pinus helipensis* can be inhibited by lead (Asati et al., 2016).

Table 1: Effect of heavy metal on seed germination during the time interval (3, 7, 10 days).

S. No	Heavy Metal	After 3 Days	After 7 Days	After 10 Days	Total no of seed	Total % of seed germination
1	Control	6 Seed	10 Seed	10 Seed	10	100%
2	Zn	2 Seed	6 Seed	8 Seed	10	80%
3	Pb	5 Seed	6 Seed	7 Seed	10	70%
4	Cu	1 Seed	1 Seed	1 Seed	10	10%

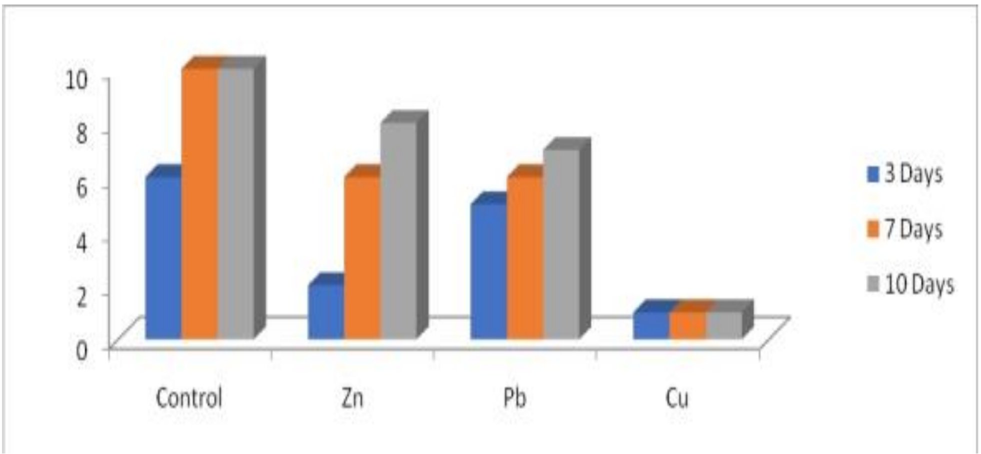

Figure 1: Effect of heavy metal on seed germination during the time interval (3, 7, 10 days).

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Effect of metallic stress on shoot length

The effects of different stresses given to seedling to analyze the growth of shoot varied accordingly to the metal and has been summarized in Figure 2; Table 2. The results displayed that the metal concentrations of Zn stimulated the shoot length in 15 days (Zn: 14.0±0.003) and 20 days (Zn: 15.8±0.010) as what of compared to the control (15 days: 14.3±0.003; 20 days: 15.5±0.01). The inhibitory effects of short-length stimulation were exhibited by Pb and Cu.

Table 2: Shoot length after 15 and 20 days

No. of plants	Control 15 days	Control 20 days	Zn 15 days	Zn 20 days	Pb 15days	Pb 20 days	Cu 15 days	Cu 20 days
1.	14.3±0.003	15±0.012	10.0±0.004	13.5±0.01	8.5±0.021	11.0±0.002	10.5±0.018	12.0±0.004
2.	13.5±0.012	14±0.011	14.0±0.003	14.5±0.012	8.8±0.031	11.2±0.011	12.6±0.014	13.0±0.002
3.	12.5±0.002	15.5±0.01	15.3±0.012	15.8±0.010	10.0±0.002	12.3±0.016	9.5±0.012	10.2±0.02
4.	11.0±0.005	12.0±0.002	11.5±0.014	14.0±0.002	10.0±0.003	13.0±0.012	11.0±0.012	12±0.003
5.	13.0±0.015	13.4±0.016	10.5±0.011	12.0±0.003	7.5±0.002	9.5±0.014	7.0±0.002	9.0±0.012

