Physiological and Biochemical Responses of Maize (*Zea mays*) to Phenanthrene Toxicity

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Abstract

Polycyclic aromatic hydrocarbons (PAHs), a class of organic pollutants, have been observed to exert deleterious effects on plant growth and various physiological processes. In this study, the effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on growth parameters, photosynthetic pigments, some antioxidant enzymes, and some biochemical compounds of the maize plant were investigated. The experiments were conducted as pot cultures of plants under controlled conditions using a completely randomized design (CRD) with three replications for each treatment. Increasing phenanthrene concentration reduced all studied growth parameters and significantly increased photosynthetic pigment contents. Phenanthrene led to the increase in catalase, peroxidase, and superoxide dismutase activities in the roots but in shoots, only the peroxidase activity was increased. Moreover, higher phenanthrene concentrations were associated with elevated levels of malondialdehyde and hydrogen peroxide, coupled with a marked reduction in soluble sugar content in both shoot and root tissues ($p < 0.05$). Also, increase in phenanthrene concentration in the shoots decreased the total phenol and flavonoid contents compared to anthocyanin. Phenanthrene treatment led to a significant reduction in the concentrations of lauric acid, meric acid, palmitic acid, and oleic acid in maize shoots. In conclusion, it seems that high concentrations of phenanthrene induce oxidative stress in the maize, and plants improve their enzymatic antioxidant system to moderate the stress condition. In addition, damage of cell membranes by phenanthrene leads to weakening of plants root system as well as disordering in water and nutrient uptake and finally reduction in the plant growth.

Keywords: Antioxidant system, Oxidative stress, Maize plant, Phenanthrene, Physiological responses, Polycyclic aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large group of organic compounds composed exclusively of carbon and hydrogen (Ansari *et al.,* 2023). These compounds form a

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33 major group of environmental pollutants, mainly produced by the incomplete fuel combustion
34 and various anthropogenic sources (Kumar *et al.,* 2021). Some compounds of PAHs are
35 carcinogenic and mutagenic and therefore may possibly be important in the context of human
36 health concerns. Due to their high persistence, long-term toxicity and accumulation in the
37 environment, PAHs have attracted more attention in recent years (Gitipour *et al.,* 2018;
38 Thacharodi *et al.,* 2023). Polycyclic aromatic hydrocarbons consist of two groups including low
39 molecular weight (LMW) and high molecular weight (HMW) compounds containing 2-3 and
40 4-7 rings, respectively. Phenanthrene (PHE), a representative of the LMW PAHs, is made of
41 three benzene rings and thus it is determined as one of the central pollutants listed in the
42 Environmental Protection Agency (Houshani and Salehi-Lisar, 2020; Tarigholizadeh *et al.,*
43 2022).

44   Plants can uptake PAHs both through their roots and leaves, subsequently transfer these
45 compounds into food chains (Jia *et al.,* 2021). A number of factors such as concentration and
46 physicochemical properties of the compound, soil type, temperature, plant species, stage of
47 ontogenesis, and lipid content of plants can influence the rate of PAHs uptake by plants
48 (Patowary *et al.,* 2017; Wu and Zhu, 2019). Indeed, PAHs can exert their influence throughout
49 the entire lifecycle of a plant, from germination to maturity (Kummerova *et al.,* 2012).
50 Accordingly, some known effects of PAHs on plants resulted in biochemical and physiological
51 changes such as alterations in enzyme activities, reduction in photosynthesis and respiration
52 rates, generation of reactive oxygen species, and damage to cellular membranes via lipid
53 oxidation (Houshani *et al.,* 2019; Sushkova *et al.,* 2020). While substantial research has
54 addressed the well-known adverse effects of PAHs on plants, there remains a critical need to
55 delve deeper into the physiological aspects of these effects and the complicated mechanisms
56 controlling plant responses to PAH contamination and toxicity. This study aims to contribute
57 to the existing body of knowledge by providing valuable insights into these crucial areas. Maize
58 (*Zea mays* L.) is a very important annual crop with thermophilic and photophilic characteristics
59 which can adapt to various types of soils. Due to the relative resistance of maize to salt, drought
60 stress, and its short growth cycle, it is usually grown as a remediation crop in various adverse
61 conditions. Due to the importance of corn as food resource for animal, bird and human feeding;
62 the entrance of pollutants into this species can lead to some concerns on food safety (Houshani
63 et al., 2019). Therefore, we examined the effects of different concentrations of phenanthrene
64 (PHE) as one of the abundant PAHs in the environment (Tarigholizadeh *et al.,* 2022) on the

65 growth of maize in this study. Moreover, the evaluation of the biochemical, lipid content and,

66 physiological responses of plants to PHE toxicity was another aim of this study.

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68 **2. Materials and methods**

69 **2.1. Experimental design**

70 The experiments were conducted as pot cultures of plants under controlled conditions using a

71 completely randomized design (CRD) with three replications for each treatment.

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73 **2.2. The treatment**

74 In order to provide different concentrations of PHE (25, 50, 75 and, 100 ppm), the suitable

75 amount of the compound was dissolved in ethanol for each treatment and then the solutions

76 were sprayed on sterile perlite in pots. Treated perlite was used for plant cultivation after

77 evaporation of ethanol for 72 h.

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79 **2.3. Plant culture**

80 The seeds of maize (*Zea mays* L.) were obtained from the East Azerbaijan Research and

81 Education Centre for Agriculture and Natural Resources (Tabriz, Iran) and stored at 4 $^{\circ}$C until

82 cultivation. Appropriate numbers of seeds were chosen with respect to their vigor and

83 uniformity, sterilized using 1% (v/v) sodium-hypochlorite solution for 5 minutes, and washed

84 sufficiently using sterile distilled water. Then, sterilized seeds were planted in uncontaminated

85 (control) and PHE-contained perlite. After 3 days, all germinated seeds were transferred to

86 growth chambers under controlled conditions (25-30°C, 16/8 h light/dark photoperiod and

87 relative humidity of 60%) for 3 weeks. The water content of the pots was adjusted to 100%

88 field capacity every two days by sterile distilled water. After 4 and 10 days, the water of pots

89 was replaced with 50% and 100% Hoagland solution respectively.

