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Differential growth and metal uptake efficiency of castor bean genotypes on a lead contaminated soil

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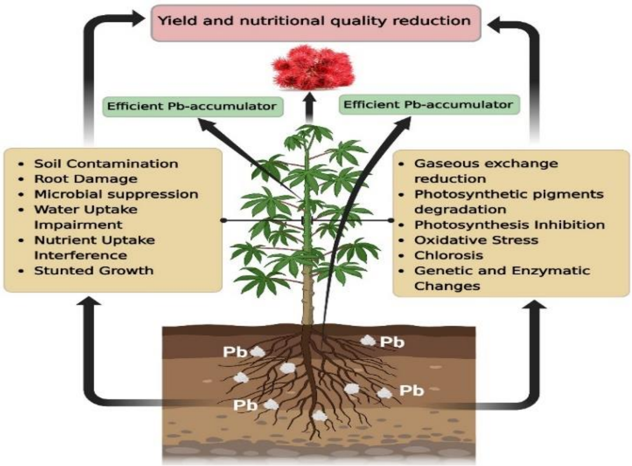
Graphical abstract



Abstract

Present study investigated the unknown responses of five castor bean genotypes to lead (Pb) contaminated soil, focusing on their growth and metal uptake efficiency. A unique aspect is the simultaneous assessment of multiple genotypes, offering a holistic view of their reactions to soil contamination. Five castor genotypes (NIAB Gold, NIAB Spineless, NIAB-2020, DS-30, and C-3) were grown in pots filled with soil spiked with varying concentrations of Pb (0, 100, 200, 400, and 800 mg kg^{-1}). Results have shown that increasing Pb concentrations led to a general decrease in plant biomass and photosynthetic pigments in all castor genotypes, however NIAB-2020 and DS-30 showcased remarkable resilience. Interestingly, the activities of antioxidant enzymes revealed a contrasting pattern. While superoxide dismutase and peroxidase activity declined, catalase and ascorbate peroxidase activity increased with higher Pb levels. Notably, DS-30 and NIAB-2020 exhibited the maximum catalase and ascorbate peroxidase activity at highest Pb concentration. Furthermore, these genotypes displayed impressive Pb uptake, particularly in their roots, exceeding the other tested genotypes. With metal translocation factor less than 1, NIAB-2020 and DS-30 showed promising potential for phytostabilization through efficient Pb accumulation, coupled with strengthened antioxidant defense mechanisms and restricted translocation, making them compelling candidates for eco-friendly remediation strategies.

Keywords: Castor bean, lead uptake, phytostabilization, antioxidants, osmolytes, water relation.

1. Introduction

In the 21^{st} century, pollution control is a major challenge due to the massive increase in pollutants produced by human activities (Adnan *et al.* 2024). Among all, heavy metals and organic pollutants are known as two major factors behind soil pollution. Technogenic and anthropogenic activities are the main drivers of heavy metal pollution in the environment. Lead is widely utilized for the manufacture of storage batteries, paints, alloy formation and casting etc. (Khan *et al.* 2018). The presence of Pb in the soil is hazardous to human health due to its long-lasting residual and chance of entry in the food chain (Mühlbachová 2011). Therefore, it is important to remediate the Pb contaminated soil to reduce the human exposure through consumption of contaminated food commodities.

Different strategies are proposed for the remediation of heavy metal contaminated soils (Wang *et al.* 2021), however, phytoremediation is considered cost-effective, environmental friendly, and easy to adopt technology with good public perception (Priya *et al*. 2023). Among the diverse array of phytoremediation techniques, each offering unique mechanisms and applications, phytostabilization stands out for its focus on immobilizing contaminants within the soil by accumulating it in their roots (Yan *et al.* 2020). This prevents heavy metals from leaching into groundwater and reduces the risk of human exposure. Plants suitable for phytostabilization should have low metal transfer rates from roots to shoots, be resistant to heavy metal stress, and exhibit rapid growth (Shackira and Puthur 2019).

Castor bean, *Ricinus communis* L., has good potential to grow well on heavy metal contaminated soils (Ali *et al.* 2022; Palanivel *et al.* 2020). Additionally, castor possesses enormous adaptive potential in diverse habitats including resistance against drought, salinity, etc. hence, suitable for marginal lands (Song *et al.* 2021). Moreover, castor is an important non-edible industrial oil crop with various non-food and industrial applications as being used in soap, cosmetics, pharmaceutical, paints and lubricants. Castor bean is a dual-purpose crop that can be used to clean up contaminated soil and also produce biofuel, making it a sustainable and environmentally friendly option (Singh *et al.* 2021). Therefore, phytostabilization of Pb contaminated soils using castor crop may be an environmental friendly and safe option that could give economic benefits from contaminated soils as well.

Various studies have reported the potential of castor bean for the phytostabilization of heavy metal contaminated soils in Brazil (Andreazza *et al.* 2013), China (Yang *et al.* 2017) and India (Boda *et al.* 2017). In a greenhouse experiment, castor plants, were grown on copper contaminated soil, showed high biomass production, a high tolerance to copper and a high ability to accumulate copper in their roots (Andreazza *et al.* 2013). Similarly, castor bean has been comprehensively evaluated as a multipurpose phytoremediation crop for phytostabilization and re-vegetation of waste disposed peri-urban contaminated soils (Boda *et al.* 2017). It was also noticed that transport from shoot to seed and oil components was not detected. These findings suggest that castor bean can be used to reclaim heavy metal-contaminated soils and also provide non-edible agricultural products.

Heavy metal pollution is becoming an adverse problem in many cities of Pakistan including Karachi (Karim *et al.* 2015), Faisalabad (Farid *et al.* 2015), Lahore (Khan *et al.* 2013), and Kasur (Afzal *et al.* 2014). Higher levels of Pb above the WHO permissible limits, are observed in soil, water and edible crops in many urban and peri-urban areas which may cause serious health issues in Pakistan. In a survey study, Afzal and coworkers (2014) determined the level of heavy metal contamination in groundwater and soil near the industrial area of Kasur. The survey results revealed that soil and groundwater in the study area are highly contaminated with all tested heavy metals specially chromium. Similarly, another study showed higher concentrations of Pb in drinking water, collected from Pearl valley of Azad Jammu Kashmir, that varies from 1.8-4.7 mg L^{-1} which is far above the permissible limit (0.01 mg L^{-1}) set by WHO (Ali *et al.* 2019). In case of soil, highest Pb concentration (1753 mg kg^{-1}) was detected under mining activities from Kohistan region, Gilgit Baltistan province (Muhammad *et al*. 2011). These findings indicate an alarming situation of heavy metals contamination of agricultural soils in the vicinities of industrial cities of Pakistan and demands urgent remedy for the removal of heavy metals from soils.