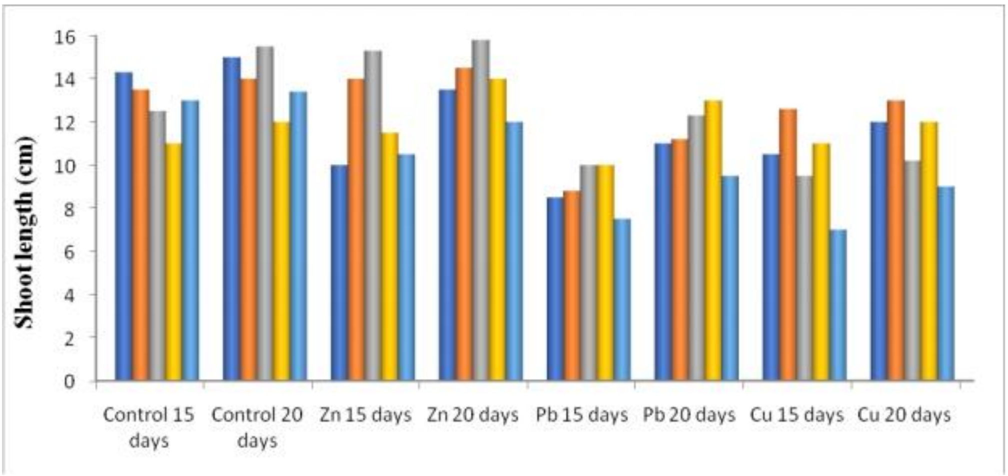

Figure 2: Shoot length after 15 and 20 days.

It was also observed that the average growth of shoot after 15 days was 12.5±0.013 (Control), 12.26±0.018 (Zn), 8.96±0.014 (Pb), 7.33±0.014 (Cu). Also, the maximum length after 15 days was 14.52±0.016 (Control), 14.23±0.014 (Zn), 12.01±0.015 (Pb), 10.02±0.013 (Cu). The authors also took the average and maximum length for 20 days observation which was 13.10±0.017 (Control), 11.95±0.016 (Zn), 9.80±0.014 (Pb), 8.37±0.013 (Cu) for 15 days; and 15.51±0.018 (Control), 14.87±0.015 (Zn), 13.04±0.019 (Pb), 11.92±0.015 (Cu) for 20 days (Table 3; Figure 3). Soils that be contaminated with Pb pollution impedes the seedling growth (Sethy and Ghosh, 2013) through several functional actions including an increase in lipid peroxidation, along with activation of superoxide dismutase (SOD) and guaiacol peroxidase (POD) and ascorbate peroxidase (APX), thereby playing a governing role in removing H_2O_2 (Pourrut et al., 2011). Other studies in past, shows that early seedling growth in species of rice, barley, soyabean, maize, tomato, and other legumes, can also be inhibited by lead exposure (Asati et al., 2016).

Table 3: Average and Maximum shoot length after 15 days and 20 day

Characters	Control	Zn	Pb	Cu
Average of Shoot length after 15 days	12.5±0.013	12.26±0.018	8.96±0.014	7.33±0.014
Max. Shoot length After 15 days	14.52±0.016	14.23±0.014	12.01±0.015	10.02±0.013
Average of Shoot length after 20 days	13.10±0.017	11.95±0.016	9.80±0.014	8.37±0.013
Max. Shoot length After 20 days	15.51±0.018	14.87±0.015	13.04±0.019	11.92±0.015

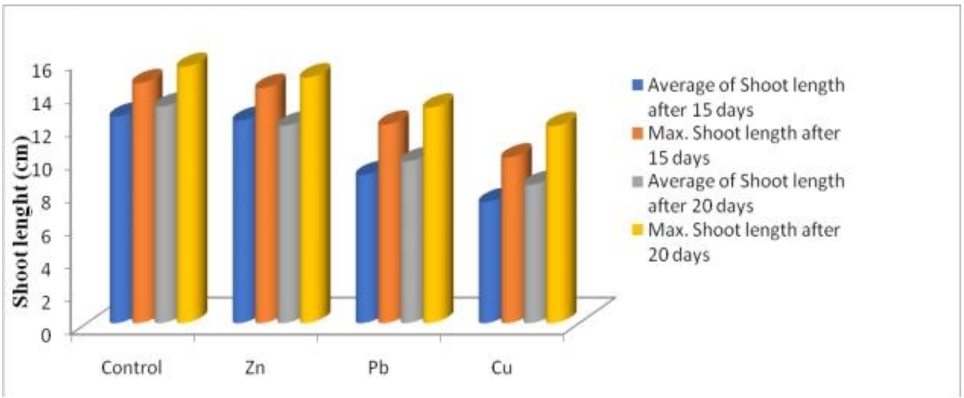

Figure 3: Average and Maximum shoot length after 15 days and 20 days.

