Novel role of polyethylene glycol to alleviate lead stress in *Raphanus sativus* (#102808)

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Novel role of polyethylene glycol to alleviate lead stress in *Raphanus sativus*

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Abstract The continuous heavy metals (HMs) contamination in our ecosystem due to industrialization, urbanization, and other anthropogenic activities has become a serious environmental constraint to successful crop production. Lead (Pb) toxicity causes ionic, oxidative, and osmotic injuries which induce various morphological, physiological, metabolic, and molecular abnormalities in plants. Polyethylene glycol (PEG) is widely used to elucidate drought stress induction and alleviation mechanisms in treated plants. Some recent studies have unveiled the potential of PEG in regulating plant growth and developmental procedures including seed germination, root and shoot growth, and alleviating the detrimental impacts of abiotic stresses in plants. Therefore, the current study aimed to assess the effects of seed priming with various concentrations (10%, 20%, 30%, and 40%) of PEG on the growth and development of radish plants growing under Pb stress (75 mg/kg soil). Pb toxicity reduced root growth (32.89%), shoot growth (32.81%), total chlorophyll (56.25%), and protein content (58.66%) in treated plants. Similarly, plants showed reduced biomass production of root (35.48%) and shoot (31.25%) under Pb stress, while 30% PEG seed priming enhanced biomass production of root (28.57%) and shoot (35.29%) under Pb contaminated regimes. On the other hand, seedlings obtained from 30% PEG priming demonstrated a notable augmentation in the concentrations of photosynthetic pigments, antioxidative activity, and biomass accumulation of the plants. PEG-treated plants showed modulations in the enzymatic activities of peroxidase (PO), catalase (CAT), and superoxide dismutase (SOD). These changes collectively played a role in mitigating the adverse effects of Pb on plant physiology. Our data revealed that PEG interceded stress extenuation encompasses numerous regulatory mechanisms including scavenging of ROS through antioxidant and non-antioxidants, improved photosynthetic

activity, and appropriate nutrition. Hence, it becomes necessary to elucidate the beneficial role of PEG in developing approaches for improving plant growth and stress tolerance.

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12   **Abstract**

13   The continuous heavy metals (HMs) contamination in our ecosystem due to industrialization,
14   urbanization, and other anthropogenic activities has become a serious environmental constraint
15   to successful crop production. Lead (Pb) toxicity causes ionic, oxidative, and osmotic injuries
16   which induce various morphological, physiological, metabolic, and molecular abnormalities in
17   plants. Polyethylene glycol (PEG) is widely used to elucidate drought stress induction and
18   alleviation mechanisms in treated plants. Some recent studies have unveiled the potential of PEG
19   in regulating plant growth and developmental procedures including seed germination, root and
20   shoot growth, and alleviating the detrimental impacts of abiotic stresses in plants. Therefore, the
21   current study aimed to assess the effects of seed priming with various concentrations (10%, 20%,
22   30%, and 40%) of PEG on the growth and development of radish plants growing under Pb stress
23   (75 mg/kg soil). Pb toxicity reduced root growth (32.89%), shoot growth (32.81%), total
24   chlorophyll (56.25%), and protein content (58.66%) in treated plants. Similarly, plants showed
25   reduced biomass production of root (35.48%) and shoot (31.25%) under Pb stress, while 30%
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30   modulations in the enzymatic activities of peroxidase (PO), catalase (CAT), and superoxide
31   dismutase (SOD). These changes collectively played a role in mitigating the adverse effects of
32   Pb on plant physiology. Our data revealed that PEG interceded stress extenuation encompasses
33   numerous regulatory mechanisms including scavenging of ROS through antioxidant and non-

34 antioxidants, improved photosynthetic activity, and appropriate nutrition. Hence, it becomes

35 necessary to elucidate the beneficial role of PEG in developing approaches for improving plant

36 growth and stress tolerance.

37 **Keywords:** Antioxidant, Plant Growth, Pb, PEG, radish

38 **Introduction**

39 Heavy metals (HMs) are metallic elements counting lead (Pb), arsenic (As), copper (Cu),

40 mercury (Hg), nickel (Ni), cadmium (Cd), chromium (Cr) having more than 5 gm cm³ density

41 (Ataie et al., 2022). Heavy metal contamination and subsequent effects of metal toxicity have

42 become a crucial factor in deteriorating our ecosystem. Trace quantities of HMs naturally exist in

43 the rhizospheric area. However, anthropogenic activity, rapid industrialization and unwise

44 application of synthetic agrochemicals have enhanced HMs concentration resulting in

45 environmental pollution (Wang et al., 2022). Metal pollution may cause negative effects on the

46 growth and development of microbes, animals, and plants.

47 Lead (Pb) is widely distributed in soils worldwide and is recognized as one of the world's most

48 hazardous metals due to its toxicity. This toxic metal severely affects cellular growth, metabolic

49 activity, and physiological progressions of plants, animals, and microbes. Seeds sown in Pb-

50 contaminated regimes exhibit poor germination percentage leading to poor crop stand and yield

51 (Erdoğan and Demirhan, 2022). Moreover, Pb toxicity triggers calcium (Ca) related mechanisms

52 thus reducing iron (Fe) interceded procedures which in turn declines seed germination, stress

53 tolerance, and biomass production (Chourasia et al., 2021). Plants affected by Pb show poor

54 photosynthetic activity and higher dilapidation of membranous polyunsaturated fatty acyl

55 residues leading to lipid peroxidation. Similarly, this toxic metal impedes the uptake and

56 translocation of essential plant nutrients and water, reduces stomatal activity, declines the

57 volume of guard cells, and induces oxidative injuries through the overproduction of reactive

58 oxygen species (ROS). Lead decreases biosynthesis and activity of catalase. However, it may

59 enhance the activity of peroxidase to scavenge ROS in some crop plants (Guo et al., 2020).

60 Plants rely on their enzymatic and non-enzymatic antioxidative machinery to alleviate stress.

61 Seed pretreatment with some chemicals called seed priming is an economical and easy approach

62 to improve plant growth and environmental stress tolerance (Bose et al. 2018, Kumar et al. 2021)

63 Seedlings raised from primed seeds mitigate stress by maintaining physiochemical, metabolic,

64 and molecular activities (Zanganch et al. 2018; Taie et al. 2019; Li Y et al. 2021). Hydropriming

65   is a common pre-sowing seed treatment that improves seed germination and seedlings' vigor

66   (Xasir et al., 2022). Priming upregulates the expression level of stress-associated genes which

67   empower nutrient translocation, cytoskeletal adjustment, cellular division, RNA/DNA synthesis,

68   and frequent germination of metal stress tolerant seedlings (Rajjou et al. 2012; Bewley et al.

69   2013; Manonmani et al. 2014; Li et al. 2021). Higher activity of protein and starch hydrolyzing

70   enzymes such as amylase and protease in the case of primed seeds assists in fast germination by

71   improving embryo nutrition (Varier et al. 2010; Di Girolamo and Barbanti 2012; Miransari and

72   Smith 2014). In the same way, priming activates the synthesis and bioactivity of antioxidative

73   enzymes. Seed priming also augments the biosynthesis of osmolytes and membranous integrity

74   (Banerjee and Roychoudhury 2018) The higher antioxidant activity in seedlings of primed seeds

75   mitigates stress and improves plant growth under metal-spiked circumstances (Chen et al. 2021;

76   Kaushik et al. 2022). Furthermore, stress tolerance induction through priming agents acts as a

77   "priming memory" which re-energizes the plant tolerance mechanism under adverse conditions

78   (Chen and Arora 2013; Srivastava et al. 2021). Besides, numerous chemical priming agents

79   including mannitol, KNO_3, KCl, $CaCl_2$, PEG, K_3PO_4, and, NaCl have shown enormous potential

80   as an osmotic agent (Dadach et al., 2022). Several scientists have reported the beneficial effects

81   of PEG as an osmopriming agent. This inert priming agent has a larger molecular size and is not

82   toxic to the proteins and developing embryos. Stress mitigation by PEG seed priming has a

83   trivial advantage over other stress-alleviating strategies attributable to its easy availability,

84   ecofriendly nature, and requirement in minute quantity to assuage stress tolerance and improve

85   plant growth. It was hypothesized that priming seeds with PEG may improve plant stress

86   tolerance (Bagavathiannan, 2021; Andrzejak, 2023).

87   Radish (*Raphanus raphanistrum subsp. sativus*) is an annual or occasionally perennial dicot

88   belonging to the family Brassicaceae. It is an economical, ancient, universal kitchen garden

89   vegetable (Luo et al., 2020). Bioactive constituents originating from diverse plant components

90   and extractions of radish demonstrate pharmacological attributes, showcasing efficacy in

91   addressing a spectrum of health concerns encompassing cardiovascular disorders and microbial

92   infections (Li, 2012; Manivannan et al., 2019; Shirasawa et al., 2020). Radish plants exhibit

93   vulnerability to metal-induced stress and display susceptibility to the higher concentrations of Cd

94   and, Pb (Verma et al., 2021; Galhaut et al., 2014). The application of PEG assists plants in

95   mitigating stress by regulating metabolic and physiological activities of plants (Uddin et al.,

96 2021), improving root and shoot growth (Lei et al., 2021), and modulating the antioxidative

97 activity of enzymes (Marimuthu et al., 2018). The study regarding PEG application for plant

98 stress tolerance is still in its early stages. Experiments for standardization of suitable PEG

99 concentrations for stress alleviation through seed priming have not been evaluated for several

100 crops. PEG priming under stress conditions augmented chlorophyll levels, heightened

101 antioxidative performance, enhanced root and foliage development, and intensified expression of

102 stress-responsive genes in paddy plants (Marimuthu et al., 2018). Yet, the beneficial effects of

103 PEG seed priming on physiochemical activities and growth of Pb-stressed radish remain

104 unknown. Consequently, it becomes mandatory to comprehend the potential of PEG in plant

105 stress alleviation to enhance plant growth and productivity.