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91 **2.4. Harvesting of plants and assays**

92 The cultivation period of plants lasted for 21 days, when the PAHs toxicity symptoms were

93 observed in plants. Biochemical and physiological assays were carried out using fresh samples

94 prior to plant harvesting. After the evaluation of shoot height and root length, the harvested

95 plants were divided into the roots and shoots. The samples were washed appropriately with

96 water, instantly dried on the towel paper and after determining the fresh weight transferred to

97 70 $^{\circ}$C chamber. The dry weight of samples was measured after 72 h.

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100 **2.5. Measurement of photosynthetic pigments content**

101 Photosynthetic pigments content (chlorophyll a, b, total chlorophyll, and total carotenoids)

102 was measured according to of Hartmut (1987). Briefly, 0.1 g of fresh leaf samples was

103 homogenized with 5 ml of >99.5% acetone using a mortar and pestle on ice bath. Homogenates

104 were filtered using a number 42 Whatman filter paper. The determination of pigment contents

105 was carried out using the absorbance of extracts at 645, 663, and 470 nm by spectrophotometer

106 (Analytic Jena, Specol 1500, and Germany).

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108 **2.6. Measurement of total protein content and antioxidant enzyme assays**

109 Approximately 0.1 g of samples were homogenized in ice-cold phosphate-buffered solution

110 (50 mM, pH = 7) using mortar and pestle. The homogenates were centrifuged at 10,000 g for

111 10 min at 4 $^{\circ}$C. The supernatants were used immediately for determination of the total soluble

112 protein content (Bradford, 1976), as well as the activities of superoxide dismutase (SOD),

113 peroxidase (POD), and catalase (CAT).

114 SOD activity was evaluated by determination of nitro-blue-tetrazolium (NBT) photoreduction

115 inhibition by extracts (Winterbourn *et al.,* 1976). The reaction mixture contained 2.7 ml sodium

116 phosphate solution (1 M, pH=7.8), 100 µl NBT (1.5 mM), 200 µl NaCN (0.3 mM), EDTA (1

117 M), 50 µl of riboflavin (0.12 mM) and 50 µl of enzyme extract. The mixture was illuminated

118 at light intensity of 40 W for 12 minutes and the absorbance of solution was recorded at 560

119 nm. The amount of the enzyme causing 50% protection of NBT photoreduction was considered

120 as one unit of SOD and the activity is expressed as U mg^{-1} protein.

121 The activity of POD was determined by Obinger and colleagues *(*1997) method. The reaction

122 mixture (1 ml) containing 300 µl of guaiacol (4 mM), 350 µl of phosphate buffer (10 mM, pH=

123 7), 300 µl of H$_2$O$_2$ (50 mM) and 50 µl of enzyme extract. The reaction was initiated by adding

124 H$_2$O$_2$ to reaction mixture and POD specific activity (expressing as U mg^{-1} protein) was

125 calculated using the extinction coefficient of 26.6 mM^{-1}cm^{-1} for guaiacol (Obinger *et al.,* 1997).

126 CAT activity was assayed according to the methods of Chance and Maehly (1955). The

127 reaction mixture contained 2.5 ml potassium phosphate buffer (50 mM, pH= 7), 1 ml H$_2$O$_2$ (10

128 mM) and 500 µl of enzyme extract. CAT activity was expressed as U mg^{-1} protein.

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130 **2.7. Measurement of malondialdehyde content (MDA)**

131 Approximately, 0.1 g of samples were homogenized with 0.1% (W/V) trichloroacetic acid

132 (TCA) and centrifuged for 5 minutes at 10000 g. Subsequently, 0.5 ml of supernatants was

133 mixed with 2 ml of 20% TCA containing 0.5% of 2-thiobarbituric acid and was heated in hot

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134 water at 95 °C for 30 minutes. Mixture was immediately transferred to ice bath and then was

135 centrifuged at 10000 g for 15 min. Finally, the absorbance of supernatant was recorded at 532

136 nm and MDA concentration was calculated according to a standard curve prepared using

137 3,1,1,3-tetraethoxy propane (0-100 nmol) and expressed as μmol g^{-1} FW (Boominathan and

138 Doran 2002).

2.8. Estimation of hydrogen peroxide (H_2O_2) content

140 0.5 ml of supernatant prepared from section 2-7 was immediately mixed with 0.5 ml of

141 phosphate buffered solution (10 mM, pH = 7) and 1 ml of KI solution (1 M) and the mixture

142 was kept at 25°C for 15 minutes. Finally, the absorbance was recorded at 390 nm and the

143 concentration of H_2O_2 was calculated according to a standard curve prepared using H_2O_2 (0-

144 120 μmol). The data was expressed as μmol g^{-1} FW (Harinasut *et al.,* 2003).

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2.9. Measurement of total flavonoid, phenol, and anthocyanin contents

147 For the measurement of total flavonoid and phenol contents, 0.1 g of sample was homogenized

148 in 80% methanol using a mortar and pestle. The homogenate was centrifuged at 10000 g for 5

149 min and then 500 μl of supernatant was mixed with 1.5 ml of 80% methanol, 100 μl of 10%

150 aluminum chloride solution, 100 μ of 1 M potassium acetate, and 2.8 ml of distilled water. After

151 40 minutes, absorbance of the mixture was measured at 415 nm compared to the control. The

152 total flavonoid content of the extract was described as mg quercetin equivalents (QE) g^{-1} FW

153 (Chang *et al.,* 2002).

154 The total phenol content was determined by the Folin–Ciocalteu method (Meda *et al.,* 2005).

155 A volume of 2.8 ml of distilled water, 100 μl of Folin–Ciocalteu reagents and 2 ml of sodium

156 carbonate 2% were added to 100 μl of supernatant and incubated for 30 minutes. The

157 absorbance of sample was measured at 720 nm compared to the control. The data was expressed

158 as mg gallic acid equivalents (GAE) g^{-1} FW.

159 To measure the total anthocyanin content, 0.02 g of dried plant sample was pulverized with 4

160 ml of hydrochloric acid containing 1% methanol in a porcelain mortar. The solution was kept

161 in a refrigerator for 24 hours and then was centrifuged for 10 minutes at 13000 g. The

162 supernatant was separated and the absorbance of the extract was measured at 530 and 657 nm

163 compared to the control (hydrochloric acid containing 1% methanol). Anthocyanin content of

164 each extract was calculated using the following equation (Mita *et al.,* 1997).