Effect of metallic stress on root length

Table 4 and figure 4 describe what effects are rought through the given metallic stresses of Zn, Pb and Cu, and all of these brought about varied values. Growth of root length was promoted by Zn (15 days: 5.5±0.013; 20 days: 8.8±0.016) as compared to that of control (15 days: 5.5±0.018; 20 days: 9.0±0.012). The repressing effects were observed in the pots with Cu (15 days: 2.0±0.015; 20 days: 4.2±0.014) and Pb (15 days: 2.0±0.016; 20 days: 4.5±0.015) as the given metallic stress. Studies reveal that metallic stresses of Pb and Cu, along with other major or minor effects, do inhibit the root elongation (Pourrut et al., 2011). In *Chloris gayana* (Rhodes grass), copper reduces root growth (Sheldon and Menzies, 2005; Pichhode and Nikhil, 2015). In addition, *allium species*, barley and *Raphanus sativas*, have shown inhibited in root and shoot elongation. Further, it majorly depends upon the concentration of lead and its ionic composition, which depicts to what extent the inhibition of root elongation, can take place (Asati et al., 2016).

The authors also took average and maximum length for 15 days and 20 days observation for root growth as well which is depicted in Table

5; Figure 5. For 15 days, average growth values were 3.76±0.011 (Control), 4.4±0.016 (Zn), 3.76±0.014 (Pb), 2.9±0.016 (Cu); and the maximum growth values were as 5.50±0.018 (Control), 5.23±0.013 (Zn), 4.31±0.019 (Pb), 3.9±0.017 (Cu). Similarly, observations were noted for 20 days. The average growth values for 20 days were 4.91±0.013 (Control), 8.00±0.013 (Zn), 5.72±0.020 (Pb), 5.81±0.013 (Cu); and the maximum growth values were as 9.0±0.012 (Control), 11.5±0.013 (Zn), 9.5±0.026 (Pb), 7.5±0.015 (Cu).

Table 4: Root length after 15 days and 20 days.

No. of plants	Control 15 days	Control 20 days	Zn 15 days	Zn 20 days	Pb 15 days	Pb 20 days	Cu 15 days	Cu 20 days
1.	5.3±0.015	9.0±0.012	4.5±0.012	8.5±0.014	4.5±0.013	6.2±0.016	2.5±0.018	4.2±0.014
2.	2.0±0.012	4.2±0.011	3.5±0.015	5.5±0.015	5.0±0.019	9.5±0.013	5.0±0.012	7.0±0.011
3.	4.0±0.014	5.0±0.013	5.5±0.013	8.8±0.016	2.0±0.016	4.5±0.015	2.5±0.016	5.3±0.013
4.	2.0±0.017	3.5±0.012	5.0±0.013	7.5±0.017	5.0±0.016	6.5±0.012	2.0±0.015	5.4±0.012
5.	5.5±0.018	7.2±0.014	3.5±0.011	6.2±0.018	2.3±0.016	5.5±0.011	2.5±0.017	6.0±0.018