Various lab and greenhouse studies reported the potential of different grasses (Ullah *et al.* 2019), vegetables (Akhtar *et al.* 2020) and oilseed crops (Qureshi *et al.* 2020) for the phytoremediation of heavy metal contaminated soil. However, a mechanism based study exploring the genotypic responses of castor bean for various morpho-physiological indices, antioxidant activities and phytostabilization capabilities on Pb-contaminated soil is lacking. To harness the potential of castor genotypes, current study investigated the genotypic responses of five castor genotypes in terms of morpho-physiological characteristics and phytostabilization capabilities on a Pb-contaminated soil.

2. Materials and methods

2.1. Experimental outline and treatment plan

An open pot experiment was conducted to evaluate the growth, physiological and phytostabilization response of different castor genotypes on Pb contaminated soil under greenhouse conditions at the Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Pakistan with coordinates 31.4504 N and 73.1350 E. Seeds of five castor genotypes (NIAB Gold, NIAB Spineless, NIAB-2020, DS-30 and C-3) were collected from Plant Breeding and Genetics Division (PBGD), NIAB. Plastic pots (20 × 22.5 cm) were filled with thoroughly mixed, air-dried and precisely sieved soil (5 g) using 2 mm mesh. The well prepared soil was spiked with different concentrations of Pb (0, 100, 200, 400, and 800 mg kg^{-1} soil) prior to 3 months of sowing to equilibrate the metal in the soil. For this, above stated Pb concentrations were firstly calculated and then applied in solution form using lead nitrate of Sigma Aldrich (purity ≥ 99.0%). Soil without any treatment of Pb was considered as control. After the accomplishment of the required time period, five pre-soaked seeds for each genotype were sown in each pot with three replications following completely randomized design (CRD) with factorial settings. Plants were grown under natural environment with average temperatures of 30–35°C (night-day). The basal dose of fertilizer (NPK) was calculated and applied to each pot. Plants were watered two times in a week to maintain the soil moisture at field capacity level.

Table 1. Physico-chemical characteristics of the experimental soil

Sand (%)	Silt (%)	Clay (%)	Texture	pH	EC (dS m^{-1})	Total N (%)	Available P(mg kg^{-1})	O.M (%)	Total Pb (mg kg^{-1})
60	35.5	4.5	Sandy loam	7.65	2.05	0.081	5.9	0.99	N.D

O.M=Organic matter, N.D= Not detected

2.2. Physicochemical analysis of the experimental soil

Soil was collected in bulk from the experimental site of NIAB and categorized for different physicochemical analysis (Table 1). Bouyoucos hydrometer protocol was followed for the determination of soil texture (Lotfy and Mostafa 2014). Saturated paste was prepared to determine the pH of the soil using pH meter (ST-5000-OHAUS-USA) whereas soil extract was used to determine

EC using EC meter (a-AB33EC, OHAUS, USA). Total nitrogen (N) and available phosphorous (P) were analyzed following Kjeldahl (Bremner 1960) and Olsen (1954) method respectively. Organic matter was determined as described by Walkley and Black (1934). Available Pb in soil was determined by the method as described by Ullah *et al.* (2020).

2.3. Gas exchange parameters

Stomatal conductance (SC), transpiration (TR) and photosynthetic rate (PR) of castor plants were recorded through Decagon method (Toro *et al.* 2019) in the morning hours using a porometer (LI-Cor Inc. LI-1600 Steady State Porometer).

Table 2. Growth attributes of various castor genotypes influenced by different levels of Pb

Pb (mg kg^{-1}) soil	Genotypes	HT (cm)	SFW (g plant^{-1})	DBM (g plant^{-1})	RDW (g plant^{-1})
0	NIAB-G	39.33 c	27.77 b	2.23 c	0.50 d
	NIAB-SPN	39.66 c	31.11 a	2.44 b	0.57 c
	NIAB- 2020	46.66 b	32.31 a	2.69 a	0.69 a
	DS-30	52.33 a	32.34 a	2.67 a	0.66 b
	C-3	45.66 b	27.93 b	2.47 b	0.57 c
100	NIAB-G	38.00 cde	24.92 cd	1.93 e	0.42 f
	NIAB-SPN	38.33 cd	24.95 cd	2.09 d	0.48 e
	NIAB- 2020	39.33 c	27.19 bc	2.21 cd	0.49 e
	DS-30	38.66 cd	26.92 bc	2.19 cd	0.49 e
	C-3	38.33 cd	26.06 bc	2.14 cd	0.43 f
200	NIAB-G	31.66 fg	21.20 ef	1.78 f	0.37 j
	NIAB-SPN	33.33 f	21.60 e	1.53 g	0.39 i
	NIAB- 2020	37.00 de	22.66 de	1.92 e	0.41 g
	DS-30	36.00 e	22.31 e	1.93 e	0.41 gh
	C-3	33.66 f	21.30 e	1.52 g	0.40 hi
400	NIAB-G	30.00 ghi	15.93 g	1.31 hij	0.30 m
	NIAB-SPN	31.00 gh	16.04 g	1.35 hi	0.32 l
	NIAB- 2020	31.00 gh	20.58 ef	1.50 g	0.33 l
	DS-30	31.00 gh	20.94 ef	1.51 g	0.35 k
	C-3	30.33 gh	18.37 f	1.43 gh	0.32 l
800	NIAB-G	23.66 k	6.31 i	1.09 k	0.13 p
	NIAB-SPN	22.00 k	6.52 i	1.19 jk	0.19 o
	NIAB- 2020	29.33 hi	8.15 hi	1.31 hij	0.26 n
	DS-30	28.00 ij	9.23 h	1.29 ij	0.20 o
	C-3	26.00 j	7.39 hi	1.25 ij	0.19 o
	LSD	2.25	2.44	0.12	0.02