165 **A= A$_{530}$-(0.25×A$_{657}$)**

166   A is the absorbance of the solution (subscripts indicate the wavelengths at which the

167   absorbance is measured).

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169   **2.10. Measurement of soluble sugars content**

170   The soluble sugar content was determined by phenol-sulfuric acid technique (Kochert 1978).

171   5 ml of ethanol (70%) was added to 50 mg of dry sample and incubated in refrigerator for one

172   week. The sample was centrifuged at 10000 g for 15 minutes at room temperature. Then, 0.5

173   ml of the plant extract was added to 1.5 ml distilled water and then 1 ml of 5% phenol and 5 ml

174   of concentrated sulfuric acid were added to the extract. The mixture was vortexes and incubated

175   for 30 minutes at room temperature. The absorption of solution was recorded at 485 nm. The

176   data was expressed as mg g^{-1} DW.

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178   **2.11. Measurement of fatty acid**

179   **2.11.1. Preparation of phenacyl esters of fatty acids**

180   The fresh plant tissues were homogenized using chloroform-methanol solvent (2: 1) and

181   filtered by a filter paper. The filtrates was poured into the decanter, 20 ml of distilled water was

182   added and shaken slightly. The lower phase was saponified with 50 ml potassium hydroxide in

183   70% ethanol and boiled in a water-bath for 1 h. After cooling, 50 ml of distilled water was

184   added and the solution was poured into a decanter. Then, 30 ml of ether was added, shaken well

185   and the ether phase was collected (for 3 times). Then, the solution was acidified using 30 ml

186   hydrochloric acid (4N) and 30 ml of diethyl ether was added and shaken to form two phases.

187   The upper phase, which is ether containing fatty acids, was separated and the content of

188   decanter was washed by 30 ml of distilled water. The solution was dehydrated by a rotary

189   evaporator at 80 °C using absolute ethanol. Finally, 10 ml of diethyl ether was added and stored

190   in the refrigerator (Hamilton *et al.,* 1992).

191   The ether of the samples was evaporated, and one ml of methanol was added to each sample.

192   Then, 100 µl phenol phthalene was added and the solutions were alkaline by adding NaOH (0.2

193   mM). The samples were evaporated and 200 µl of 18-crown-6 (200 µl 2mg ml^{-1} in acetonitrile)

194   and 200 µl of 4-bromophenacyl bromide (200 µl 2mg ml^{-1} in acetone) were added. The samples

195   were heated hot water-bath at 80 °C for 15 min. 600 µl acetonitrile was added to all samples

196   and kept in -20 °C until analyses (Bodoprost and Rosemeyer, 2007; Rioux *et al.,* 1999; Hanist

197   *et al.,* 1988).

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200 **2.11.2. Fatty acid phenacyl ester separation on HPLC**

201 To investigate changes in fatty acids in plants treated with PHE, 6 available fatty acid

202 standards including lauric acid (C12: 0), meristic acid (C14: 0), palmitic acid (C16: 0), oleic

203 acid (C18: 1), linoleic acid (C18: 2), and arachidonic acid (C20: 4) were considered. Fatty acids

204 were prepared as a phenyl derivative and then injected into the high-performance liquid

205 chromatograph (HPLC) and standard chromatograms were obtained. Fatty acid phenacyl esters

206 were analyzed using a HPLC (KNAUER, Germany) equipped with a UV detector and a Φ 4.6

207 × 150 mm reverse phase C18 column. The UV-detector wavelength was set at 242 nm and the

208 column temperature was maintained at 40 °C. The mobile phase with flow-rate 0.8 mL min^{-1}

209 and with a gradient of methanol/acetonitrile/water started at 80:10:10 (v/v/v) which increased

210 linearly to 86:10:4 (v/v/v) in 30 min, then increased linearly to 90:10:0 (v/v/v) in 10 min holding

211 at 90:10:0 (v/v/v) for 5 min and returning to the initial conditions (80:10:10) in 5 min. the whole

212 running time for each injection was set to 50 min (Bodoprost and Rosemeyer, 2007; Rioux *et*

213 *al.,* 1999; Hanist *et al.,* 1988).

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215 **2.12. Statistical analysis**

216 All measurements were conducted with three replications and data were reported as mean ±

217 standard deviation (SD). The data were analyzed using GLM procedure by SPSS software

218 (Ver.16) and Tukey's multiple range tests was used for mean comparisons at 1 % probability

219 level. Microsoft excel 2013 software was used for the preparation of figures.

220

221 **3. Results**

222 **3.1. Growth parameters**

223 The findings obtained from this study indicate a significant decline in all assessed growth

224 parameters with an increase in PHE concentration compared to the control (*p*<0.05) (Table 1).

225 Accordingly, treatment of plants with 100 ppm of PHE led to a 68.89% and 56.07% reduction

226 in the shoot and root length. The fresh weights of shoot and root were also decreased by 80%

227 and 72.28%, respectively. Also, by the exposure to 100 ppm of PHE, dry weights of shoots and

228 roots were decreased by 85.78% and 74.83%. However, no significant differences in root dry

229 and fresh weights were seen among 50, 75 and 100 ppm of PHE treatments.

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235 **Table 1.** The effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on the growth
236 parameters of maize.

Phenanthrene (ppm)	Shoot Length (cm)	Root Length (cm)	Shoot FW (mg)	Root FW (mg)	Shoot DW (mg)	Root DW (mg)
0	0.25 [a] ± 39.1	1.59 [a] ±28	64.75 [a] ±995	35.45 [a] ±888	16.99 [a] ± 197	24.03 [a] ± 155
25	1.03 [b] ± 30.6	0.03 [b] ± 19.6	38.64 [b] ±648	17.25 [b] ±601	5.46 [b] ± 93	12.16 [ab] ±80
50	0.06 [c] ± 20.3	0.51 [b] ± 18.3	58.52 [c] ±388	13.91 [c] ± 260	9.07 [c] ± 62.6	1.36 [b] ± 52.3
75	0.21 [d] ± 13.3	0.51 [c] ± 10.6	45.3 [d] ± 220	16.13 [c] ±235	2.25 [d] ± 43.6	4.73 [b] ±47.3
100	0.26 [d] ± 13.3	0.26 [e] ± 12.3	18.81 [d] ±199	19.33 [e] ± 201	1.94 [e] ±28	2.28 [b] ± 39