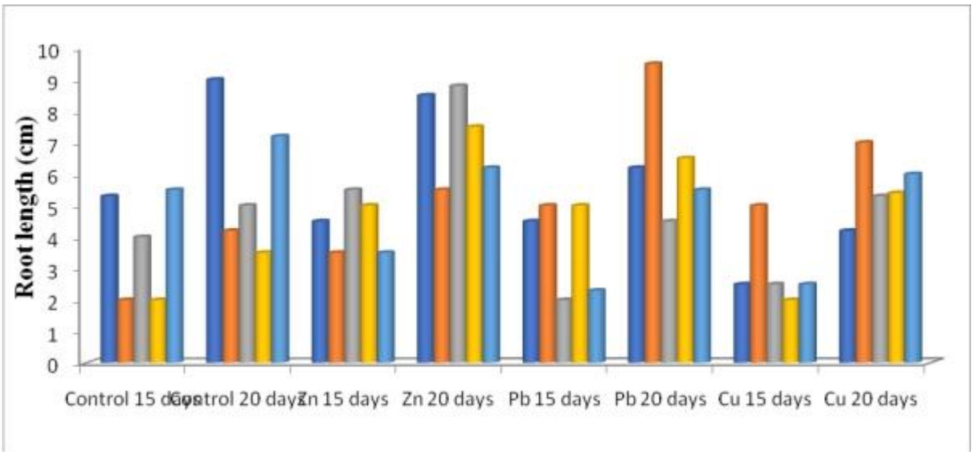

Figure 4. Root length after 15 days and 20 days.

Comparison between Fresh and Dry Weight of the seedlings

As the experiment proceeded, the fresh weight and dry weight for the plant under control and Zn, Pb, Cu metallic exposures was recorded as in Table 6; Figure 6. The observations were taken for plants after 15 days, 20 days, and 30 days. In comparison to control, the plants with metallic exposure especially in the case of Pb has initially lesser fresh and dry weight in early 15-20 days, but gradually increased when recorded at 30 days. Also, after 30 days, the control has fresh and dry weight as 0.960±0.012 and 0.896±0.016 which in comparison to exposure plant was higher such that weights (fresh/dry) of them were Zn (0.214±0,012/ 0.154±0.017), Pb (0.342±0.016/ 0.284±0.014), Cu (0.233±0.011/ 0.181±0.009). These values which were observed were more han half of the values observed for the control plants.

Table 5: Average and Maximum Root length after 15 days and 20 days

Characters	Control	Zn	Pb	Cu
Average of Root length after 15 days	3.76±0.011	4.4±0.016	3.76±0.014	2.9±0.016
Max. Root length after 15 days	5.50±0.018	5.23±0.013	4.31±0.019	3.9±0.017
Average of Root length after 20 days	4.91±0.013	8.00±0.013	5.72±0.020	5.81±0.013
Max. Root length after 20 days	9.0±0.012	11.5±0.013	9.5±0.026	7.5±0.015

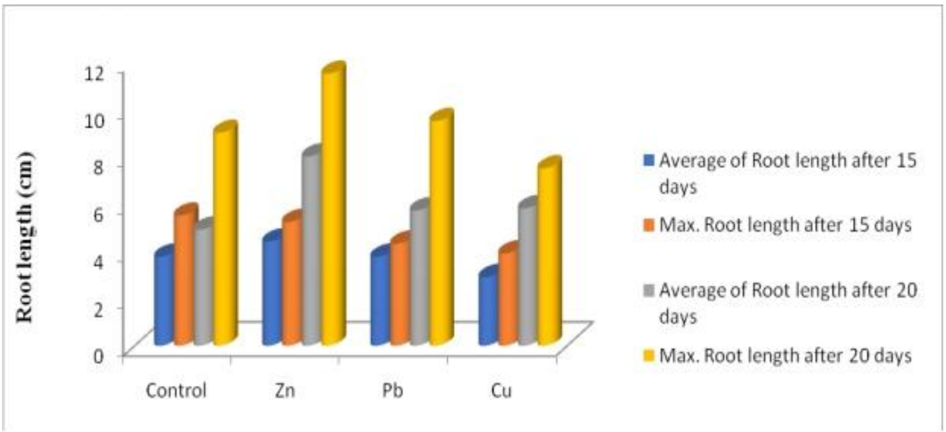

Figure 5. Average and Maximum Root length after 15 days and 20 days

Table 6: Fresh and Dry weight of plant *Linum usitatissimum* after 15 days and 20 days