106 Therefore, the core objective of this research work was to examine the influences of Pb stress on

107 morpho-physiological features of radish and divulge the probable mechanisms related to the

108 PEG-interceded Pb stress alleviation in radish.

109 **Materials and methods**

110 **Seed priming and growth conditions**

111 The seeds of radish (Variety: Mino Early) were obtained from RSC (Roshan Seed Corporation)

112 Lahore, Pakistan. Firstly, the seeds were surface sterilized with 1% sodium hypochlorite for 10

113 minutes and air dried in an ambient environment after washing with distilled water thrice. Lead

114 acetate (Pb $(C_2H_3O_2)_2$ as a source of Pb and polyethylene glycol (PEG) were purchased from

115 Sigma-Aldrich (USA). The seeds were primed in varying concentrations of PEG solutions in

116 four beakers in which 100ml of 10%, 20%, 30%, and 40% PEG were taken and 30 seeds in each

117 beaker were placed for 16 hours, under low light exposure at 25° C. After priming seeds were

118 washed thrice with distilled water and dried under shade for 12 hours (Shatpathy et al., 2018).

119 Non-primed seeds were considered as control. A pot experiment was performed in a wire-house,

120 Botanical Garden, Institute of Botany, Quid-e-Azam campus, University of the Punjab, Lahore

121 (N 31° 30' 4.3236", E 74° 18' 5.4684"). During this study, there was 18 ± 3 °C/12 ± 2 °C

122 (day/night), 56–68% relative humidity, 11 h photoperiod. Loamy soil obtained from 0-12″ depth

123 of Botanical Garden, University of the Punjab composed of 139, 510, 284, and 20 g/ kg sand,

124 clay, silt, and organic matter, respectively, at 6.8 pH was used for the current study. Moisture

125 concentration of soil at field capacity was 72% and its electrical conductivity was 0.19 dS m^{-1}.

126 While, it had 9.6 mg P, 2.39 g Si, 2.87 mg Ca, 1.65 mg K, 5.07 g N, and 5.37 mg Mg in kg^{-1}.

127 Soil was contaminated with 75mg/kg Pb and kept for 15 days before sowing of radish seeds.

128 Afterward, 2 Kg of Pb-spiked-soil and Pb-free soil were filled in 10″ plastic pots. Then five

129 radish non-primed and primed seeds were sown in the amended soil and each pot was

130 supplemented with 100mL 50% Hoagland's solution on each seventh day. Thinning was

131 performed after 15 days and three seedlings were allowed to grow further.

132 **Germination test**

133 Over 10 10-day period, the primed seeds underwent germination defined by the emergence of a 2

134 mm long radical following the criteria outlined by Kava et al. (2006). The germination process

135 was meticulously monitored with observations recorded at 24-hour intervals. Each treatment

136 underwent 3 replications to ensure precision in statistical analysis. The 20-day-old seedlings

137 were systematically examined to assess various growth and germination characteristics,

138 including overall germination percentage and seedling vigor index. Furthermore, an extensive

139 range of parameters about growth, nutritional content, and physiological traits underwent

140 comprehensive analysis, conducted 90 days following the germination initiation. This approach

141 was employed to ensure a thorough evaluation of the seed's developmental progression.

142 The germination assessment procedures outlined in the authoritative manual of the Seed Analysis

143 Association (1984) were implemented to ascertain the number of viable seeds that successfully

144 underwent germination. The quantification of germinated seeds was executed on the 9th day

145 following seed deployment, and the germination percentage was determined using the

146 established conventional methodology (Shakirova et al., 2003) as follows:

147
$$Germination\ Percentage\ (GP) = \frac{N1}{N2} \times 100$$

148 Here, N1 is the number of germinated seeds, while N2 is the total number of seeds sown.

149 **Seedling vigor index (SV1)**

150 The computation of the SVI was executed by employing the formula specified by AOSA in 1990

151 as delineated below:

152
$$SVI = (Root\ length + Shoot\ length) \times GP$$

153 **Assessment of morphological parameters**

154 Following 90 days of germination, morphological parameters including pod and seed counts,

155 plant shoot length, and plant root length from 3 randomly selected plants of each treatment were

156 evaluated.

157 **Determination of fresh and dry weight of plants**

158 The fresh shoot weight of three randomly selected seedlings (60 days following germination)

159 from each treatment was analyzed by electric weighing balance. Before the weighing process,

160 any surplus moisture present on the surfaces of the plants was eliminated using tissue paper. The

161 mean values were presented as the total weight of plans.t in its fresh state. Subsequently, the

162 plants were subjected to a controlled drying process within a thermal chamber set at 60°C for 48

163 hours. Once the drying process reaches a point where there is no discernible alteration in the

164 weight of the plants, the dry weight is duly documented and recorded. Subsequently, the average

165 value for all plant specimens was then computed.

166 **Estimation of gas exchange parameters**

167 IRGA (Li-COR Inc: Biosci., USA) was used to examine gas exchange properties such as net

168 photosynthesis rate, transpiration rate, and stomatal conductivity, from fully stretched leaves at

169 10 a.m.

170 **Determination of chlorophyll and carotenoid pigments**

171 Using a spectrophotometer set to measure at 440, 465, and 663 nm, the amounts of chlorophyll a,

172 chlorophyll b, and total chlorophyll were determined according to Khaleghi et al. (2012). Fresh

173 plant leaves were crushed and mixed with 80% acetone. The test tube extract was then

174 transferred to the cuvette. Carotenoid content was estimated by using the Lichtenthaler-Wellburn

175 (1983) approach as follows:

$$Total\ Chlorophyll\left(\frac{mg}{g-1\ freshweight}\right) = \frac{V}{100} \times W$$

176

177 V= volume of sample and w= weight of sample

178 **Evaluation of plant mineral status**

179 The plant samples (400 mg) were subjected to digestion using Aqua regia at a 3: 1: 1 ratio

180 (HNO_3: $HCLO_4$: H_2SO_4, V/V). After cooling, the digested components were filtered through

181 filter paper, and a final volume of 100 mL was achieved by adding distilled water. The

182 quantification of mineral content in plant samples was determined using atomic absorption

183 spectroscopy (Nitrogen) and a flame photometer (phosphorus, potassium) (Allen et al., 1976).

184 **Estimation of proline**

185 The extraction and evaluation of free proline in radish plants were conducted following the acid

186 ninhydrin method described by Bates et al. (1973). For this purpose, 100 mg frozen leaves were

187   homogenized with 2 mL of sulphosalicylic acid. The samples were agitated at a rotational speed

188   of 750 revolutions per minute (rpm) for 30 minutes. A 0.8 mL volume of supernatant was mixed

189   with an equal volume of acid-ninhydrin reagent. The resultant mixture underwent vigorous

190   agitation and was subsequently allowed to equilibrate at ambient temperature for 25 minutes,

191   during which phase separation occurred. The absorbance of toluene was measured at a

192   wavelength of 520 nm with pure toluene serving as the reference.

193   **Assessment of accumulation coefficient and metal tolerance index**

194   Aqua regia an acidic mixture was used to decompose the desiccated plant material. The resulting

195   mixture was filtered and diluted with distilled H_2O to reach a volume of 100 mL. The

196   concentration of Pb in both root and shoot tissues was quantified using an atomic absorption

197   spectrophotometer (Allen et al.,1976). The accumulation Coefficient was calculated using the

198   following formula proposed by Al Farraj et al. (2010):

199   $$Accumulation\ coefficient = \frac{Concenteration\ (Shoot\ or\ Root)}{Concenteration\ of\ soil}$$

200   To calculate the Metal Tolerance Index (MTI), the following equation used by Balin et al. (2007)

201   was used:

202   $$\%\ MTI = \frac{Dry\ weight\ of\ treated\ plants}{Dry\ weight\ of\ untreated\ plants} \times 100$$

203   **Estimation of total soluble proteins and antioxidant enzymes**

204   The present study employed cryogenic grinding with liquid nitrogen to process fresh plant

205   material, thereby enabling subsequent analysis of protein content and enzymatic activities. After

206   cryogenic grinding, the resulting finely ground plant material was immersed into a 3 mL

207   phosphate buffer solution. Subsequently, the sample underwent centrifugation at 14000 rpm for

208   30 minutes at 4°C. The resultant supernatant from the centrifuged mixture was meticulously

209   collected, and subsequently stored at 0°C. This strategic storage condition was implemented to

210   facilitate the subsequent quantitative assessment of protein content, as well as enzymatic

211   activities of POD, CAT, and SOD.