237 The data represent the mean of three replications ± SD and similar upper-case letters indicates no significant
238 difference at $p < 0.05$. DW: Dry Weight, FW: Fresh Weight.
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3.2. Photosynthetic pigments content

241 Chlorophyll a content exhibited significant increases in plants treated by 50, 75, and 100 ppm
242 of PHE in comparison with the control plants, with the most significant elevation (+128.85%)
243 observed at 100 ppm PHE treatment ($p < 0.05$). In contrast, all applied levels of PHE
244 significantly decreased chlorophyll b content. Such content in the plants treating with 25, 50,
245 75, and 100 ppm of PHE were decreased to 27.04, 72.26, 75.71, and 76.36 %, respectively.
246 Moreover, the highest value of carotenoids (+126.98 %) was recorded in plants exposed to 100
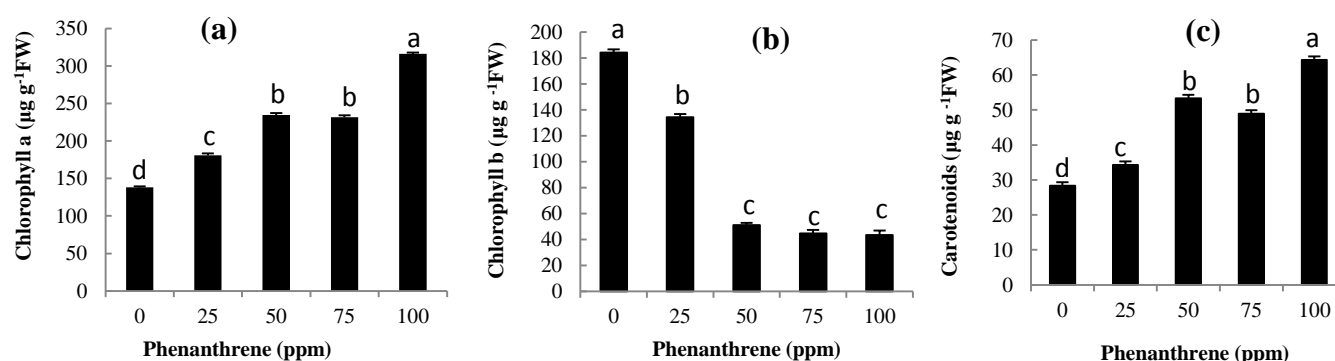247 ppm of PHE (Fig. 1).
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250 **Fig 1.** The effects of different concentrations of phenanthrene on photosynthetic pigments content of maize plants.
251 (a) Chlorophyll a, (b) Chlorophyll b, and (c) Carotenoids. The data represent the mean of three replications and
252 error bars indicate SD. The same letters above the bars indicate no significant differences ($p<0.05$).
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3.3. The content of malondialdehyde (MDA)

255 Malondialdehyde was measured as an indicator to assess the severity of oxidative stress
256 induced by PHE. A statistically significant increase in MDA content was found after exposure
257 to PHE ($p<0.05$). The results also indicated that MDA content in the shoots of treated plants
258 exceeded that in the roots. Compared to the control, MDA contents of the shoots were increased
259 by 86.1, 104.4, 115.15, and 128.81% when plants treated with 25, 50, 75, and 100 ppm PHE,
260 respectively. A similar trend was also seen in the MDA content in the roots, with the highest

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261 increase occurring at 50 ppm PHE exposure (Fig. 2). Differences in MDA contents were not

262 statistically significant among treatments with higher concentrations of PHE.
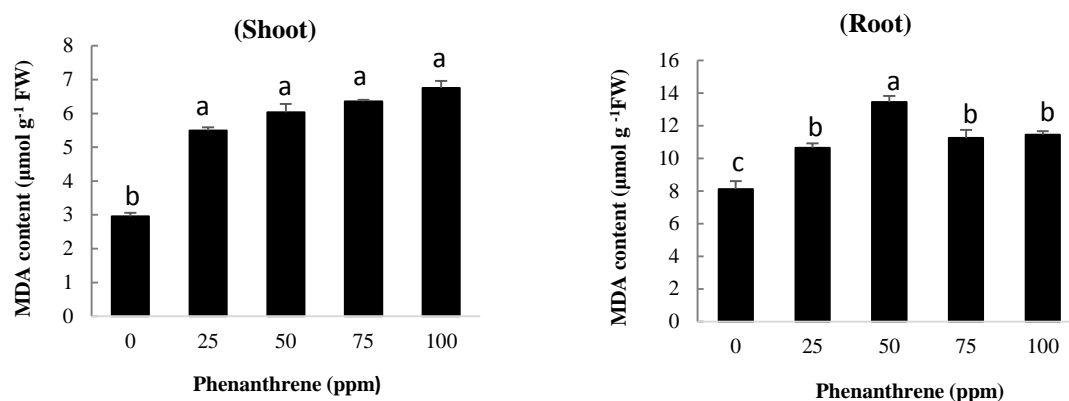
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Fig 2. The effects of different concentrations of phenanthrene on malondialdehyde (MDA) contents of maize plants. The data represent the mean of three replications and error bars indicate SD. The same letters above the bars indicate no significant differences ($p<0.05$).

3.4. Hydrogen peroxide (H_2O_2) content

269 Phenanthrene had a significant effect on the content of H_2O_2 in treated plants. The enhanced

270 content of H_2O_2 in plants had been observed through the increasing concentrations of PHE

271 ($p<0.05$). The H_2O_2 contents in the shoots of plants treated with 25, 50 75, and 100 ppm of PHE

272 were increased by 160.67, 171, 171.58, and 207.21%, respectively. The values for the roots of