Data represent the mean of three replicates. Means within the same column having different letters are significantly different at $p \leq 0.05$ according to LSD. (Pb=lead); (NIAB-G= NIAB GOLD, NIAB-SPN=NIAB SPINELESS) and (Ht= Plant Height, SFW= Shoot fresh weight, DBM=Dry Biomass, RDW=Root dry weight)

2.4. Estimation of relative water content and electrolyte leakage

Relative water content (RWC) of the castor genotypes was determined using the method as described by Teulat *et al.* (2003). Randomly selected fresh leaves from top of the plants were collected. After measuring the fresh weight (FW), leaves were dipped in distilled water for about 24 hours in an incubator (FOC 2251, VELP, Italy). After the completion of required time period, leaves were dried on a filter paper and turgid weight (TW) was measured. Finally, samples were placed in an oven (Lenton, UK) at 70°C for about 24 hours to record the dry weight (DW) of the leaves. RWC was calculated by using the given formula:

$$RWC\ (\%) = FW - DW\ /\ TW - DW\ x\ 100$$

For the calculation of electrolyte leakage (EL), the leaves were cut into smooth discs and shifted to test tubes holding 25 mL of deionized water. Test tubes were vortex (VM3, CAT, Germany) gently and preliminary conductivity (EC_0) of the solution was noted. The tubes were kept in an incubator overnight at 4°C to assess EC_1. Lastly, to determine EC_2 the test tubes were placed in an autoclave (RAYPA, AES-75) for 20 minutes at 121°C. Electrolyte leakage of the castor genotypes was determined by the method as described by Yang *et al.* (1996).

$$EL\ (\%) = EC_1 - EC_0\ /\ EC_2 - EC_0 x\ 100$$

2.5. Plant growth parameters

Plants were harvested after 3 months and data regarding plant height (PH) and shoot fresh weight (SFW) were recorded. Roots were washed thoroughly with tap water using plastic mesh to remove the soil particles and then with deionized water. Shoot and root samples were oven dried at 70°C for 72 hours to record dry weight.

2.6. Determination of photosynthetic activity

Arnon (1949) protocol was opted to determine photosynthetic pigments in fresh leaves of castor genotypes. Approximately 0.2 g of leaf sample was chopped and placed in an incubator at 4°C for 24 hours with 80% acetone (v/v) for complete bleaching of leaves. The extract was centrifuged at 13000 rpm for about 10 minutes. Supernatant was collected for the determination of chlorophyll *a*, chlorophyll *b* and carotenoids at 663nm, 645 nm and 470 nm, respectively, using a spectrophotometer (VIS-1100, BMS, 204592, Canada).

Table 3. Influence of Pb contamination on physiological attributes of castor genotypes

Pb (mg kg^{-1}) soil	Genotypes	RWC (%)	EL (%)	SC (mmol m^{-2} s^{-1})	TR (mmol m^{-2} s^{-1})	PR (umol m^{-2} s^{-1})
0	NIAB-G	99.00 a-d	9.16 qr	11.36 c	2.55 ab	383.53 cd
	NIAB-SPN	99.40 abc	8.42 rs	12.40 b	2.52 abc	427.16 bc
	NIAB- 2020	100.00 a	6.67 s	12.87 b	2.63 a	454.50 ab
	DS-30	100.00 a	7.23 s	15.76 a	2.58 ab	485.89 a
	C-3	99.80 ab	10.81 pq	12.20 b	2.52 abc	346.52 de
100	NIAB-G	95.62 a-f	12.80 mno	10.64 cd	2.44 b-e	287.41 fgh
	NIAB-SPN	96.02 a-f	13.85 lmn	10.91 cd	2.45 b-e	304.20 efg
	NIAB- 2020	98.80 a-d	11.49 op	11.26 c	2.50 abc	332.52 e
	DS-30	97.93 a-e	12.47 nop	11.26 c	2.49 a-d	325.09 ef
	C-3	96.90 a-f	14.49 klm	10.99 cd	2.46 a-e	314.71 ef
200	NIAB-G	93.37 a-f	15.36 kl	9.38 fg	2.31 efg	242.76 hi
	NIAB-SPN	93.82 a-f	16.19 jk	9.39 fg	2.32 d-g	245.29 hi
	NIAB- 2020	94.25 a-f	14.75 kl	10.38 de	2.42 b-e	251.62 hi
	DS-30	95.01 a-f	14.49 klm	9.78 ef	2.42 b-e	268.39 ghi
	C-3	93.82 a-f	17.19 ij	9.62 efg	2.37 c-f	230.30 ij
400	NIAB-G	91.04 f	37.68 f	7.00 jk	2.06 hij	193.41 jkl
	NIAB-SPN	91.87 ef	24.54 g	7.68 ij	2.11 hi	183.64 klm
	NIAB- 2020	93.06 b-f	17.36 ij	8.85 gh	2.14 ghi	223.93 ijk
	DS-30	92.82 c-f	18.25 i	8.09 hi	2.20 fgh	194.73 jkl
	C-3	92.58 def	20.24 h	7.92 i	2.05 h-k	177.87 lm
800	NIAB-G	80.40 g	109.92 b	5.51 m	1.92 jkl	140.63 mn
	NIAB-SPN	78.69 g	223.59 a	6.09 lm	1.88 kl	157.45 lmn
	NIAB- 2020	90.79 f	81.85 e	6.65 kl	2.00 ijk	165.68 lm
	DS-30	82.26 g	102.36 d	6.80 kl	1.97 i-l	173.37 lm
	C-3	70.59 h	106.26 c	5.44 m	1.81 l	119.66 n
	LSD	6.76	1.81	0.81	0.18	44.91

Data represent the mean of three replicates. Means within the same column having different letters are significantly different at p ≤ 0.05 according to LSD. (Pb=lead); (NIAB-G= NIAB GOLD, NIAB-SPN=NIAB SPINELESS) and (RWC=Relative Water content, EL= Electrolyte Leakage, SC= Stomatal Conductance, TR= Transpiration rate, PR= Photosynthetic rate)

2.7. Determination of antioxidants enzymes

Antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) were determined spectrophotometrically. After five weeks of germination, fresh samples (0.2 g) of castor leaves were ground and homogenized in 0.05 M phosphate buffer solution (pH 7.8). The homogenized mixture was filtered and centrifuged at 12,000 rpm for 10 minutes at 4°C.