212   The Biuret technique developed by Racusen and Johnstone (1961) was employed to estimate

213   soluble protein concentration. To initiate the reaction, 2 mL of Biuret reagent was mixed with 2

214   mL of the supernatant. Subsequently, the optical density at 545 nm was measured using a

215   spectrophotometer. The quantification of protein concentration was achieved by referencing a

216   standard curve consisting of pre-established protein values as follows:

217
$$Protein\ contents\left(\frac{mg}{g}\right) = \frac{Curve\ value \times Total\ extract}{Extract\ used\ \times Weight\ of\ tissue\ used} \times 1000$$

218   The approach established by Luck (1974) was used to evaluate POD activity. The experimental

219   setup involved a buffer solution, guaiacol, enzyme extract, and hydrogen peroxide solution. The

220   quantification of POD was achieved by ascertaining the duration necessary to attain a 0.1-unit

221   increment in absorbance at 240nm.

222
$$Peroxidase\ activity\left(\frac{mg}{g}\right) = \frac{Absorbance \times df}{Weight\ of\ tissue\ used \times EU} \times 1000$$

223   df= dilution factor EU=extraction unit

224   The CAT activity assessment was conducted using the Beers and Sizer (1952) technique, in the

225   presence of two buffer solutions. For this purpose, a reaction mixture comprising 2.9 mL of

226   buffer B and 0.2 mL of enzyme extract was used. In contrast, the control group contained just 3

227   mL of buffer A. The duration required for the reduction of absorbance (at a wavelength of 240

228   nm) from 0.45 to 0.40 was utilized for CAT activity evaluation. This activity was subsequently

229   quantified as units per mL of the enzyme. This method ensures a rigorous and standardized

230   approach, contributing to the robustness and reliability of the scientific investigation.

231
$$Catalase\ activity\left(\frac{units}{mL\ enzyme}\right) = \frac{3.45 \times df}{Min \times 0.1}$$

232   Maral et al. (1977) conducted a study wherein they employed spectrophotometric analysis to

233   measure the activity of SOD. This was achieved by evaluating its capacity to hinder the

234   photochemical reduction of nitroblue tetrazolium. For this purpose, two test tubes containing a

235   mixture of sodium cyanide, NBT methionine, riboflavin, and EDTA as a substrate were

236   employed in the technique. The absorbance of the illuminated tube at a wavelength of 560 nm

237   was compared to that of the non-illuminated mixture. The measurement of SOD activity was

238   conducted in mg of protein.

239
$$\%Inhibition = \frac{absorption\ of\ control\ sample - absorption\ of\ experimental\ sample}{absorption\ of\ experimental\ sample} \times 100$$

240   **Determination of hydrogen peroxide**

241   Hydrogen peroxide extraction from plant tissues was induced following the methodology

242   proposed by Patterson et al. (1984). The pulverization of fresh leaves was conducted, in

243   conjunction with the utilizing TCA (Trichloroacetic acid) and activated charcoal. The sample

244   underwent centrifugation at 3000rpm for 20 minutes at a temperature of 4 °C. The filtrate was

245   then divided into 1 mL aliquots. One of these containers was filled with 81 g of CAT and kept at

246   ambient temperature for 10 min. A volume of 1 mL of colorimetric reagent was introduced to

247   both the aliquots and was allowed to incubate for 10 min at a temperature of 30 °C. The

248   spectrophotometric measurement was conducted to determine the absorbance at 505 nm.

249   **Statistical analysis**

250   The data obtained represents the mean value of three replicates. RStudio software was employed

251   to conduct principal component analysis (PCA) and determine Pearson correlation coefficients

252   among the observed variables. SPSS Ver. 20 was used to calculate the analysis of variance.

253   Subsequently, the Tukey Honestly Significant Difference (HSD) test was employed to assess the

254   treatment means, enabling the ranking and comparison of significantly distinct treatments at a

255   probability level of $P < 0.05$.

256   **Results**

257   **Morphological parameters**

258   **Germination (%) and seedling vigor index (SVI)**

259   An observable increase in the germination % and SVI has been noted due to the Pb and PEG

260   applications. The absence of the PEG application caused a significant decline in the germination

261   % (36.6%) and the SVI (59.13%) was noted under Pb stress conditions compared to control

262   plants. Table 4 presents the observed fluctuations in germination percentage and morphological

263   parameters in response to the imposition of Pb-induced stress. The seedlings subjected to Pb-

264   induced stress exhibited a reduced germination percentage (47.7%) compared to plants in the

265   control group. Values for maximum growth performance (GP) and SVI were recorded at the

266   PEG-30% treatment, exhibiting elevated germination compared to both the control group and

267   Pb-contaminated treatment, as well as other PEG treatments. The germination rate in the PEG-

268   30% treatment showed a notable increase of 19.04% compared to the control. Under the

269   influence of Pb stress, reduction is observed in various growth attributes compared to the control

270   group. Specifically, there is a reduction of shoot length (48.83%) and root length (32.89%) under

271   Pb stress. The recorded data under Pb stress conditions revealed that shoot and root length

272   significantly increased in the presence of PEG-30%. Specifically, a remarkable increase of

273   62.79% in shoot length and 50.98% in root length was observed compared to the conditions

274   solely subjected to Pb stress.

275   **Biomass assessment**

276   The radish plants subjected to Pb-induced stress showed a notable decline in the fresh weights of

277   both their shoot and root, with a reduction of approximately 32.89% and 35.48%, respectively,

278   when compared to the control seedlings. The observation revealed that the PEG-primed seeds

279   revealed a distinct positive impact on both the production of fresh and dry biomass, in contrast to

280   plants subjected to Pb-induced stress. Lead stress reduced shoot biomass (by 60%) and root

281   biomass (by 50%) in exposed seedlings. Growth parameters increased with increased PEG

282   concentrations. Nevertheless, it is important to mention that an increase in the concentration of

283   PEG (up to 40%) resulted in a slight decrease in growth. This decline, though relatively minor,

284   was still comparable to the control group and showed no significant difference when compared

285   to plants subjected to Pb-induced stress. The application of PEG-30% led to a significant

286   augmentation in the fresh weight of both the shoot (54.54%) and root (40%), in comparison to

287   plants that did not undergo priming and were exposed to Pb-induced stress. The seed that

288   underwent priming with PEG-30% showed a notable reduction in fresh and dry biomass

289   production compared to the control group (Table 1).

290   Maximum pods and highest seed number per pod in treated plants were counted in PEG-30%+Pb

291   treated plants, which were 7.29% and 30% higher, respectively, compared with the plants grown

292   in a stress-free condition (control). Pb toxicity resulted in a notable decrease in the quantity of

293   pods and the number of seeds per pod. The observed pod count per plant in Pb stress conditions

294   exhibited a 65.62% reduction compared to the number of pods per plant in the control group.

295   Additionally, the number of seeds per pod in plants treated with Pb was 70% lower than that of

296   the control group.

Estimation of gas exchange parameters

298   Plants subjected to Pb stress exhibited a notable reduction in gas exchange traits, including net

299   photosynthetic activity (A), transpiration rate (Ei), and stomatal conductivity (gs), as compared to

300   the control group. Specifically, the observed decreases were 46.15%, 36.36%, and 49-25% for

301   stomatal conductivity, transpiration rate, and net photosynthetic rate, respectively, compared to

302   the control plants. Polyethylene glycol (PEG) primed seeds exhibited enhanced gas exchange

303   parameters, thereby mitigating the unfavorable effects of Pb in Radish. Specifically, when

304   exposed to a combination of PEG-30% and Pb, these plants demonstrated an 85.71% increase in

305   stomatal conductivity, 57.14% enhancement in transpiration, and a 93.59% increase in net

306　photosynthetic rate in contrast, plants that were solely treated with Pb did not exhibit such

307　improvements (Figure 1).

308　**Determination of photosynthetic pigments**

309　The radish plants exhibit an apparent decline in their photosynthetic pigments when subjected to

310　soil contaminated with Pb. Compared to control, Pb reduced chlorophyll *b* synthesis (0.2 mg/g

311　FW) more than chlorophyll *a* (0.4 mg/g FW). Our results showed a decrease of 45.94% in

312　carotenoids, 56.25% in total chlorophyll, and 60% in chlorophyll *b* content. Significant

313　enrichments were noted in the levels of chlorophyll content and carotenoids within the plant that

314　underwent pre-treatment with PEG, under both Pb-stressed and non-stressed soil conditions.

315　The application of PEG has been found to enhance the synthesis of photosynthetic content.

316　Notably, the most optimal results were obtained when utilizing PEG at a concentration of 30%.

317　This concentration increased Chl *a* by 60%, Chl *b* by 61%, total chlorophyll by 56.25%, and

318　carotenoids by 58.33% under Pb-induced stress.