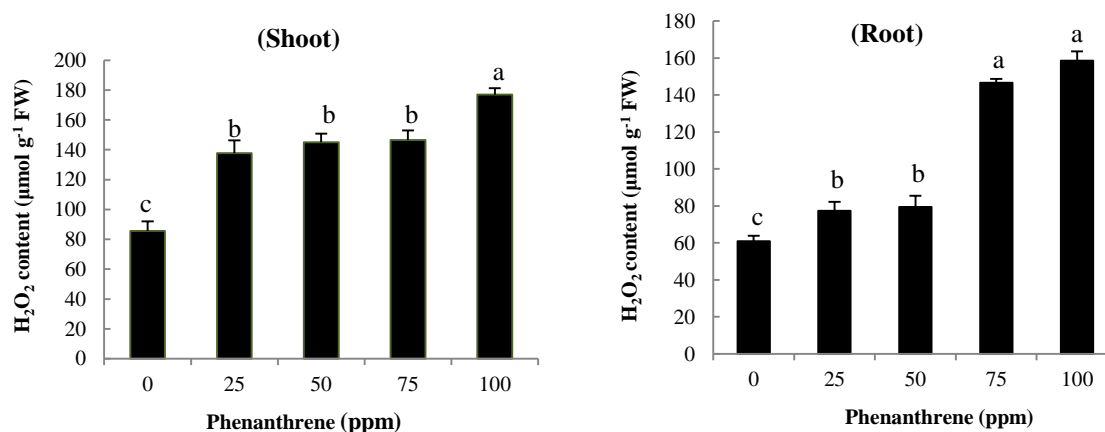273 the same plants recorded increases of 126.73, 130.01, 240.38, and 260.17%, respectively (Fig.

274 3).

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Fig 3. The effects of different concentrations of phenanthrene on hydrogen peroxide (H_2O_2) contents of maize plants. The data represent the mean of three replications and error bars indicate SD. The same letters above the bars indicate no significant differences (p<0.05).

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3.5. Soluble sugars content

The results revealed that PHE had significant effect on the content of soluble sugars ($p<0.05$). The content of soluble sugars of both shoots and roots were decreased at various levels of phenanthrene in comparison with the control, and the greatest reduction in soluble sugars was observed in the shoots and roots of plants subjected to 50 (38.77%) and 100ppm (46.07%), respectively($p<0.05$) (Fig. 4).
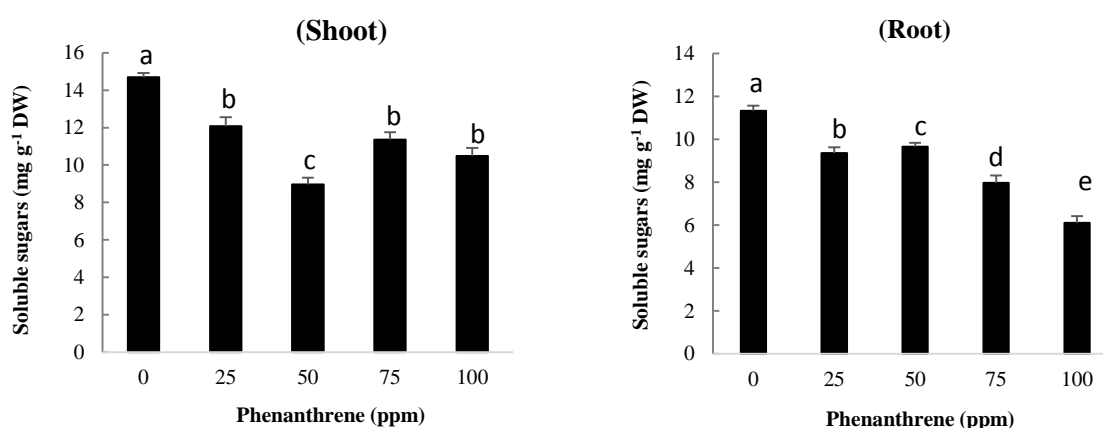

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Fig 4. The effects of different concentrations of phenanthrene on soluble sugars contents of maize plants. The data represent the mean of three replications and error bars indicate SD. The same letters above the bars indicate no significant differences ($p<0.05$).

3.6. Activity of antioxidant enzymes

The changes in the activity of CAT, POD, and SOD within the shoot and root of maize plants after treatment with PHE were shown in Table 2. The CAT activity was declined in the shoots of the plants exposed to 50, 75, and 100 ppm of PHE. Conversely, in the roots, CAT activity responded differently, with significant increases of 91.55%, 86.88%, and 48.44% observed at 50, 75, and 100 ppm of phenanthrene, respectively, compared to the control ($p<0.05$).

POD activity in the shoots of treated plants with various concentrations of PHE was significantly higher in comparison with the control. The highest POD activities were observed in plants treated by 50 (+112%) and 75 ppm (+128%) of PHE, respectively ($p<0.05$). In roots only significant increase in POD activity was seen at 75 ppm (+57.26%) of phenanthrene (Table 2).

PHE treatments affected SOD activity in the shoots. SOD activity was increased at a low concentration (25 ppm) of PHE and then declined at higher levels (75 and 100 ppm). Up to 28% increase in SOD activity was detected in plants treated with 25 ppm of PHE, but in plants treated by 75 and 100 ppm of PHE, SOD activity was decreased by 47.16 and 54.91%, respectively,

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309 compared with the control. Interestingly, SOD activity in the root was significantly higher in

310 plants treated with different PHE concentrations and the highest activity was found in plants

311 treated with 75 ppm of phenanthrene ($p < 0.05$) (Table 2).

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313 **Table 2**. The effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on antioxidant
314 enzymes activity (U mg^{-1} protein) in the shoot and root of maize plants.

Phenanthrene (ppm)	Shoot			Root		
	CAT	POD	SOD	CAT	POD	SOD
0	0.002 [a] ± 0.183	0.052 [d] ± 2.5	0.112 [b] ± 15.9	0.032 [d] ± 0.452	7.69 [b] ±80.5	1.6 [c] ± 47.8
25	0.051 [a] ± 0.183	0.031 [c] ±3.5	2.35 [a] ± 20.5	0.029 [c] ± 0.576	5.98 [bc] ± 65.7	3.25 [c] ± 47.8
50	0.021 [c] ± 0.108	0.022 [a] ± 5.3	1.95 [b] ± 16.1	0.033 [a] ± 0.862	2.45 [b] ± 79.6	2.33 [b] ±81.3
75	0.019 [bc] ± 0.121	0.041 [a] ±5.7	1.22 [c] ± 8.43	0.127 [a] ± 0.841	6.26 [a] ± 126	6.9 [b] ±90.7
100	0.014 [b] ± 0.137	0.045 [ab] ± 4.4	0.521 [c] ± 8.61	0.012 [b] ± 0.668	7.33 [c] ± 46.6	3.1 [a] ±117

315 The data represent the mean of three replications ±SD and similar upper case letters indicates no significant
316 difference at $p < 0.05$. CAT: catalase, POD: peroxidase and SOD: superoxide dismutase.