Superoxide dismutase activity was analyzed by following Giannopolitis and Ries (1977) protocol at 560 nm. The reaction solution (3.0 mL) consisted of Nitroblutetrazolium (NBT) (75 µM), EDTA-Na_2 (100 µM), methionine (130 µM), riboflavin (20 µM) and enzyme extract (100 µL). The reaction was started by placing the test tubes under fluorescent lamp for 20 minutes. Superoxide dismutase activity was determined by the photoreduction of NBT. Pütter (1974) procedure was opted to determine peroxidase (POD) activity. The reaction mixture (3.0 mL) consisted of enzyme extract (100 µL), guaiacol (100 µL 1.5%, v/v), H_2O_2 (100 µL 300 mM) and 2.7 mL 25 mM phosphate buffer (pH 7.0). The increase in absorbance due to the oxidation of guaiacol was measured spectrophotometrically at 470 nm.

Catalase (CAT) activity was determined by monitoring the reduction in the absorbance at 240 nm using the procedure as described by Aebi (1984). The assay mixture (3.0 mL) comprised of 100 µL enzyme extract, 100 µL H_2O_2 (300 mM), and 2.8 mL 50 mM phosphate buffer with 2 mM EDTA (pH 7.0). Ascorbate peroxidase (APX) activity was determined by the method as described by Nakano and Asada (1981). The reaction mixture consisted of 100 µL enzyme extract, 100 µL ascorbate (7.5 mM), 100 µL H_2O_2 (300 mM) and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The oxidation of ascorbate was observed at 290 nm using UV-VIS-Spectrophotometer (MetaSpec Pro, China).

2.8. Determination of total soluble proteins, total soluble sugars and total phenolic contents

Total soluble protein (TSP) and total soluble sugars (TSS) were analyzed spectrophotometrically by the procedure as described by Bradford (1976) and Riazi *et al.* (1985) procedure. Micro-colorimetric method was followed for the determination of total phenolic contents (TPC). The assay solution consisted of 100 µL leaf extract, 100 µL 10% (v/v) Folin-Ciocalteau reagent and 1.2 mL 700 mM Na_2CO_3. Absorbance for TPC was read at 765 nm on a spectrophotometer (Ainsworth and Gillespie 2007).

2.9. Lead concentration in shoot and root

Ground and oven-dried shoot and root samples were digested with nitric acid and perchloric acid (8:2 v/v) on a hot plate until the sample become colorless. After cooling, the digested samples were diluted to 50 mL with distilled water and filtered through Whatman No. 42 filter paper. The filtered extract was stored in plastic bottles. Reagent blanks were also included to ensure the precision and accuracy of the analysis. The metal analysis was carried out using atomic absorption spectroscopy (Analytikjena, contrAA 800D, Germany). Translocation factor (TF) and bio concentration factor (BCF) were also calculated (Ullah *et al.* 2020).

TF = Pb in aerial parts/Pb in roots

BCF shoot = Pb concentration in shoot/Pb in treated soil

BCF root = Pb concentration in root /Pb in treated soil

2.10. Statistical analysis

The data obtained were analyzed using analysis of variance and the means were compared by applying the least significance difference test $p \leq 0.05$ (Steel *et al.* 1980). Data represent the mean of three replicates. The statistical analysis was conducted using the statistical software Statistix 8.1. Mean data of all parameters in study were subjected to perform Principal Component Analysis (PCA).

Table 4. Photosynthetic pigments of castor genotypes influenced by different levels of Pb

Pb (mg kg^{-1}) soil	Genotypes	Chl a mg g^{-1}/FW	Chl b mg g^{-1}/FW	Total Chl mg g^{-1}/FW	Carotenoids mg g^{-1}/FW
0	NIAB-G	5.42 ab	4.65 ab	10.0 abc	4.15 b
	NIAB-SPN	5.40 ab	4.64 ab	10.0 abc	4.15 b
	NIAB- 2020	5.47 a	4.72 a	10.1 a	4.20 a
	DS-30	5.47 a	4.65 ab	10.1 ab	4.16 ab
	C-3	5.40 ab	4.63 ab	10.0 abc	4.14 b
100	NIAB-G	5.13 def	4.58 b	9.72 d	4.04 c
	NIAB-SPN	5.13 def	4.59 ab	9.71 d	4.04 c
	NIAB- 2020	5.23 bcd	4.62 ab	9.85 cd	4.05 c
	DS-30	5.35 abc	4.59 ab	9.94 bc	4.05 c
	C-3	5.19 cde	4.27 c	9.46 e	4.04 c
200	NIAB-G	4.98 f-j	4.24 c	9.23 fgh	4.01 c
	NIAB-SPN	5.02 e-i	4.23 c	9.24 fgh	4.02 c
	NIAB- 2020	5.11 d-g	4.26 c	9.37 ef	4.04 c
	DS-30	5.07 d-h	4.25 c	9.32 efg	4.03 c
	C-3	4.97 f-k	4.23 c	9.20 fgh	3.96 d
400	NIAB-G	4.89 h-l	4.16 c	9.05 h	3.71 f
	NIAB-SPN	4.86 i-l	3.88 d	8.74 i	3.90 e
	NIAB- 2020	4.92 g-k	4.19 c	9.11 gh	3.95 de
	DS-30	4.97 f-k	4.21 c	9.18 fgh	3.92 de
	C-3	4.87 i-l	3.82 de	8.69 ij	3.68 f
800	NIAB-G	4.66 m-p	3.76 def	8.42 klm	3.50 h
	NIAB-SPN	4.72 l-o	3.76 def	8.47 jkl	3.58 g
	NIAB- 2020	4.84 i-m	3.77 def	8.61 ijk	3.59 g
	DS-30	4.82 j-m	3.76 def	8.58 ijk	3.59 g
	C-3	4.78 k-n	3.75 def	8.54 i-l	3.58 g
	LSD	0.19	0.13	0.21	0.05

Data represent the mean of three replicates. Means within the same column having different letters are significantly different at p ≤ 0.05 according to LSD. (Pb=lead); (NIAB-G= NIAB GOLD, NIAB-SPN=NIAB SPINELESS) and (Chl a=Chlorophyll a, Chl b= Chlorophyll b, Total Chl= Total Chlorophyll).