319　**Evaluation of plant mineral status**

320　The table 3 shows data on nitrogen (N), phosphorus (P), and potassium (K) uptake found in the

321　leaf tissues of *R. sativus* plants. *R. sativus* plants subjected to Pb treatment exhibited significant

322　reduction in their N, P, and K levels. It was noted that the N content decreased by 43.47%, the P

323　content by 28.96%, and the K content by 30.35% compared to the control plants. Leaf samples

324　exhibited significantly elevated N, P, and K levels in plants subjected to PEG-30%+Pb treatment

325　over stress plants. PEG-30%+Pb upsurges the N by 50.93%, P by 46.4%, and K by 38.18% over

326　Pb-induced seedlings.

327　**Estimation of proline content**

328　When PEG was applied as a seed pre-treatment, proline concentration in radish plants under Pb

329　stress conditions significantly increased. In the presence of Pb-induced stress, PEG-30% showed

330　the highest proline concentration, measuring about 5.24 mg/g. The findings of this experiment

331　indicate that the proline concentration of radish plants was significantly higher in the treatment

332　group when different PEG concentrations (10%, 20%, 30%, and 40%) were used alone or in

333　combination with Pb. Upon subjecting plants solely to Pb, a substantial and prominent increase

334　in proline content of 43.28% was observed compared to the control group (Figure 3).

335　**Assessment of Pb uptake**

336   Table 4 indicated that plants grown in Pb-enriched soil showed enhanced Pb uptake by 3.9 µg/g

337   DW in plant roots and 0.12 µg/g DW in plant shoots. Nevertheless, PEG treatment inhibited Pb

338   translocation and uptake in both roots and shoots. Pb concentrations in roots grown under 10, 20,

339   30, and 40% PEG were 3.48, 2.40, 3.77, and 2.73 µg/g DW, and in PEG-treated plant shoots,

340   they were 0.336, 0.493, 0.246, and 0.166 µg/g DW. PEG 20 and 30% reduced the Pb uptake by

341   50% and 21% in roots and 16.86% and 53.51% in shoots, respectively.

342   **Assessment of metal tolerance index and accumulation coefficient**

343   The plants cultivated in soil contaminated with Pb exhibited AC of 0.05 and MTI of 42.33%.

344   Application of 20% PEG and 30% PEG resulted in significantly increased MTI values by

345   66.32% and 58.77 %, respectively compared to plants affected by Pb contamination. The seed

346   primed with 20% PEG under Pb stress, resulted in a significant reduction of the apparent

347   conductivity (AC) value by 40% (Table 4).

348   **Estimation of soluble protein content**

349   The presence of Pb hinders the plant's ability to synthesize proteins, leading to a subsequent

350   decrease in various growth parameters. Upon comparing plants subjected solely to Pb treatment

351   with the control group, a notable and significant reduction of 60% in their protein content was

352   observed. Application of pre-treatment of PEG exhibited a distinct positive impact on the protein

353   concentrations in radish plants subjected to Pb stress conditions. PEG-30% demonstrates the

354   highest protein concentration by 1.3 mg/g under Pb-induced stress. This concentration closely

355   resembles the control values, indicating the Pb mitigating effects of PEG (Figure 3).

356   **Estimation of antioxidant enzyme activities**

357   The current investigation explains the physiological reactions of antioxidant enzymes in the

358   presence of Pb and PEG treatments. According to studies, the utilization of various

359   concentrations of PEG (10%, 20%, 30%, and 40%) in isolation, as well as in combination with

360   Pb, exhibited a notable enhancement in the superoxide dismutase (SOD) activity of the radish

361   plant when compared to the control group. Similarly, the levels of catalase (CAT), hydrogen

362   peroxide (H_2O_2), and peroxidase (POD) activities were found to be elevated. The comparative

363   investigation among plants treated with Pb and control plants demonstrated that the Pb

364   significantly upsurge the levels of SOD, POD, CAT, and H_2O_2 by 86.08%, 44.07%, 54.47%, and

365   78.62%, respectively (Figure 2). The Pb+ PEG-20% treatment revealed the most elevated

366   superoxide dismutase (SOD) activity. This treatment demonstrated a notable increase of 86.50%

367 compared to the control group, and a slight increment of 0.22% compared to plants treated solely

368 with Pb. The Pb+PEG-40% treatment exhibited the highest level of POD activity, surpassing that

369 of plants treated with Pb by 2.91% and the control by 48.34%. The plants subjected to the

370 combination of Pb+PEG-30% showed the most significant elevation in CAT activity (55.26%),

371 over the plants exposed solely to Pb stress. Conversely, the plants treated exclusively with Pb

372 demonstrated the highest levels of hydrogen peroxide content, showing a considerable 81.48%

373 increase compared to the control treatment.

374 **Correlation between various growth and physio-biochemical attributes with Pb uptake and**

375 **accumulation**

376 A Pearson's correlation analysis was employed to identify the association between the growth

377 and physio-biochemical characteristics of PEG-treated *R. sativus* cultivated in soil contaminated

378 with Pb (Figure 4). The concentration of Pb in the shoot exhibited a positive correlation with the

379 accumulation coefficient in the shoot and the proline content in plants of the species *R. sativus*.

380 In contrast, the concentration of Pb in the shoot exhibited a negative correlation with various

381 factors including metal tolerance index, potassium, root dry weight, total phenol content,

382 stomatal conductance, germination percentage, total chlorophyll, transpiration rate, shoot dry

383 weight, photosynthetic rate, protein content, fresh weight of root and shoot, root length, and

384 carotenoids. According to these findings, PEG showed efficacy in reducing the negative impacts

385 of Pb stress by enhancing the absorption of vital nutrients and modifying antioxidant enzymes.

386 **Principal component analysis**

387 The cultivation of *R. sativus* on soil contaminated with Pb, supplemented with PEG, exhibited a

388 correlation between growth and various physiological and biochemical traits. This relationship

389 was elucidated through the utilization of loading plots derived from principal component

390 analysis, as depicted in Figure 5. The initial two principal components, Dim1 and Dim2,

391 constitute the predominant proportion of all components and encompass over 79.0% of the entire

392 dataset. Dimension 1 constitutes 57.2% of the dataset, while Dimension 2 accounts for 21.8% of

393 the dataset. A positive correlation was observed among various variables, including potassium,

394 magnesium, sodium, zinc, total phenol content, and stomatal conductance. The proline content,

395 metal tolerance index, and accumulation coefficient exhibited a significant negative correlation.

396 The largest impact on the development and antioxidant capacity of *R. sativus* was seen under Pb

397 stress. Plant growth was negatively correlated with it, but antioxidant capacity was positively

398 correlated. PEG primarily increased plant height, aboveground biomass, and POD, CAT, and

399 SOD activity to boost plant growth and antioxidant capacity (Figure 5).

400 **Discussion**

401 Lead (Pb) is a noxious element and its increased contamination has alarmed the researchers to

402 find ways to reduce the toxicity impacts of this heavy metal on our ecosystem. Priyanka et al

403 (2021) reported that Pb pollution reduces crop growth and productivity. According to Diaconu et

404 al. (2020), plants acquire Pb through their root system, with a minor uptake occurring through

405 the leaves. Primarily Pb is accumulated in the root system. However, a fraction of this element is

406 also transported to the aboveground plant parts including foliage, shoot, and reproductive tissues

407 (Yadav et al., 2022, Bassegio et al., 2020). Upon entering into cellular compartments, Pb elicits

408 inhibitory effects on the actions of various growth-related enzymes (Shu et al., 2012). Reduced

409 enzymatic activity instigates perturbations in mineral nutrition and the equilibrium of water

410 homeostasis. Furthermore, Pb induces modifications in hormonal synthesis exerting noticeable

411 impacts on the structural integrity and permeability of cellular membranes (Zulfiqar et al., 2019;

412 Usman et al., 2020). These multifaceted cellular responses highlight the harmful influence of Pb

413 on various physiological processes, as validated by the pertinent literature.

414 During the present study, radish seeds were cultivated in soil contaminated with Pb to

415 systematically evaluate the consequences of Pb toxicity on seed germination and subsequent

416 plant growth. Our study unveiled a detrimental impact of Pb toxicity on compromised seed

417 germination, reduced seed vigor index, and diminished weights of both roots and shoots. A

418 significant decrease in germination percentage (Table 1) of seeds present in Pb spiked soil

419 correlates with the findings of Nas et al. (2018). Sengar et al. (2009) and Kadoll (2021) also

420 described the same inhibitory effect of heavy metals on the germination percentage of *Oryza*

421 *sativa, Spartina alterniflora, Zea mays,* and *Hordeum vulgare.* The presence of Pb also affects

422 the cytokinesis by disrupting microtubules leading to a decline in cell division (Nabi et al., 2019)

423 resulting in decreased germination percentage and seedling vigor.

424 Pb imposes deleterious impacts on food reserves and causes a significant change in the

425 enzymatic activity of amylases and proteases which are involved in seed germination of

426 numerous higher plants (Osman et al., 2023; Singh et al., 2011, Sengar et al., 2009; Yang et al.,

427 2020).