Characters	Control	Zn	Pb	Cu
Fresh Weight after 15 days	0.076±0.016	0.060±0.012	0.048±0.013	0.064±0.014
Dry Weight after 15 days	0.012±0.017	0.010±0.011	0.0096±0.012	0.012±0.015
Fresh Weight after 20 days	0.096±0.012	0.086±0.014	0.067±0.015	0.051±0.014
Dry Weight after 20 days	0.012±0.011	0.010±0.015	0.009±0.016	0.008±0.012
Fresh Weight after 30 days	0.960±0.012	0.214±0,012	0.342±0.016	0.233±0.011
Dry Weight after 30 days	0.896±0.016	0.154±0.017	0.284±0.014	0.181±0.009

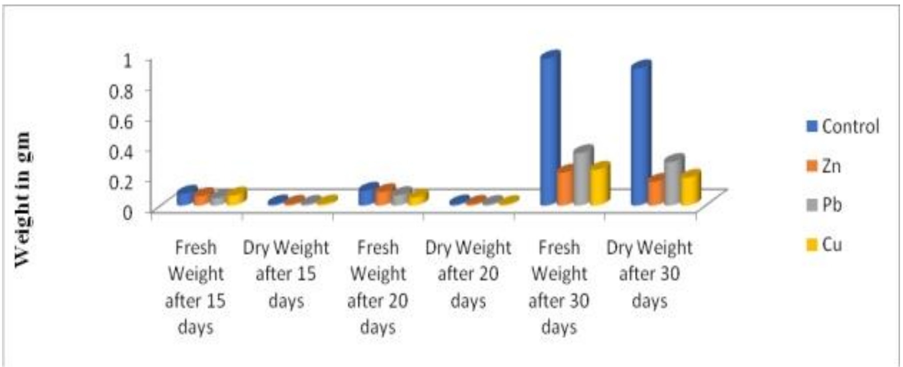

Figure 6. Fresh and Dry weight of plant *Linum usitatissimum* after 15 days and 20 days.

Table 7: Antioxidant profiling of plant biomass *Linum usitatissimum*

	Phenol (µg/ml)	Flavanoid (µg/ml)	Flavanol (µg/ml)	Tannin (µg/ml)
Control 15 days	0.117±0.011	0.049±0.001	0.234±0.012	0.015±0.002
Zn 15 days	0.041±0.01	0.02±0.003	0.162±0.023	0.012±0.005
Pb 15 days	0.053±0.013	0.038±0.009	0.193±0.014	0.01±0.003
Cu 15 days	0.073±0.017	0.028±0.006	0.201±0.018	0.009±0.003
Control 20 days	0.153±0.019	0.067±0.01	0.289±0.012	0.026±0.005
Zn 20 days	0.032±0.015	0.013±0.01	0.136±0.019	0.007±0.001
Pb 20 days	0.021±0.013	0.021±0.002	0.167±0.014	0.006±0.003
Cu 20 days	0.053±0.016	0.016±0.003	0.184±0.018	0.008±0.001
Control 30 days	0.258±0.012	0.093±0.006	0.321±0.012	0.038±0.003
Zn 30 days	0.055±0.013	0.04±0.002	0.198±0.019	0.016±0.004
Pb 30 days	0.114±0.013	0.063±0.001	0.214±0.012	0.019±0.002
Cu 30 days	0.092±0.017	0.075±0.001	0.232±0.023	0.015±0.003
Control 45 days	0.301±0.013	0.13±0.007	0.386±0.014	0.045±0.006
Zn 45 days	0.083±0.018	0.096±0.013	0.256±0.018	0.02±0.002
Pb 45 days	0.152±0.017	0.084±0.008	0.251±0.02	0.024±0.007
Cu 45 days	0.132±0.014	0.095±0.017	0.298±0.013	0.027±0.002
Control 60 days	0.365±0.011	0.167±0.005	0.398±0.011	0.051±0.003
Zn 60 days	0.126±0.013	0.121±0.01	0.301±0.013	0.027±0.004
Pb 60 days	0.183±0.012	0.143±0.009	0.294±0.016	0.031±0.005
Cu 60 days	0.173±0.013	0.153±0.006	0.312±0.013	0.037±0.002

Antioxidant Profiling:

In the current research, the methanolic extract of the plant *Linum usitatissimum*, demonstrated different antioxidant activity when the plants were treated with metallic exposures. Total phenolic content (TPC) was expressed in gallic acid equivalents (GAE) wherein the concentration in µg/ml of phenol was observed to be lesser in the plant extract which was treated with Zn than compared to control and plants treated with Pb and Cu. The highest and lowest phenolic content was observed after 60 days (0.183±0.012) in the plant treated with Pb (Table 7; Figure 7). We can assume that the lead concentrations do not affect the levels of phenolic extract as Zn does. The plants like flaxseed have been identified since years due to the characters possessed by them. The identification of phytochemicals and their antioxidant profiling found in the plant extracts can assist to acknowledge their therapeutic potential. Amid all the phytochemicals, the antioxidant found to be a good amount is the phenolic compounds present in the plants. These act as hydrogen donators, reducing agents, or free radical scavengers (Elshamy et al., 2019; Lahmadi et al., 2019a; Lahamadi et al., 2019b; Srivastava and Soni, 2019).

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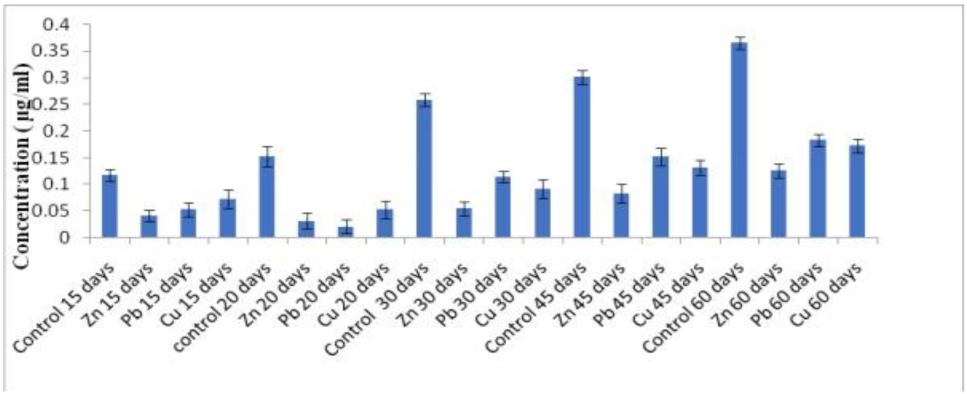

Figure 7. Phenol profile of *Linum usitatissimum* plant biomass

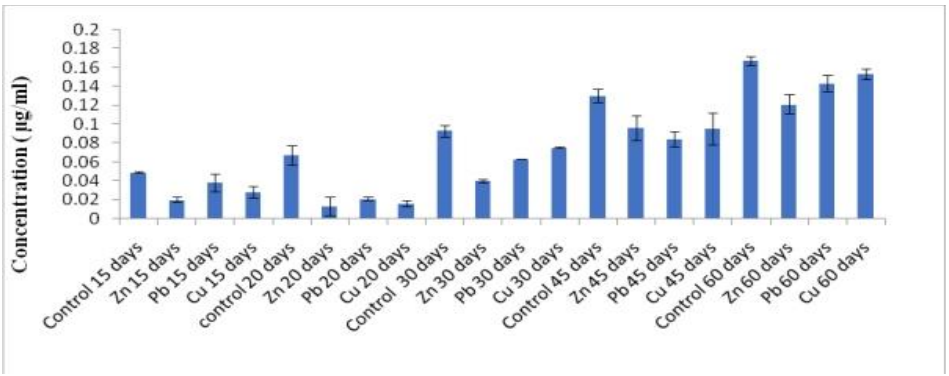

Figure 8. Flavanoid profile of *Linum usitatissimum* plant biomass

The flavonoid content varied from 0.013±0.01 and 0.02±0.003 in early 15-20 days to 0.143±0.009 and 0.153±0.006 in 60 days observation (Table 7; Figure 8). As per the results, the flavanol content in plants treated with metallic exposures displayed the lowest values in Zn treated than compared to others (Table 7; Figure 9). Tannin contents were expressed in micrograms of tannic acid equivalent (TAE) per ml. The lowest tannin content found was 0.006±0.003 (20^{th}-day observation) and the highest tannin content was 0.037±0.002 (60^{th}-day observation) (Table 7; Figure 10).