3. Results

3.1. Effect of Pb contamination on plant growth attributes

The analysis of variance showed a significant influence of Pb treatments on the growth attributes of different castor bean genotypes. Plant height was significantly decreased ($p \leq 0.05$) with the increasing concentrations of Pb in the soil. At 800 mg Pb kg^{-1} soil, NIAB-2020 showed better performance against Pb with an average height of 29.3 cm followed by DS-30 (28.0 cm) as compared to other genotypes. Shoot fresh biomass (SFW), root dry weight (RDW) and shoot dry weight (SDW) were decreased due the presence of Pb. At 800 mg Pb kg^{-1} soil, the highest SFW up to 9.23 g plant^{-1} was observed in DS-30 followed by NIAB-2020 and C-3 i.e. 8.15 and 7.39 g plant^{-1} whereas maximum SDW and RDW i.e. 1.31 and 0.26 g plant^{-1} were recorded in NIAB-2020, respectively followed by DS-30 (Table 2).

3.2. Effect of Pb contamination of soil on physiological parameters

Table 3 illustrates the effect of Pb on the physiological parameters of different castor genotypes. The RWC of all genotypes were slightly affected with the increasing concentrations of Pb ($p \leq 0.05$). Comparing the varieties against each level of Pb, RWC showed a faint decreasing trend from 0 to 200 mg kg^{-1}. At 800 mg Pb kg^{-1} soil, NIAB-2020 showed maximum retention of water in leaves (90%) followed by DS-30 (82%), NIAB Gold (80%), NIAB spineless (78%) and C-3 (70%) due to the production of osmolytes. Similarly, EL was increased significantly with the increasing concentration of Pb in the soil ($p \leq 0.05$). At 800 mg Pb kg^{-1} soil, maximum EL was observed in NIAB spineless and NIAB Gold as compared to other genotypes. Observing the gas exchange parameters, SC, TR and PR were significantly reduced with the increasing concentrations of Pb ($p \leq 0.05$). A gradual decrease in SC due to 100 to 800 mg kg^{-1} Pb in soil disturbed the TR and PR in castor genotypes. At 800 mg kg^{-1}, significant reduction in PR was observed in C-3 and NIAB Gold i.e. 119.6 and 140.63 mmol m^{-2} s^{-1} respectively, while highest PR was recorded in DS-30 and NIAB-2020.

3.3. Effects of Pb on leaf pigment contents

The effect of Pb on the pigment content of the castor genotypes resulted in significant decreasing trend ($p \leq 0.05$) with increasing metal stress. Interestingly, NIAB-2020 exhibited highest total chlorophyll and carotenoid content up to 8.61 and 3.59 mg g^{-1} respectively, as compared to other genotypes at 800 mg kg^{-1} (Table 4).

Table 5. Influence of Pb contamination on the antioxidants activity of castor genotypes

Pb (mg kg^{-1}) soil	Genotypes	SOD (U/g FW)	POD (mM/g FW)	CAT (mM/g FW)	APX (mM/g FW)
0	NIAB-G	2196.7 cde	8.19 bc	3.86 i	30.73
	NIAB-SPN	2300.7 c	8.29 bc	1.86 j	31.53 j
	NIAB- 2020	3284.0 a	12.07 a	4.63 hi	38.10 i
	DS-30	2979.5 b	11.75 a	4.70 hi	38.50 i
	C-3	2251.1 cd	8.39 b	4.20 i	29.29 k
100	NIAB-G	1993.3 ef	6.41 ef	5.10 ghi	40.77 h
	NIAB-SPN	2045.8 de	5.97 fg	5.60 gh	40.53 h
	NIAB- 2020	2142.2 cde	7.32 d	5.70 gh	53.37 f
	DS-30	2078.3 cde	7.46 cd	5.83 fgh	53.40 f
	C-3	2032.5 de	7.01 de	5.10 ghi	42.26 g
200	NIAB-G	1583.8 g-j	5.39 ghi	7.03 ef	57.24 e
	NIAB-SPN	1780.4 fgh	5.66 fgh	8.20 de	59.12 d
	NIAB- 2020	1801.4 fg	5.82 fg	8.40 d	59.38 cd
	DS-30	1794.7 fg	5.78 fg	8.30 de	59.13 cd
	C-3	1663.0 ghi	5.30 g-j	6.30 fg	53.58 f
400	NIAB-G	1454.9 i-l	4.51 jk	8.80 cd	59.75 bcd
	NIAB-SPN	1548.4 ijk	4.58 ijk	8.40 d	59.64 bcd
	NIAB- 2020	1568.5 h-k	5.27 g-j	9.20 bcd	60.73 b
	DS-30	1582.8 g-j	5.14 g-j	9.30 bcd	60.70 b
	C-3	1520.8 ijk	4.90 hij	9.13 bcd	60.26 bc
800	NIAB-G	1059.6 n	3.03 m	9.80 bc	63.45 a
	NIAB-SPN	1278.3 lmn	2.17 n	9.96 bc	64.02 a
	NIAB- 2020	1388.1 j-m	3.92 kl	12.13 a	64.31 a
	DS-30	1357.5 klm	3.56 lm	12.23 a	64.43 a
	C-3	1182.8 mn	3.24 lm	10.33 b	63.45 a
	LSD	224.4	0.83	1.29	1.13

Data represent the mean of three replicates. Means within the same column having different letters are significantly different at p ≤ 0.05 according to LSD. (Pb=lead); (NIAB-G= NIAB GOLD, NIAB-SPN=NIAB SPINELESS) and (SOD= Superoxide dismutase, POD= Peroxidase, CAT= Catalase, APX= Ascorbate Peroxidase).