428 On the other hand, Galhaut et al. (2014) described the alleviative activity of PEG against Pb-
429 induced toxic effects. They reported that increased germination rate and seedling growth in PEG-
430 primed seeds correlated with our findings. In our experiment, the stress-ameliorative potential of
431 30% PEG was exhibited through improved chlorophyll synthesis in radish plants under Pb stress
432 (Table 2). Laxman et al. (2020) and Pagano et al. (2023) also described the efficacy of PEG in
433 enhancing seed germination percentage ascribed to modification of seed water relationship. Our
434 findings in the context of improved germination are also similar to the results of Nagarajan et al.
435 (2005) which showed an increased germination percentage of seeds in tomatoes when primed
436 with PEG as priming with PEG enhanced tissue hydration of seeds which increased the efficacy
437 of seeds against stress agents.

438 Lead accumulation causes a disturbance in cellular division and elongation resulting in reduced
439 plant growth and biomass accumulation (Khan et al. 2020; Diaconu et al. 2020) which are allied
440 with our current findings (Table, 1, 5).

441 Data presented in Table 1 showed decreased root dry weight in radish plants exposed to Pb.
442 Similar findings were reported in the case of wheat, cotton, sunflower, and brassica subjected to
443 metal stress (Ramana et al., 2021; Kanwal et al., 2020; Bassegio et al., 2020; Alaboudi et al.,
444 2018). Ghori et al. (2019) reported that a reduction in biomass and growth of plants is associated
445 with a decline in photosynthetic activity resulting from retardation in the activity of photosystem
446 II and nitrogen assimilation which is in agreement with our findings (Table 2, 3).

447 The present study elucidates the pronounced influence of Pb on gas exchange parameters,
448 corroborating earlier studies that consistently underscore the deleterious effects of this noxious
449 metal on the photosynthetic rate (Lamhamdi et al., 2013). This phenomenon is attributed to
450 disruptions in the chloroplast ultrastructure, diminished chlorophyll synthesis, interference with
451 electron transport, and inhibition of crucial enzymes in the Calvin cycle. The closure of stomatal
452 pores leads to a reduction in stomatal conductance, carbon dioxide levels, and rate of
453 photosynthesis is greatly responsible for reduced rates of transpiration has been evidenced and
454 substitution of Mg^{2+} ions within the chlorophyll molecule induces structural damage as it is a
455 pivotal component of chloroplast, thereby diminishing photosynthetic efficiency. Therefore, the
456 results of our study correlating with the former investigations showed reduced stomatal
457 conductance, chlorophyll content (Table 2), and photosynthetic efficiency (Figure 1). Similarly,
458 the affinity of Pb toward proteins essential for light reactions, Calvin cycle, and in photosystems

459 adversely affects the photosynthetic apparatus, consistent with prior studies demonstrating

460 alterations in chlorophyll content in *Spinacia oleracea* (Lamhamdi et al., 2013) under Pb

461 toxicity. Additionally, activation of chlorophyllase, excessive ROS accumulation, inhibition of 8-

462 aminolevulinate dehydrogenase, and alterations in thylakoid membrane lipid composition

463 contribute to reduced chlorophyll levels and all these significant outcomes of former research

464 work suggest the reason for reduced photosynthesis and chlorophyll content in our current

465 findings. The present study showed enhanced photosynthetic activity in radish plants primed

466 with PEG under Pb stress which aligned with the study of Salah et al. (2015) who described an

467 increased photosynthetic pigment level in rice seeds primed with PEG.

468 Rizvi et al. (2020) showed a reduced absorption of essential elements that play a crucial role in

469 the metabolism of plant biomass production in Pb-contaminated regimes that is by reduced N, P,

470 and K uptake in the seedlings grown in the Pb regime. Our data exhibited that Pb toxicity

471 diminished the concentrations of N, P, and K in various tissues of radish plants. Kanwal et al.

472 (2020) also observed a decreased level of N in plants subjected to higher concentrations of Cd,

473 Pb, and Zn. HM's induced stress affects N uptake, transportation, and assimilation in plant

474 tissues Laxman et al. (2020) The adverse impact of Pb on ionic homeostasis declines K uptake

475 and translocation, resulting in poor plant growth (Naciri et al., 2021) which correlates our study

476 in reduced uptake of minerals (Table 3).

477 Proline, an amino acid that accumulates in response to Pb toxicity, is essential for the induction

478 of plant defense mechanisms (Mishra et al., 2006). The higher accumulation of proline is

479 proposed to function as a protective mechanism, particularly for proteins involved in cellular

480 redox maintenance, and proline playing a role as a beneficial solute is suggested to be involved

481 in the synthesis of proteins. These findings align with the outcomes of our current investigation

482 as shown in Figure 3.

483 The presence of Pb perhaps led to a significant reduction in soluble protein concentration

484 compared to stress-free conditions. Comparable findings were reported by Xalxo and

485 Keshavkant (2020) in their study on *Talinum triangulare*, suggesting that diminished protein

486 content may result from suppressed synthesis and increased oxidation induced by Pb toxicity.

487 Our data showed that radish plants growing in Pb-spiked soils exhibited higher levels of ROS,

488 proline, hydrogen peroxide, and antioxidants. Our results align with studies of Priyanka et al.

489 (2021) and Yan et al (2021), illustrating that HM's induced stress elevated lipid peroxidation,

490 disrupted cellular metabolic homeostasis, and triggered free radical production. Higher ROS

491 generation stimulated non-enzymatic and enzymatic antioxidant mechanisms as adaptive

492 responses to mitigate metal stress (Sharma et al., 2020). The outcome of our study aligns with

493 the observations made by Sidhu et al. (2016), who reported an increase in the antioxidant

494 activities of *Lepidium coronopus* when exposed to Pb. Similarly, in a study conducted

495 on *Zygophyllum fabago*, it was observed that exposure to Pb resulted in elevated levels of

496 catalase (Salah et al., 2015). Taken together, the results of our study showed that PEG may act as

497 a secondary messenger to alleviate the metal-induced stress in plants. Seed priming with PEG

498 mitigated Pb stress in radishes through decreasing Pb uptake, increasing chlorophyll content, and

499 regulating synthesis and/or activation of enzymatic and nonenzymatic antioxidants which is in

500 resemblance with the outcomes of Syaiful et al., (2015) explained that priming with PEG

501 increases membranes stability and effective translocation of amino acids, soluble sugars and

502 proteins which probably enhance stress tolerance ability. Nevertheless, the stress ameliorative

503 action of signaling molecules triggered by PEG to mitigate Pb-induced toxicity remains

504 mysterious. Hence, further studies are required to recognize the precise mechanism of the PEG-

505 induced extenuation of Pb toxicity through increasing the physiological, biochemical, molecular,

506 and growth tactics.

507 **Conclusion**

508 In conclusion, Pb toxicity negatively affected physiochemical activity, gas exchange parameters,

509 germination, nutrition, and growth of radish, nonetheless, it increased MDA and ROS levels. Pb-

510 induced stress at the same time activated the tolerance mechanism of plants to detoxify ROS and

511 reduce the biosynthesis of MDA. However, plants remained incapable of managing Pb-triggered

512 cell injuries as perceived through higher levels of ROS and MDA and decreased synthesis of

513 photosynthetic pigments, root growth, shoot growth, and biomass production. Pb-stressed plants

514 exhibited elevated levels of proline biosynthesis besides enhanced activity of antioxidative

515 enzymes. Yet, 30% PEG seed priming abridged the stress injuries through increased activity of

516 SOD, POD, and CAT enzymes besides increasing the scavenging level of H_2O_2 to detoxify ROS.

517 Similarly, PEG improved photosynthetic activity by protecting the deterioration of

518 photosynthetic pigments by adjusting water equilibrium. The modulated activity of antioxidant

519 enzymes, enhanced biosynthesis of soluble proteins, and higher production of osmoprotectants

520 (proline) and total soluble sugar--- through PEG seed application maintained cellular osmotica

521 and cellular integrity. Moreover, PEG-applied plants showed decreased Pb accretion and

522 increased levels of mineral nutrients (N, P, K). Collectively, 30% PEG seed priming enhanced

523 nutrition, gas attributes, photosynthetic activity, growth, and biomass production of radish under

524 Pb stress. In forthcoming studies, the detailed biosynthesis pathways, and omics modulations

525 related to the stress tolerance mechanism through PEG application should be explored. A minute

526 amount of stress ameliorator is required for seed priming. Hence, this approach, exhibiting

527 promising results in further field trials, might be extensively employed for crop production under

528 abiotic stress conditions.

529 **Acknowledgement:**

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849 **Figure 1: Effect of PEG on gaseous exchange parameters of *R. sativus* seedlings under lead
850 stress.** The data represent the mean standard deviation (SD) of three replicates.
851 Distinct letters indicate a notable difference between the treatments at a significance
852 level of P<0.05. C=control group. Pb=75 mg/kg, PEG-1=10% PEG, PEG-2-20%
853 PEG, PEG-3=30% PEG, PEG-4-40% PEG, PEG-1-Pb-PEG-10%+Pb, PEG-2+Pb-
854 PEG-20%+Pb, PEG- 3+Pb-PEG-30%+Pb, PEG-4+Pb=PEG-40%+Pb.