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3.7. Total flavonoids, phenols and anthocyanin contents

319 By increasing the PHE concentration, total flavonoids and phenol contents in shoots were

320 significantly reduced compared to the control plants ($p < 0.05$). In roots, flavonoids content was

321 increased after exposure to 25 ppm of PHE, but no statistically significant difference was

322 evident between the plants treated with higher levels of PHE and the control plants. Except for

323 the level of 50 ppm, there was no significant increase detected in root phenol content at the

324 other concentrations. It was also seen that anthocyanin content was increased in the shoots

325 compared to the control, with the highest value (121.31%) recorded at the concentration of 100

326 ppm, but anthocyanin in the roots was decreased through the increasing levels of PHE up to 75

327 ppm (Table 3).

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329 **Table 3.** The effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on total flavonoid
330 (mg EQ g^{-1} FW), total phenol (mg EGA g^{-1} FW) and, total anthocyanin contents (mg g^{-1} FW) in the shoot and root
331 of maize plant.

Phenanthrene (ppm)	Shoot			Root		
	Total Flavonoid	Total Phenol	Total Anthocyanin	Total Flavonoid	Total Phenol	Total Anthocyanin
0	0.002 [a] ±0.292	0.106 [a] ± 4.30	0.002 [c] ± 0.61	0.001 [b] ± 0.027	0.184 [b] ± 2.86	0.001 [b] ± 1.01
25	0.009 [b] ± 0.207	0.076 [b] ± 3.72	0.012 [a] ± 1.25	0.009 [a] ± 0.040	0.051 [b] ± 2.71	0.049 [bc] ± 0.86
50	0.004 [b] ± 0.189	0.159 [b] ± 3.47	0.019 [a] ± 1.33	0.005 [b] ± 0.030	0.092 [a] ± 3.18	0.031 [c] ± 0.61
75	0.003 [c] ± 0.149	0.184 [c] ± 2.99	0.012 [a] ± 1.25	0.001 [b] ± 0.028	0.106 [c] ± 1.98	0.089 [bc] ± 0.79
100	0.005 [d] ± 0.128	0.046 [d] ± 2.69	0.048 [a] ± 1.35	0.002 [b] ± 0.028	0.205 [c] ± 2.81	0.096 [a] ± 1.3

332 The data represent the mean of three replications ±SD and similar upper case letters indicates no significant
333 difference at $p < 0.05$.

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3.8. Fatty acid concentration

336 Samples of both shoots and roots from control maize plants and those exposed to PHE at 50

337 ppm were injected to HPLC in three replications. The results of fatty acid analysis showed that

338 oleic acid is the most abundant fatty acid in both shoots and roots of maize. Alongside oleic

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339 acid, other fatty acids were also detected in maize tissues including lauric acid, meristic acid,

340 and palmitic acid and due to the lack of identification of arachidonic acid and linoleic acid fatty

341 acids; it was not possible to investigate the changes in the concentration of these fatty acids.

342 PHE treatment significantly decreased the concentrations of lauric acid, meric acid, palmitic

343 acid, and oleic acid in shoots of maize plants compared to the control plants ($p \leq 0.05$). In

344 contrast, the roots of PHE-treated plants exhibited a significant increase in the concentrations

345 of lauric acid, meric acid, and palmitic acid by 79.79, 36.36, and 23.58%, respectively.

346 However, oleic acid concentration in the roots decreased in response to phenanthrene treatment

347 ($p \leq 0.05$) (Table 4).

348
349 **Table 4.** The effect of concentration of phenanthrene (50 ppm) on fatty acid concentration ($\mu g\ g^{-1}FW$) in the
350 shoot and root of maize plant.

Fatty acid ($\mu g\ g^{-1}FW$)	Phenanthrene (ppm)	Shoot	Root
Lauric acid	0	$0.485^{a} \pm 0.012$	$0.268^{b} \pm 0.013$
	50	$0.098^{b} \pm 0.028$	$3.665^{a} \pm 0.554$
Myristic acid	0	$0.716^{a} \pm 0.025$	$0.885^{b} \pm 0.007$
	50	$0.047^{b} \pm 0.003$	$1.306^{a} \pm 0.125$
Palmitic acid	0	$0.543^{a} \pm 0.002$	$0.106^{b} \pm 0.004$
	50	$0.007^{b} \pm 0.009$	$0.131^{a} \pm 0.005$
Oleic acid	0	$196.83^{a} \pm 7.57$	$174.80^{a} \pm 5.07$
	50	$62.151^{b} \pm 6.21$	$158.85^{b} \pm 9.45$

351 The data represent the mean of three replications ±SD and similar upper-case letters indicates no significant
352 difference at $p < 0.05$.
353

3.9. Analysis correlation

355 The correlation analysis conducted to assess the relationship between MDA and H_2O_2

356 contents, as well as between CAT and SOD activities in both shoots and roots (at significance

357 levels of 1% and 5%), revealed a negative correlation coefficient between enzyme activities

358 and MDA and H_2O_2 contents. These findings demonstrated that CAT and SOD involving in

359 plants resistance to oxidative stress are induced by PHE toxicity. Moreover, no correlation was

360 seen between POD activity and MDA and H_2O_2 contents of both shoots and roots of maize

361 (Table 5).

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Table 5. Statistical analysis for correlation between the activity of antioxidant enzyme and MDA and H_2O_2 contents in the shoot and root of maize plant.

	CAT Shoot	CAT Root	POD Shoot	POD Root	SOD Shoot	SOD Root	MDA Shoot	MDA Root	H_2O_2 Shoot	H_2O_2 Root
H_2O_2 Root	-0.222^{ns}	-0.308^{ns}	-0.024^{ns}	-0.267^{ns}	-0.736^{**}	-0.341^{ns}	0.599^{*}	0.547^{*}	0.747^{**}	1
H_2O_2 Shoot	-0.541^{*}	0.002^{ns}	-0.185^{ns}	-0.181^{ns}	-0.830^{**}	-0.435^{ns}	0.904^{**}	0.822^{**}	1	
MDA Root	-0.690^{**}	0.314^{ns}	0.009^{ns}	0.189^{ns}	-0.893^{**}	-0.434^{ns}	0.892^{**}	1		
MDA Shoot	-0.539^{*}	0.110^{ns}	-0.180^{ns}	-0.011^{ns}	-0.816^{**}	-0.391^{ns}	1			
SOD Root	0.140^{ns}	0.300^{ns}	0.647^{**}	-0.078^{ns}	0.555^{*}	1				
SOD Shoot	0.624^{*}	-0.075^{ns}	-0.306^{ns}	-0.166^{ns}	1					
POD Root	-0.274^{ns}	0.697^{**}	0.246^{ns}	1						
POD Shoot	-0.267^{ns}	0.438^{ns}	1							
CAT Root	-0.446^{ns}	1								
CAT Shoot	1									

Notes: **Correlation is significant at 0.01 levels, *Correlation is significant at 0.05 levels, ^{ns} correlation is not significant.