3.4. Effect of Pb on the biochemical profile of castor bean

Results revealed that in all genotypes, SOD and POD showed negative correlation whereas an increasing trend of CAT and APX were recorded with increasing concentrations of Pb ($p \leq 0.05$) as shown in Table 5. Interestingly, at 800 mg kg^{-1}, highest CAT and APX activities were recorded in DS-30 (12.23 and 64.43 mM/g FW) followed by NIAB-2020 (12.13 and 64.31 mM/g FW), respectively (Table 5). A vigorous increase in TSS and TPC from 200 to 800 mg kg^{-1} were observed in all castor genotypes. At 800 mg kg^{-1}, NIAB-2020, DS-30 and NIAB Spineless showed an equal production of TSS of 18.6 mg g^{-1}. With reference to TPC, NIAB-2020 produced two times more phenolic contents up to 0.49 mg g^{-1} as compared to other genotypes. Total soluble proteins showed negative relation to Pb concentrations. At 800 mg kg^{-1}, DS-30 produced 8.44 mg g^{-1} TSP followed by NIAB-2020, NIAB spineless, NIAB Gold and C-3 (Table 6).

3.5. Lead concentration in castor genotypes

Results have shown a significant increase in the concentration of Pb in plant tissues of all castor genotypes ($p \leq 0.05$). Elemental analysis have shown higher uptake of Pb in roots as compared to shoots. At 800 mg kg^{-1}, highest Pb uptake in the roots were recorded in NIAB-

2020 (302.0 mg kg^{-1}) followed by DS-30 (299.3 mg kg^{-1}). The highest translocation factor of 0.99 was observed in NIAB-2020. The bioconcentration factor of roots (BCFroot) increased with the increasing concentration of Pb as compared to bioconcentration factor of shoots (BCFshoot). At 800 mg kg^{-1}, NIAB-2020 showed the maximum value of 3.59 followed by DS-30 (3.29) (Table 7).

Table 6. Osmolytes production in castor genotypes influenced by Pb contamination

Pb (mg kg^{-1})	Genotypes	TSP (mg/g)	TSS (mg/g)	TPC (mg/g)
0	NIAB-G	11.33 b	13.99 e	0.14 k
	NIAB-SPN	11.60 ab	13.99 e	0.14 k
	NIAB- 2020	11.90 ab	14.01 e	0.15 k
	DS-30	12.10 a	14.09 de	0.15 k
	C-3	11.60 ab	14.01 e	0.14 k
100	NIAB-G	10.00 c	14.09 de	0.15 k
	NIAB-SPN	10.03 c	14.12 de	0.19 j
	NIAB- 2020	11.23 b	14.20 d	0.21 hi
	DS-30	11.25 b	14.20 d	0.20 ij
	C-3	11.23 b	14.12 de	0.19 j
200	NIAB-G	9.02 efg	18.42 c	0.21 ghi
	NIAB-SPN	9.04 efg	18.42 c	0.22 gh
	NIAB- 2020	9.94 cd	18.44 bc	0.23 g
	DS-30	9.66 cde	18.44 bc	0.23 g
	C-3	9.26 def	18.42 c	0.21 ghi
400	NIAB-G	8.48 ghi	18.44 bc	0.28 f
	NIAB-SPN	8.71 fgh	18.54 abc	0.30 ef
	NIAB- 2020	8.90 fgh	18.54 abc	0.31 de
	DS-30	9.01 efg	18.59 ab	0.31 de
	C-3	8.90 fgh	18.54 abc	0.31 de
800	NIAB-G	7.59 j	18.59 ab	0.32 d
	NIAB-SPN	7.83 ij	18.69 a	0.34 c
	NIAB- 2020	8.21 hij	18.69 a	0.49 a
	DS-30	8.44 ghi	18.69 a	0.38 b
	C-3	6.77 k	18.59 ab	0.34 c
	LSD	0.68	0.15	0.02

Data represent the mean of three replicates. Means within the same column having different letters are significantly different at p ≤ 0.05 according to LSD. (Pb=lead); (NIAB-G= NIAB GOLD, NIAB-SPN=NIAB SPINELESS) and (TSS= Total Soluble sugars, TSP=Total Soluble Proteins, TPC=Total Phenolic contents).

3.6. Principal component analysis

Principal component analysis was performed to analyze the visual comparison of castor genotypes, and to collect information about the inter-relationship between variables at different levels of Pb as shown in Figure 1. Morpho-physiological parameters, pigment content, antioxidant activities, osmolytes, and Pb uptake were investigated using PCA. Biplot showed that among 8 principal components, 4 have Eigenvalues >1, while remaining components have not been discussed due to Eigenvalues <1.

At 0 mg kg^{-1}, EL showed strong association with NIAB Gold, NIAB Spineless and C-3 whereas RDW, TF, TSS, CAT, DBM, RWC, FBM, Pb in root and shoot, chlorophyll *a*, total chlorophyll and bioconcentration factor in roots were linked to DS-30. Ascorbate peroxidase, SC, PR, SOD, TPC, and chlorophyll *b* co-occurred with NIAB-2020 (Figure 1-a). It have been observed that with the increase in Pb contamination in soil, major agronomic, physiological and biochemical parameters were strongly connected to DS-30 and NIAB-2020 as compared to NIAB Gold, NIAB Spineless and C-3. At 100 and 200 mg kg^{-1}, CAT, TPC, RWC, TSS, APX, TSP, SOD and POD along with other parameters aids in the survival of DS-30 and NIAB-2020 under Pb stress (Figure 1-b, c). Similar responses were observed at 400 and 800 mg kg^{-1} in DS-30 and NIAB-2020 (Figure 1-d, e).

4. Discussion

Lead exposure have a variety of negative effects on plant growth including physiological disruptions, decreased biomass production, membrane and structural damage and reduced pigment contents. The extent of these effects depends on the type of plant and the concentration of Pb in soil.

Plant height was decreased with the increasing concentrations of Pb. Similar trend was reported in *Helianthus annuus* (Saleem *et al.* 2018). This suggests that Pb interfered more strongly with the plant metabolic processes over time. It has been observed that shoot fresh and dry biomass declined in *Parthenium hysterophorus* (Javaid *et al.* 2023) due to Pb contamination. Comparable outcomes in terms of shoot fresh and dry biomass in the present study under higher level of Pb contamination were recorded in castor bean. Likewise, a declining trend was observed in RDW with increasing levels of Pb contamination. Similar effects of Pb contamination on RDW have also been reported in *Sesamum indicum* (Naveed *et al.* 2023). A possible explanation for poor root growth is decrease in calcium levels in the root tips which leads to a decline in either cell division or cell elongation processes (Duan *et al.* 2020).