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856 **Figure 2: Effect of PEG on SOD, POD, CAT and H$_2$O$_2$ activity of *R. sativus* seedlings
857 under lead stress.** The data represent the mean standard deviation (SD) of three
858 replicates. Distinct letters indicate a notable difference between the treatments at a
859 significance level of P<0.05. C=control group. Pb=75 mg/kg, PEG-1=10% PEG,
860 PEG-2-20% PEG, PEG-3=30% PEG, PEG-4-40% PEG, PEG-1-Pb-PEG-10%+Pb,
861 PEG-2+Pb-PEG-20%+Pb, PEG- 3+Pb-PEG-30%+Pb, PEG-4+Pb=PEG-40%+Pb.

862 **Figure 3: Effect of PEG on total soluble proteins, proline contents of *R. sativus* seedlings
863 under lead stress.** The data represent the mean standard deviation (SD) of three
864 replicates. Distinct letters indicate a notable difference between the treatments at a
865 significance level of P<0.05. C=control group. Pb=75 mg/kg, PEG-1=10% PEG,
866 PEG-2-20% PEG, PEG-3=30% PEG, PEG-4-40% PEG, PEG-1-Pb-PEG-10%+Pb,
867 PEG-2+Pb-PEG-20%+Pb, PEG- 3+Pb-PEG-30%+Pb, PEG-4+Pb=PEG-40%+Pb.

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869 .**Figure 4: Correlation between growth and physio-biochemical attributes of PEG primed
870 *R. sativus* grown in lead contaminated soil. The figure employs various abbreviations to
871 represent specific parameters, includes:** SL (shoot length), RL (root length), SFW (shoot fresh
872 weight),RFW (root fresh weight),   SDW (shoot dry weight),RDW (root dry weight), SC
873 (stomatal conductance), PR (photosynthetic rate), TR (transpiration rate), T proteins (soluble
874 protein content), SOD (Superoxide dismutase Activity), POD (Peroxidase Activity), Caro
875 (carotenoids), CAT (Catalases activity), HP (Hydrogen peroxides activity), Caro (carotenoids),
876 chl b (chlorophyll b),chl *a* (chlorophyll a), TC (total chlorophyll), pods/pla (Pods per plants),
877 seeds/pod(Seeds per plants), SVI (Seed vigor index), N(Nitrogen), P(Phosphorus), K

878   (potassium), Pro (Proline content in radish plants), Pb in root (Lead in radish Roots), Pb in shoot

879   (Lead in radish shoot), AC (Accumulation Coefficient), MTI (metal tolerance index), GP

880   (germination percentage).

881

882   **Figure 5: Principal component analysis plots to illustrate the relationship between**

883   **growth and physio-biochemical characteristics of PEG treated *R. sativus* cultivated in**

884   **lead-contaminated soil. The figure employs various abbreviations to represent specific**

885   **parameters, include:** SL (shoot length), RL (root length), SFW (shoot fresh weight),RFW

886   (root fresh weight),   SDW (shoot dry weight),RDW (root dry weight), SC (stomatal

887   conductance), PR (photosynthetic rate), TR (transpiration rate), T proteins (soluble protein

888   content), SOD (Superoxide dismutase Activity), POD (Peroxidase Activity), Caro

889   (carotenoids), CAT (Catalases activity), HP (Hydrogen peroxides activity), Caro

890   (carotenoids), chl *b* (chlorophyll *b*), Chl *a* (chlorophyll *a*), TC (total chlorophyll), pods/pla

891   (Pods per plants), seeds/pod(Seeds per plants), SVI (Seed vigor index), N(Nitrogen),

892   P(Phosphorus), K (potassium), Pro (Proline content in radish plants), Pb in root (Lead in

893   radish Roots), Pb in shoot (Lead in radish shoot), AC (Accumulation Coefficient), MTI

894   (metal tolerance index), GP (germination percentage).

895 **Table 1: Effect of PEG on the growth and biomass production of *R. sativus* under lead stress.**

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Treatments	Growth Traits					
	Shoot length (cm)	Root length (cm)	Shoot FW (g plant^{-1})	Root FW (g plant^{-1})	Shoot DW (g plant^{-1})	Germination Percentage
C	6.4± 0.43 ab	7.6 ± 0.29 ab	0.32± 0.02 ab	0.31± 0.04 a	0.05±0.001 ab	70 ± 0.88 b
Pb	4.3± 0.43 d	5.1 ± 0.26 d	0.22± 0.00 c	0.2± 0.02 a	0.02± 0.001 f	36± 1.98 e
PEG-1	4.5± 0.29 cd	7.1 ± 0.14 abc	0.26± 0.01 bc	0.26± 0.01a	0.03± 0.001 def	59± 1.20 cd
PEG-2	5.3± 0.47 bcd	7.2 ± 0.23 abc	0.28± 0.00abc	0.28± 0.01 a	0.03± 0.001 ef	67± 1.35 bc
PEG-3	6.1± 0.08 abc	7.4 ± 0.23 abc	0.33±0.02 ab	0.27± 0.03 a	0.05± 0.001 a	85± 1.19 a
PEG-4	4.4± 0.14 d	6.5 ± 0.11 c	0.29±0.01 abc	0.26± 0.00 a	0.03± 0.001 cde	65± 2.55 bc
PEG-1 + Pb	5.4± 0.17 abcd	7.2 ± 0.05 abc	0.29±0.00 abc	0.25± 0.00 a	0.04± 0.002 bc	57± 0.98 e
PEG-2 + Pb	6.2± 0.43 ab	7.3 ± 0.31 abc	0.3± 0.00 ab	0.26± 0.00 a	0.04± 0.002 abc	65± 1.14 bc
PEG-3 + Pb	7± 0.11 a	7.7 ± 0.23 a	0.34± 0.01a	0.28± 0.02 a	0.05±0.00 ab	84± 2.21 a
PEG-4 + Pb	3.9± 0.29 d	6.6 ± 0.12 bc	0.3± 0.00 ab	0.25± 0.00 a	0.04± 0.00 cd	61± 1.39 cd

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898 The data represent the mean standard deviation (SD) of three replicates. Distinct letters indicate a notable difference between
899 the treatments at a significance level of $P<0.05$. C=control group. Pb=75 mg/kg, PEG-1=10% PEG, PEG-2=20% PEG, PEG-
900 3=30% PEG, PEG-4=40% PEG, PEG-1-Pb=PEG-10%+Pb, PEG-2+Pb=PEG-20%+Pb, PEG-3+Pb=PEG-30%+Pb, PEG-
901 4+Pb=PEG-40%+Pb.

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904   **Table 2: Effect of PEG on photosynthetic pigments, total chlorophyll and**
905   **carotenoids of *R. sativus* under lead stress.**
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Treatments	Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Total Chlorophyll (mg/g FW)	Carotenoids (mg/g FW)
C	0.9 ± 0.00 b	0.5 ± 0.01 a	1.6 ± 0.01 ab	3.7 ± 0.14 a
Pb	0.4 ± 0.03 e	0.2 ± 0.01e	0.7 ± 0.01 c	2.0 ± 0.00 f
PEG-1	0.8 ± 0.01 c	0.3 ± 0.00 cd	1.2 ± 0.00 bc	4.5 ± 0.01c
PEG-2	0.8 ± 0.01 c	0.3 ± 0.00 cd	1.3 ± 0.01abc	4.7 ± 0.02 b
PEG-3	1.0 ± 0.01 a	0.4 ± 0.01 bc	1.6 ± 0.01 ab	4.9 ± 0.01 a
PEG-4	0.6 ± 0.01 d	0.3 ± 0.01de	1.6 ± 0.01 abc	3.1 ± 0.01 a
PEG-1 + Pb	0.6 ± 0.01 d	0.3 ± 0.00de	1.1 ± 0.01 abc	3.9 ± 0.01 d
PEG-2 + Pb	0.8 ± 0.03 bc	0.3 ± 0.03de	1.3 ± 0.01 ab	4.3 ± 0.02 b
PEG-3 + Pb	1.0 ± 0.02 a	0.5 ± 0.01 ab	1.6 ± 0.01a	4.8 ± 0.01 a
PEG-4 + Pb	0.6 ± 0.02 d	0.3 ± 0.01de	1.0 ± 0.01 ab	4.6 ± 0.01 e

908
909   The data represent the mean standard deviation (SD) of three replicates. Distinct letters indicate a notable difference between
910   the treatments at a significance level of $P<0.05$. C=control group. Pb=75 mg/kg, PEG-1=10% PEG, PEG-2=20% PEG, PEG-
911   3=30% PEG, PEG-4=40% PEG, PEG-1-Pb=PEG-10%+Pb, PEG-2+Pb=PEG-20%+Pb, PEG-3+Pb=PEG-30%+Pb, PEG-
912   4+Pb=PEG-40%+Pb.
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921   **Table 3: Effect of PEG on nitrogen, phosphorus and potassium of**
922   ***R. sativus* under lead stress.**

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Treatments	N (mg/g DW)	P^+ (mg/g DW)	K^+ (mg/g DW)
C	41.6 ± 0.61 b	11.7 ± 0.17 b	43.1 ± 1.21 bc
Pb	23.5 ± 1.03 e	6.7 ± 0.15 h	30.6 ± 1.02 e
PEG-1	27.8 ± 1.28 de	8.5 ± 0.17 f	35.2 ± 1.56 de
PEG-2	33.5 ± 1.07 cd	9.7 ± 0.12 e	39.9 ± 0.56 cd
PEG-3	41.1 ± 0.73 b	10.6 ± 0.08 cd	46.3 ± 2.23 ab
PEG-4	26.9 ± 1.21 e	7.6 ± 0.20 g	30.5 ± 0.95 e
PEG-1 + Pb	38.0 ± 0.94 bc	10.5 ± 0.17 de	35.0 ± 0.84 de
PEG-2 + Pb	40.4 ± 1.27 b	11.4 ± 0.17 bc	43.0 ± 0.82 bc
PEG-3 + Pb	47.9 ± 1.56 a	12.5 ± 0.18 a	49.5 ± 1.10 a
PEG-4 + Pb	33.5 ± 1.89 cd	7.5 ± 0.18 g	31.9 ± 1.44 e

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925   The data represent the mean standard deviation (SD) of three replicates. Distinct letters indicate a notable difference
926   between the treatments at a significance level of $P<0.05$. C=control group. Pb=75 mg/kg, PEG-1=10% PEG, PEG-
927   2=20% PEG, PEG-3=30% PEG, PEG-4=40% PEG, PEG-1-Pb=PEG-10%+Pb, PEG-2+Pb=PEG-20%+Pb, PEG-
928   3+Pb=PEG-30%+Pb, PEG-4+Pb=PEG-40%+Pb.