4. Discussion

PAHs constitute a substantial class of pollutants recognized for their detrimental effects on both animals and plants. These compounds can adversely affect plants growth, disrupt photosynthesis, and inhibit enzyme activities (Ahammed *et al.,* 2012; Sushkova *et al.,* 2021). In the current study, PHE treatments negatively affected the growth parameters, especially at higher concentrations. The reduction of growth and biomass production in the presence of PAHs was consistent with earlier studies involving different plant species such as *Arabidopsis thaliana* (Alkio *et al.,* 2005; Liu *et al.,* 2009), rice (Li and Yi, 2012), and tomato (Ahammed *et al.,* 2012). PHE and possibly other compounds of this group can disrupt root development in the early stages of plant growth. Since roots play a crucial role in mineral nutrition, this disruption can lead to growth impairment (Dupuy *et al.,* 2016).

Photosynthetic pigments content was changed in a different way by phenanthrene concentrations. Accordingly, chlorophyll a and carotenoids contents were increased and chlorophyll b content was decreased. Elevated levels of reactive oxygen species (ROS) can contribute to chlorophyll decline, thereby impairing photosynthesis. This is associated with the significant reduction of the light-harvesting complex protein within photosystem II under stressful conditions. Part of this complex protein, chlorophyll b, is embedded in the chloroplast membrane, and increased ROS levels within the chloroplast, resulting from oxidative stress, accelerate chloroplast membrane degradation. Thus, stress-induced degradation of complex protein results in a reduction of chlorophyll b (Sharma *et al.,* 2020; Moustakas *et al.,* 2022). On the other hand, pigments accumulation could be due to negative effect of PHE on growth. As plant growth decreases, the concentration of the pigmentation tends to increase.

13

399   Our results have shown that H_2O_2 and MDA contents in both shoots and roots enhanced

400   through increasing PHE levels. Therefore, MDA accumulation resulting from oxidative stress

401   and ROS accumulation was a reliable marker for determining of the negative effect of PHE on

402   the growth of maize plants. Generally, plants equipped with enzymatic and non-enzymatic

403   mechanisms for scavenging ROS accumulation under oxidative stress (Tarigholizadeh *et al.,*

404   2021). Several enzymes like SOD, CAT, and POD as enzymatic mechanisms play important

405   roles during seed germination. SOD, as the first line of defense against ROS, acts on superoxide

406   radicals producing under stress conditions, catalyzing their conversion into H_2O_2 and O_2

407   (Hasanuzzaman *et al.,* 2021). CAT is the primary H_2O_2 scavenging enzyme in plant cells (Li

408   and Yi, 2012). In this study, CAT, POD, and SOD activities were increased in the roots, while

409   CAT and SOD activities were decreased in the shoots by different levels of PHE. Also, the

410   correlation analysis (Table 4) demonstrated a negative correlation between H_2O_2 and MDA

411   contents, as well as between CAT and SOD activities, in both the shoot and root of maize,

412   emphasizing the role of these enzymes in ROS detoxification to mitigate stress conditions and

413   enhance plant resistance to oxidative stress. Moreover, the decrease in SOD and CAT activities

414   induced by PHE occurred exclusively in the shoots, while phenol and flavonoid levels

415   contributed to this decrease in shoots and roots, consequently reducing the H_2O_2 scavenging

416   ability and leading to lipid peroxidation. Therefore, it can be said that in the maize plant, the

417   antioxidant system of maize in the shoots was not strong enough to eliminate all produced ROS

418   at high concentrations, resulting in oxidative damage, diminished photosynthesis, reduced

419   soluble sugar content, and compromised growth parameters. Similar results have been reported

420   for sunflower, alfalfa, and wheat plants (Salehi-Lisar and Deljoo, 2015).

421   PHE significantly influenced soluble sugar content ($p < 0.05$), with reduced levels detected in

422   both shoots and roots. This reduction can be linked to the decreased demand for photosynthetic

423   materials due to reduced root growth (Table 1). In addition, lower carbohydrate content in roots

424   can be attributed to higher consumption of energy for resistance of PHE toxicity. Carbohydrates

425   in plants, in addition to energy production, regulate various gene expressions (Rolland *et al.,*

426   2006) and may have antioxidant activity (Lang-Mladek *et al.,* 2010).

427   Regarding fatty acids, the decrease in oleic acid concentration in the roots of maize plants

428   treated with PHE, compared to the control plants, may be caused by the oxidative stress induced

429   by PHE. Therefore, the increases of saturated fatty acids are likely to moderate stress conditions

430   of root. Moreover, their concentrations treated with pollutants decreased in the shoots and this

431   can be attributed to lipid peroxidation in the shoots confirming by MDA results. On the other

432 hand, induced stress in the shoots of plants treated with contaminants decreased photosynthesis

433 and soluble sugars, which may be due to carbon dioxide fixation decrease in the Calvin cycle.

434 The triosphosphates of this cycle by changing into acetyl-CoA provide the primary substrate

435 required for biosynthesis of fatty acids (Du *et al.*, 2020). Therefore, a decrease in photosynthesis

436 and thiophosphates may also be another reason for the reduction in fatty acids in the shoots.