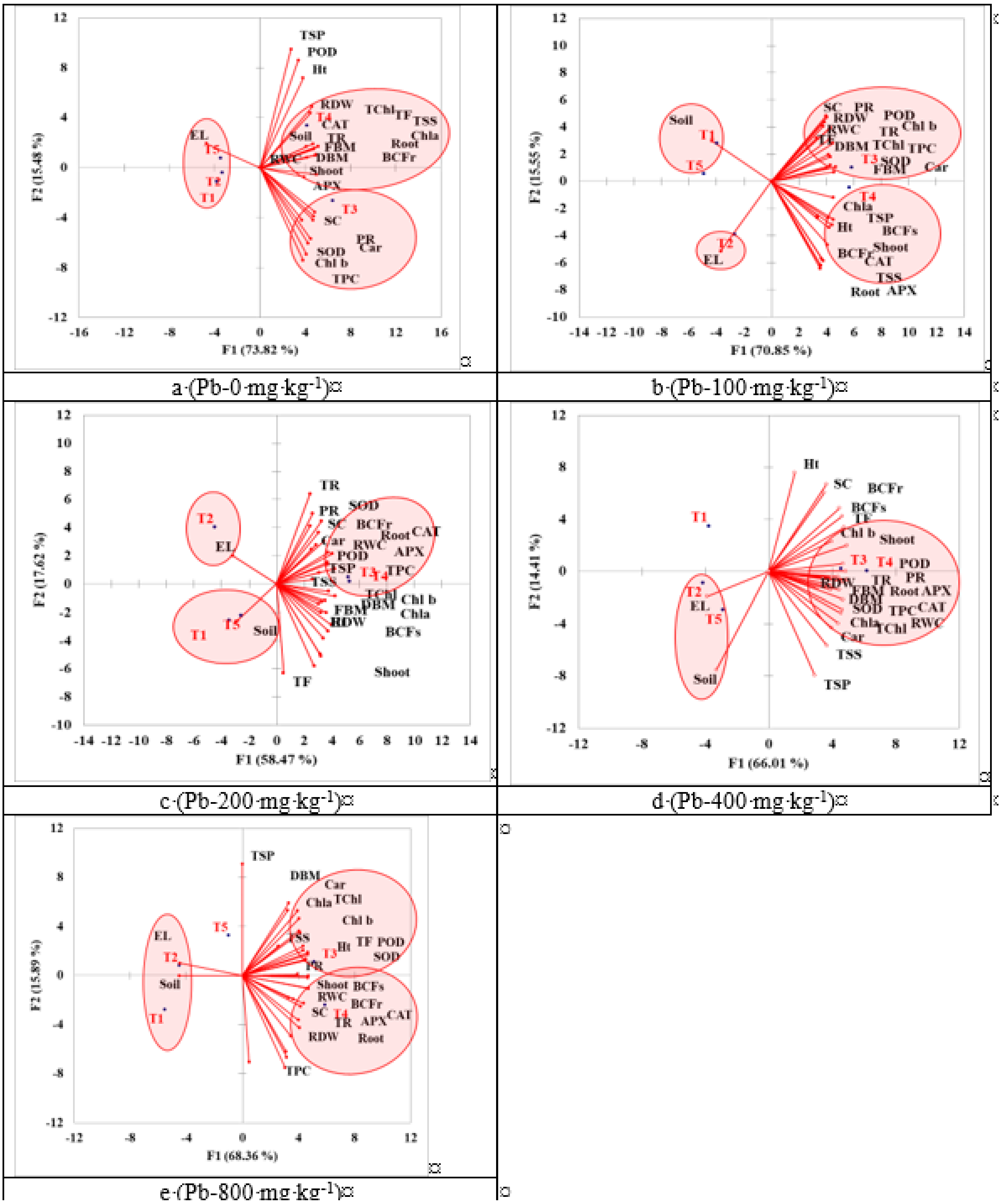


Figure 1. Principal component analysis for studied traits of castor genotypes at various levels of Pb

Relative water content is an important parameter in water relation studies, as it allows the calculation of the osmotic potential at full turgor (Siddique and Helen 2020). Upon exposure to Pb stress, relative water content was decreased with the increasing Pb concentrations. Opposing, EL was increased with the increasing Pb concentrations in soil. Our findings are consistent with the previous studies which reported that exposure to Ni and Pb can increase electrolyte leakage in *Coronopus didymus* (Sidhu *et al.* 2017). Such disorders in cell water relation may result from changes in cytoplasmic membranes induced even by very low concentrations of metal ions where the biological functions of cell membranes may be disabled as a result of a changed permeability to water and ions. Observing the gas exchange attributes, SC, TR and PR were declined with the increasing concentrations of Pb in soil as reported in *Corchorus capsularis* (Saleem *et al.* 2020). Exposure to Pb can have a significant impact on the amount and type of pigments synthesis in plants. The concentrations of chlorophyll and carotenoids were reduced as Pb disrupted the structure and function of enzymes involved in chlorophyll synthesis and directly interact with the components of photosystem II (PSII) (Rai *et al.* 2016).