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936 **Table 4: Effect of PEG seed priming on Pb uptake, accumulation coefficients and metal tolerance index, vigor index, number of Pods**
937 **per plant, and number of seeds pod^{-1} of *R. sativus* under lead stress.**

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Treatments	Pb uptake in root (µg/g 1 DW)	Pb uptake in shoot (µg/g 1 DW)	AC	No of Pods/plant	No of seeds/pod
C	0	0	0	9.6 ± 0.88 ab	10±0.57 abc
Pb	3.9 ± 0.67 a	0.59 ± 0.10 a	0.05 ± 0.008 a	3.3 ± 0.33 d	3 ± 0.88 f
PEG 10%	0	0	0	6.0 ± 0.57 cd	7 ± 0.57cde
PEG 20%	0	0	0	7.3 ± 0.33 abc	9 ± 0.57 bcd
PEG 30%	0	0	0	10.0 ±0.57 ab	11 ± 0.57 ab
PEG 40%	0	0	0	6.6 ± 0.88 bcd	4 ± 1.20 ef
Pb+PEG 10%	3.5 ± 0.35 ab	0.33 ± 0.06 ab	0.05 ±0.004 ab	5.6 ±0.66 cd	7 ± 0.66 cde
Pb+PEG 20%	2.4 ± 0.25 b	0.49 ± 0.11 ab	0.03 ± 0.003 b	7.0 ± 1.0 abc	9 ± 0.57 bcd
Pb+PEG 30%	3.8 ± 0.22 ab	0.24 ± 0.03 ab	0.05 ± 0.002 ab	10.3 ± 0.88a	13 ± 0.57 a
Pb+PEG 40%	2.7 ± 0.15 b	0.16 ± 0.03 b	0.04 ± 0.002 b	7.0 ± 0.57 abc	6 ± 0.57 def

940

941 The data represent the mean standard deviation (SD) of three replicates. Distinct letters indicate a notable difference between the
942 treatments at a significance level of $P<0.05$. C=control group. Pb=75 mg/kg, PEG-1=10% PEG, PEG-2=20% PEG, PEG-3=30% PEG,
943 PEG-4=40% PEG, PEG-1-Pb=PEG-10%+Pb, PEG-2+Pb=PEG-20%+Pb, PEG-3+Pb=PEG-30%+Pb, PEG-4+Pb=PEG-40%+Pb.
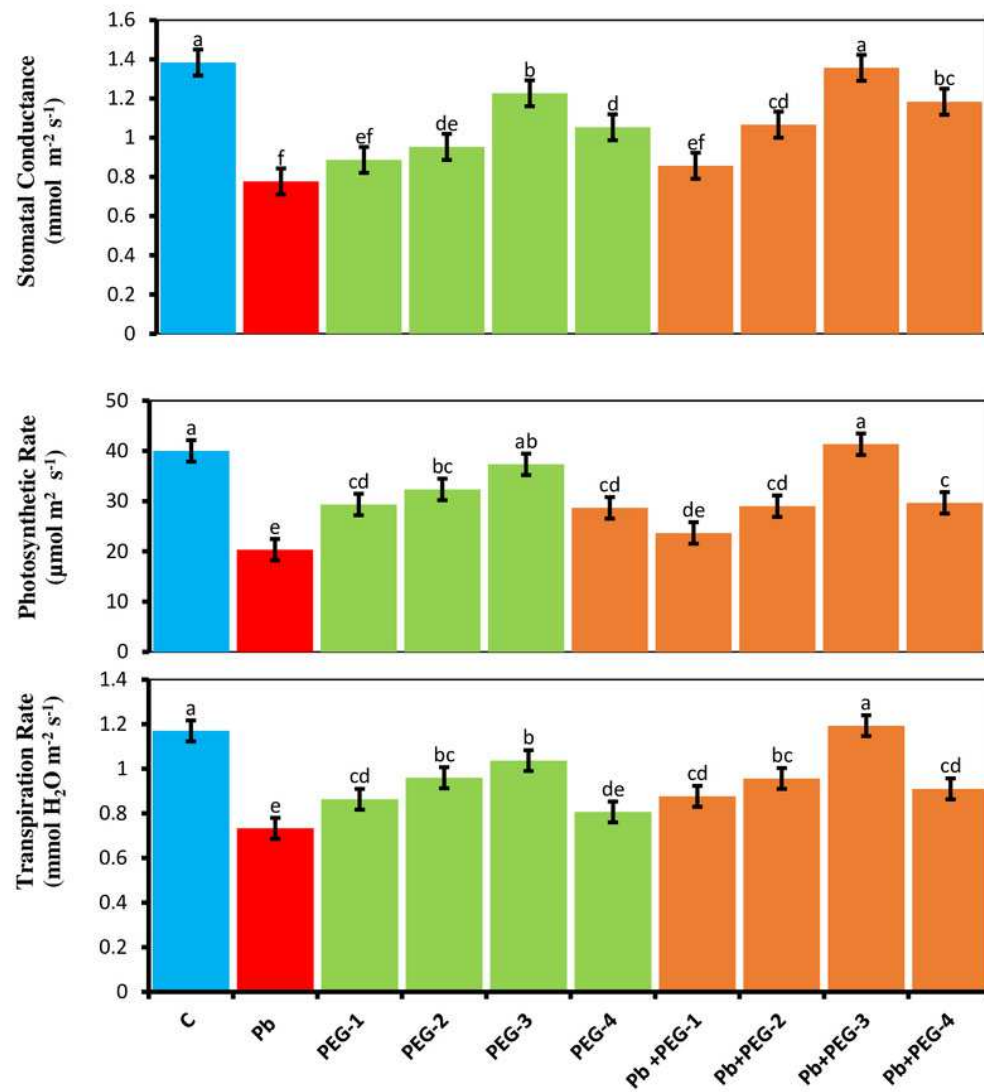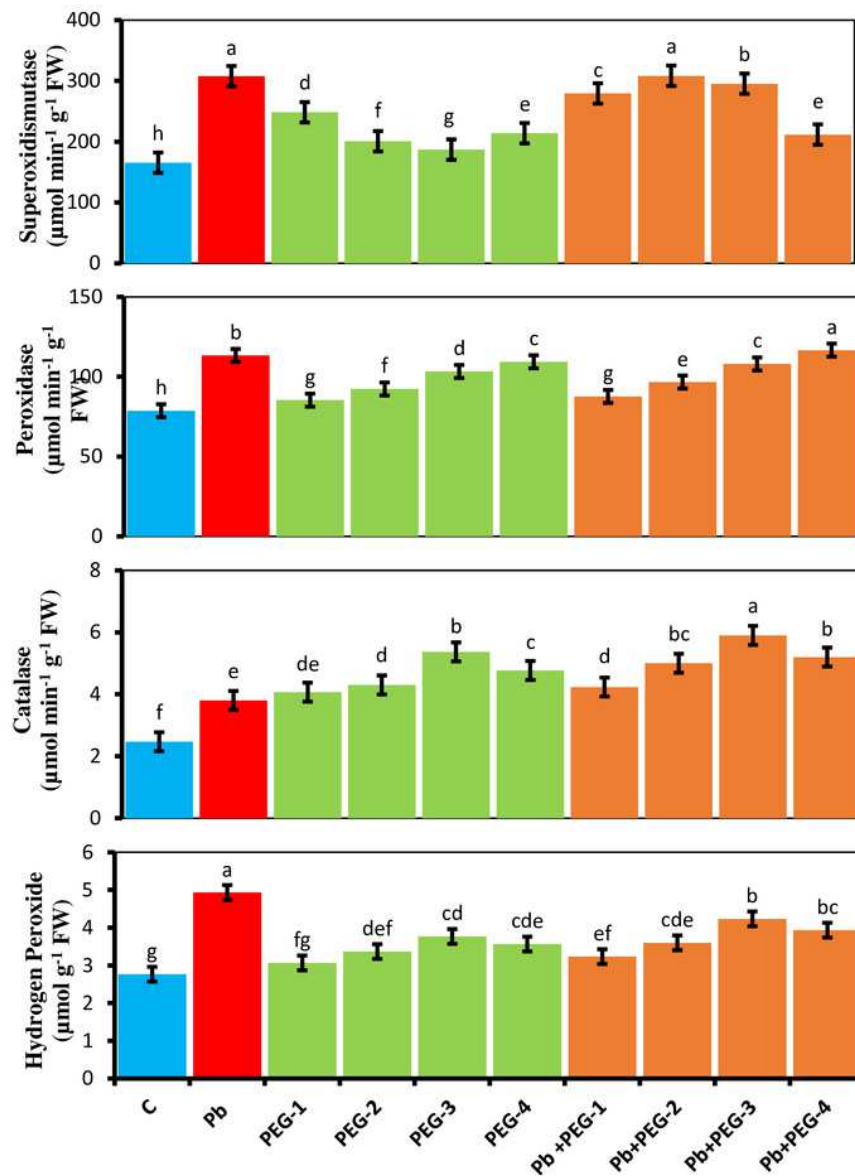
944

Figure 1

Effect of PEG on gaseous exchange parameters of *R. sativus* seedlings under lead stress.