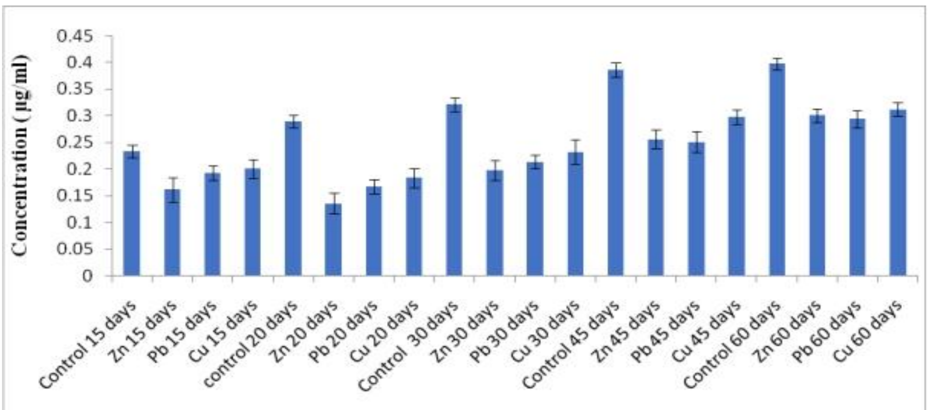

Figure 9. Flavanol profile of *Linum usitatissimum* plant biomass

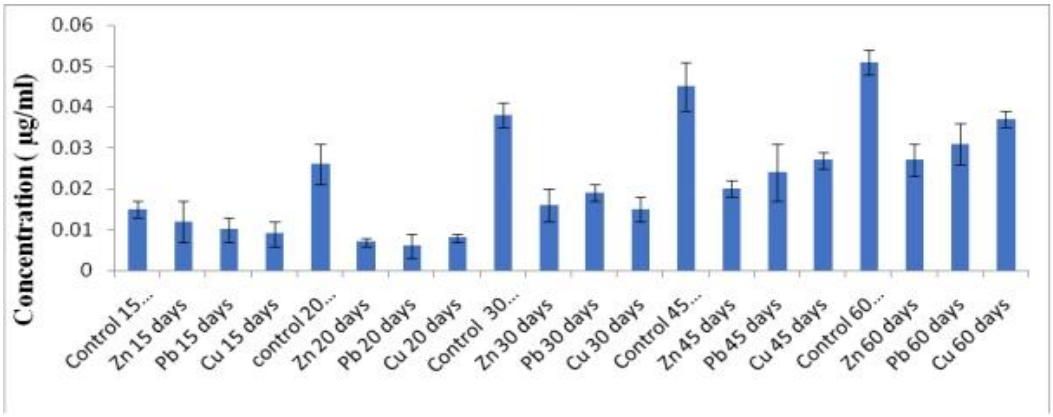

Figure 10. Tannin profile of *Linum usitatissimum* plant biomass

Conclusion

Growing on heavy metal polluted soil, the plants result in growth inhibition, which is led by alterations in their physiological and biochemical behavior. In the present study, our results showed quite a varied response of flax seeds and seedlings to the presence of toxic metals. Our experiment indicated that metals Zn, Pb and Cu have different effects on the seed germination and seedling growth in the order of inhibition under metallic exposure as Pb > Cu > Zn. In general, while the study of shoot length and root length was done, Pb was found to be more toxic than Cu and Zn. Additionally, the study also reveals that when phytochemicals were analyzed, antioxidants under metallic exposure were quite less than the control values and a more toxic effect on antioxidant profiling was given by Zn > Pb and then Cu. We can conclude that further findings can also be done on the plant species through the individual metallic stress of different concentrations given to the plant and allowed to exhibit growth properties. Also, we can analyze to what degree the cultivars of *Linum usitatissimum* can tolerate the metallic stresses and can grow which can be compared to the ones growing in the natural habitats containing heavy metals in soils.

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