Table 7. Phytoremediation characteristics of castor genotypes at different levels of Pb

Pb (mg kg^{-1})	Genotypes	Shoot (mg kg^{-1})	Root (mg kg^{-1})	TF	BCFshoots	BCFroots
0	NIAB-G	N/D	N/D	N/D	N/D	N/D
	NIAB-SPN	N/D	N/D	N/D	N/D	N/D
	NIAB- 2020	N/D	N/D	N/D	N/D	N/D
	DS-30	N/D	N/D	N/D	N/D	N/D
	C-3	N/D	N/D	N/D	N/D	N/D
100	NIAB-G	28.67 o	43.67 o	0.63 m	0.93 o	1.38 l
	NIAB-SPN	29.83 o	50.33 n	0.64 lm	0.94 o	1.71 k
	NIAB- 2020	33.67 n	51.50 n	0.65 klm	1.20 mn	1.81 k
	DS-30	37.00 m	50.50 n	0.66 kl	1.30 m	1.80 k
	C-3	32.00 n	43.67 o	0.64 klm	1.09 n	1.41 l
200	NIAB-G	81.00 j	90.33 m	0.68 i	1.86 k	2.45 j
	NIAB-SPN	78.33 k	99.67 kl	0.68 ij	1.75 kl	2.64 hi
	NIAB- 2020	83.67 i	103.00 j	0.71 gh	2.07 ij	2.70 gh
	DS-30	82.00 ij	100.67 k	0.69 hi	2.05 j	2.66 h
	C-3	71.00 l	98.00 l	0.66 jk	1.70 l	2.52 ij
400	NIAB-G	122.67 h	190.00 hi	0.72 g	2.12 ij	2.87 ef
	NIAB-SPN	123.00 h	189.00 i	0.78 f	2.18 hi	2.82 fg
	NIAB- 2020	135.00 f	196.00 g	0.81 e	2.39 fg	2.92 ef
	DS-30	139.00 e	199.00 f	0.86 d	2.50 ef	2.96 de
	C-3	125.67 g	191.67 h	0.81e	2.29 gh	2.88 ef
800	NIAB-G	252.00 d	288.00 e	0.86 d	2.58 de	2.98 de
	NIAB-SPN	253.00 d	290.00 d	0.89 c	2.68 cd	3.05 cd
	NIAB- 2020	299.23 a	302.00 a	0.99 a	2.92 a	3.59 a
	DS-30	289.17 b	299.33 b	0.96 b	2.89 ab	3.29 b
	C-3	272.80 c	293.00 c	0.94 b	2.78 bc	3.16 c
	LSD	2.06	1.71	0.02	0.12	0.12

Data represent the mean of three replicates. Means within the same column having different letters are significantly different at $p \leq 0.05$ according to LSD. (Pb=lead); (NIAB-G= NIAB GOLD, NIAB-SPN=NIAB SPINELESS) and (N/D= Not Detected, TF=Translocation factor, BCF=Bio concentration factor).

Lead toxicity can cause oxidative stress in plants by triggering the production of reactive oxygen species (ROS) such as hydrogen peroxide and malondialdehyde (Rahim *et al.* 2022). Superoxide dismutase is especially important in providing first-line resistance to the toxic impact of ROS. In general, antioxidant activity is promoted under lower levels of heavy metal stress, but it decreases at higher levels of stress. In this study, SOD and POD was decreased with the increasing concentrations of Pb. The decline in SOD and POD in our study may be assigned to Pb stress that damaged the antioxidant enzymatic systems. Conversely, CAT and APX were increased upon exposure to Pb stress. For example, in *E. argyi,* a concentration-dependent increase in CAT activity was observed in response to Pb toxicity. This suggests that CAT activity may be one of the mechanisms by which castor bean plants detoxify Pb. The increase in CAT activity can be explained by an increase in its substrate (H_2O_2) and could be an adaptive mechanism of the plant to balance H_2O_2 level (Khan *et al.* 2018).

The production of TSP in castor bean has been decreased under Pb stress. Previously, under Pb stress *Brassica rapa* have shown a decline in concentration of TSP (Ahmed *et al.* 2023). It was found that phenols in *Ficus nitida* can prevent oxidative damage by scavenging active oxygen species and breaking the radical chain reaction during lipid peroxidation. Our results have shown that TPC have been increased under Pb stress. Likewise, concentration of TSS in plants increased markedly from 200-800 mg kg^{-1}. This is a possible mechanism that plants use to alleviate the negative effects of Pb stress by maintaining the stability of the membrane (Aldoobie and Beltagi 2013).

Castor bean is well known for its phytostabilizing efficiency to uptake various heavy metals from soil. Results showed that accumulation of Pb was higher in roots of castor as compared to shoots. The plant extensive root system aids in binding and immobilizing Pb ions, thereby preventing the leaching into groundwater and minimizing the risk of entry into the food chain (Wani et al. 2023). Moreover, the secretion of various organic compounds by the plant's roots fosters the formation of stable complexes with Pb ions contributing to the long-term stabilization of contaminated soils. Phytostabilization offers a relative low concentration of Pb in the soil owing to root uptake and immobilization by root exudates. Castor bean, due to its phytostabilization capabilities, provides a safe ground for cultivation of edible crops, thus could be used as a rotation crop to get dual benefits of food and non-food industrial uses from heavy metal contaminated soils (Khan et al. 2023; Liu et al. 2023). These findings are consistent with previous reports, which showed that roots are able to accumulate higher concentrations of heavy metals than shoots due to decreased translocation of Pb. The accumulation of Pb is known to increase in a concentration-dependent manner, but its translocation from root to shoot is low in plants such as Brachiaria mutica (Khan et al. 2018). Hence, castor bean could be a promising tool for the phytostabilization/immobilization of heavy metals in contaminated soils. This can help certify the security of agricultural products, such as fruits and vegetables which are an important part of the human food. A relative low concentration of Pb was also recorded in shoot of castor plants due to translocation from root and caused a net dilution in metal concentration of soil. The management of contaminated biomass is really a potential risk for the environment (Khan et al. 2021). However, from other perspective, it can be converted into value added products through processing, for instance, the extraction and recovery of metalloids and metals from heavy metals contaminated biomass is one of the most attractive uses (Simonnot et al. 2018; Tisserand et al. 2021). Other beneficial products that can be obtained include biochar, compost, solid composites, fragrant products, and plant based fabric and fiber production (Khan et al. 2023).

5. Conclusion

Lead toxicity negatively impacts several key factors in castor bean including growth, physiological processes, pigment content and certain osmolytes. This occurs due to increased levels of ROS and reduced antioxidant capacity. Notably, Pb accumulation was significantly higher in roots as compared to the shoots of all tested genotypes, however, NIAB-2020 and DS-30 exhibited the best potential for Pb accumulation and immobilization by the roots when compared with NIAB Gold, NIAB Spineless and C-3. In conclusion, current findings suggested NIAB-2020 a suitable genotype for phytoremediation/ phytostabilization of Pb contaminated soils.

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Statements and Declarations

Declarations

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

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