The data represent the mean standard deviation (SD) of three replicates. Distinct letters indicate a notable difference between the treatments at a significance level of P<0.05. C=control group. Pb=75 mg/kg, PEG-1=10% PEG, PEG-2-20% PEG, PEG-3=30% PEG, PEG-4-40% PEG, PEG-1-Pb-PEG-10%+Pb, PEG-2+Pb-PEG-20%+Pb, PEG- 3+Pb-PEG-30%+Pb, PEG-4+Pb=PEG-40%+Pb.

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Figure 2

Effect of PEG on SOD, POD, CAT and H_2O_2 activity of *R. sativus* seedlings under lead stress.

The data represent the mean standard deviation (SD) of three replicates. Distinct letters indicate a notable difference between the trea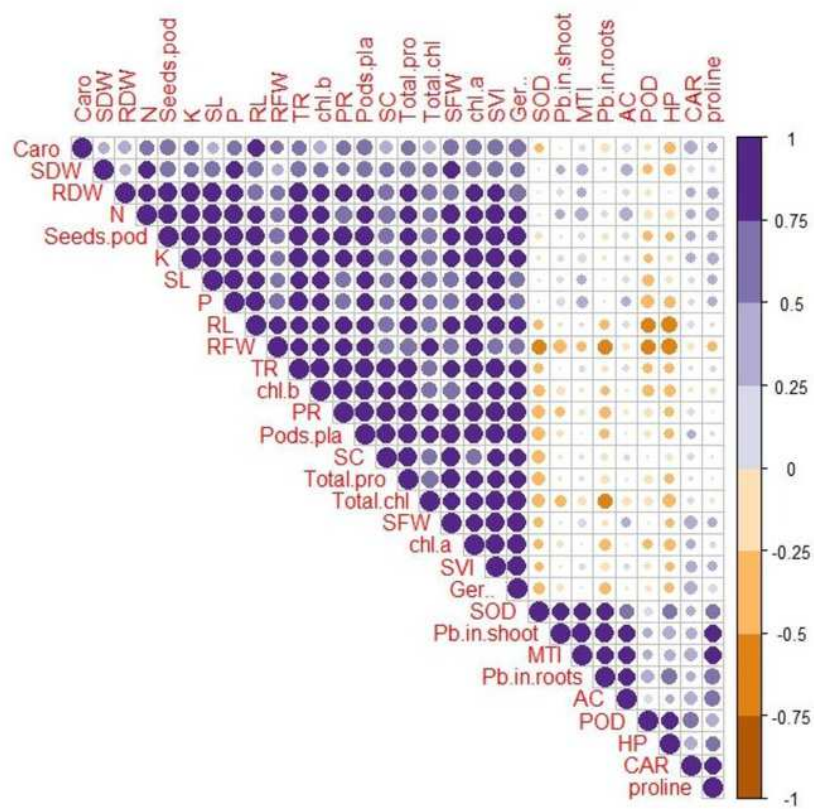tments at a significance level of $P<0.05$. C=control group. Pb=75 mg/kg, PEG-1=10% PEG, PEG-2-20% PEG, PEG-3=30% PEG, PEG-4-40% PEG, PEG-1-Pb-PEG-10%+Pb, PEG-2+Pb-PEG-20%+Pb, PEG- 3+Pb-PEG-30%+Pb, PEG-4+Pb=PEG-40%+Pb.

Figure 3

Effect of PEG on total soluble proteins, proline contents of *R. sativus* seedlings under lead stress.

The data represent the mean standard deviation (SD) of three replicates. Distinct letters indicate a notable difference between the treatments at a significance level of P<0.05. C=control group. Pb=75 mg/kg, PEG-1=10% PEG, PEG-2-20% PEG, PEG-3=30% PEG, PEG-4-40% PEG, PEG-1-Pb-PEG-10%+Pb, PEG-2+Pb-PEG-20%+Pb, PEG- 3+Pb-PEG-30%+Pb, PEG-4+Pb=PEG-40%+Pb.
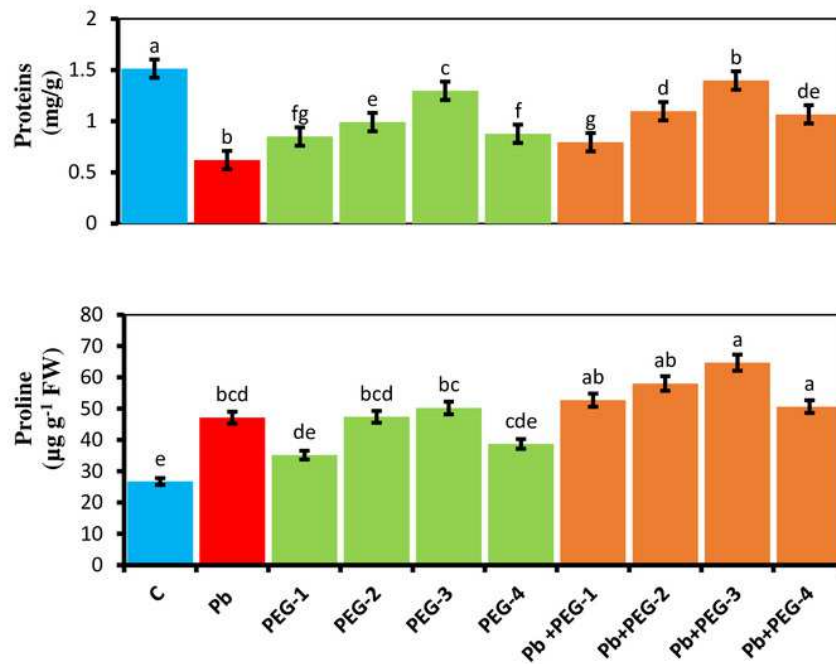
Figure 4

Correlation between growth and physio-biochemical attributes of PEG primed *R. sativus* grown in lead contaminated soil.

The figure employs various abbreviations to represent specific parameters, includes: SL (shoot length), RL (root length), SFW (shoot fresh weight),RFW (root fresh weight), SDW (shoot dry weight),RDW (root dry weight), SC (stomatal conductance), PR (photosynthetic rate), TR (transpiration rate), T proteins (soluble protein content), SOD (Superoxide dismutase Activity), POD (Peroxidase Activity), Caro (carotenoids), CAT (Catalases activity), HP (Hydrogen peroxides activity), Caro (carotenoids), chl b (chlorophyll b),chl *a* (chlorophyll a), TC (total chlorophyll), pods/pla (Pods per plants), seeds/pod(Seeds per plants), SVI (Seed vigor index), N(Nitrogen), P(Phosphorus), K (potassium), Pro (Proline content in radish plants), Pb in root (Lead in radish Roots), Pb in shoot (Lead in radish shoot), AC (Accumulation Coefficient), MTI (metal tolerance index), GP (germination percentage).

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Figure 5

Principal component analysis plots to illustrate the relationship between growth and physio-biochemical characteristics of PEG treated *R. sativus* cultivated in lead-contaminated soil.

The figure employs various abbreviations to represent specific parameters, include: SL (shoot length), RL (root length), SFW (shoot fresh weight),RFW (root fresh weight), SDW (shoot dry weight),RDW (root dry weight), SC (stomatal conductance), PR (photosynthetic rate), TR (transpiration rate), T proteins (soluble protein content), SOD (Superoxide dismutase Activity), POD (Peroxidase Activity), Caro (carotenoids), CAT (Catalases activity), HP (Hydrogen peroxides activity), Caro (carotenoids), chl *b* (chlorophyll *b*), Chl *a* (chlorophyll *a*), TC (total chlorophyll), pods/pla (Pods per plants), seeds/pod(Seeds per plants), SVI (Seed vigor index), N(Nitrogen), P(Phosphorus), K (potassium), Pro (Proline content in radish plants), Pb in root (Lead in radish Roots), Pb in shoot (Lead in radish shoot), AC (Accumulation Coefficient), MTI (metal tolerance index), GP (germination percentage).