437

5. Conclusions

439 Based on the obtained results, high concentrations of phenanthrene (75 and 100 ppm) have

440 inhibitory effects on plants. Reduced growth, lowere chlorophyll b content, and elevated

441 contents of MDA and H_2O_2 in root are some negative effects of phenanthrene. Further, the

442 increase in the activity of antioxidant enzymes due to phenanthrene treatment indicated the

443 oxidative stress caused by this compound. However, the decrease in the activity of CAT and

444 the non-destruction of the produced hydrogen peroxide due to stress indicated a decrease in the

445 resistance of the maize plant to this compound. On the other hand, the decrease in the amount

446 of MDA in the shoot and then the increase in the roots also demonstrated higher accumulation

447 of phenanthrene in the roots leading to weakening of plants root system as well as disordering

448 in water and nutrient uptake and finally reduction in the plant growth.

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References

455 1. Ahammed, G.J., Yuan, H.L., Ogweno, J.O., Zhou, Y.H., Xia, X.J., Mao, W.H., Shi, K. and
456 Yu, J.Q. 2012. Brassinosteroids alleviates phenanthrene and pyrene phytotoxicity with
457 increasing detoxification activity and photosynthesis in tomato. *Chemosphere*; **86:** 546-555.

458 2. Ansari, F., Ahmad, A. and Rafatullah, M. 2023. Review on bioremediation technologies of
459 polycyclic aromatic hydrocarbons (PAHs) from soil: Mechanisms and future perspective. *Int.*
460 *Biodeter. Biodegr;* **179:** 105582.

461 3. Bodoprost, J. and Rosemeyer, H. 2007. Analysis of phenacyl ester derivatives of fatty acids
462 from human skin surface sebum by reversed-phase HPLC: chromatographic mobility as a
463 function of physic-chemical properties. *Int J Mol Sci*; **8:** 1111-1124.

464 4. Boominathan, R. and Doran, P.M. 2002. Ni induced oxidative stress in root of the Ni hyper
465 accumulator, *Alyssum bertoloni*. *New Phytol;* **156:** 202-205.

466 5. Bradford, M.M. 1976. A rapid and sensitive method for the quantization of microgram
467 quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem;* **72:** 248-254.

468 6. Chance, B. and Mealy, A.C. 1955. Assay of catalases and peroxidases. *Methods Enzymol;*
469 **11:** 764-755.

470 7. Chang, C., Yang, M., Wen, H. and Chern, J. 2002. Estimation of total flavonoid content in
471 proposes by two complementary colorimetric methods. *J. Food. Drug .Anal;* **10:** 178-182.
472 8. Du,Y., Zhao,Q., Chen,L., , Yao, X., Zhang, W., Zhang, B. and Xie, F. 2020. Effect
473 of drought stress on sugar metabolism in leaves and roots of soybean seedlings. *Plant Physiol.*
474 *Biochem;* **146:** 1-12.
475 9. Dupuy, J., Legliz, P., Vincent, Q., Zelko, I., Mustin, Ch., Ouvard, S. and Sterckeman, T.
476 2016. Effect and localization of phenanthrene in maize roots. *Chemospher;* **149:** 130-136.
477 10. Gitipour, S., Sorial, G.A., Ghasemi, S. and Bazyari, M. 2018. Treatment technologies for
478 PAH-contaminated sites: a critical review. *Environ. Monit. Assess;* **190:** 1-17.
479 11. Hamilton, S., Hamilton, R.J. and Sewell, P. 1992. Extraction of lipids and derivative
480 formation. In: Lipid Analysis, pp. 47-48. Eds. S. Hamilton and R. J. Hamilton. Oxford
481 University Press. NY.
482 12. Hanis, T., Smrz, M., Klir, P., Macek, K., Klima, J., Base, J. and Deyl, Z. 1988.
483 Determination of fatty acids as phenacyl esters in rat adipose tissue and blood vessel walls by
484 high-performance liquid chromatography. *J. Chromatogr;* **452:** 443-457.
485 13. Harinasut, P., Poonsopa, D., Roengmongkol, K. and Charoensalaporn, R .2003. Salinity
486 effects on antioxidant enzymes in mulburg cultivar. *Sci. Asia;* **29:**109-113.
487 14. Hartmut K.L. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic
488 biomembranes, *Methods in Enzymology*, Lester Packer, R.D., Ed., New York: Academic Press,
489 pp. 350-382.
490 15. Hasanuzzaman, M., Raihan, M.R.H., Masud, A.A.C., Rahman, K., Nowroz, F. and
491 Rahman, M. 2021. Regulation of reactive oxygen species and antioxidant defense in plants
492 under salinity. *Int. J. Mol. Sci;* **22:** 9326-9340.
493 16. Houshani, M. and Salehi-Lisar, Y. 2020. Agronomic crop responses and tolerance to
494 polycyclic aromatic hydrocarbon toxicity. In: Hasanuzzaman M (ed) Agronomic Crops: Stress
495 responses and tolerance. Springer, Nature Singapore, pp: 265-284.
496 17. Jia, J., Bi, C., Jin, X., Zeng, Y., Deng, L., Wang, X. and Chen, Z. 2021. Uptake,
497 translocation, and risk assessment of PAHs in contaminated soil-air-vegetable systems based
498 on a field simulation experiment. *Environ. Pollut;* **271:** 116361- 116368.
499 18. Kochert, G., Helebust, J.A. and Craig, J.S. 1978 Physiological methods: Carbohydrate
500 determination by the phenol sulfuric acid method, *Physiological methods*, Kochert G, Eds.,
501 Cambridge University: Press Cambridge, pp. 13-16.
502 19. Kumar, M., Bolan, N. S., Hoang, S. A., Sawarkar, A. D., Jasemizad, T., Gao, B. and
503 Rinklebe, J. 2021. Remediation of soils and sediments polluted with polycyclic aromatic
504 hydrocarbons: to immobilize, mobilize, or degrade? *J.Hazard. Mater;* **420:** 126534.
505 20. Kummerova, M., Zezulka, S., Vanova, L. and Fiserova, H. 2012. Effect of organic
506 pollutant treatment on the growth of pea and maize seedlings. *Cent. Eur. J. Biol;* **7:** 159-166.
507 21. Lang-Mladek, C., Popova, O., Kiok, K., Berlinger, M., Rakic, B. and Aufastez, W. 2010.
508 Transgenerational inheritance and resetting of stressinduced loss of epigenetic gene silencing
509 in Arabidopsis. *Mol. Plant;* **3:** 594-602.
510 22. Li, L.H. and Yi, H.L. 2012. Effect of sulfur dioxide on ROS production, gene expression
511 and antioxidant enzyme activity in Arabidopsis plants. *Plant Physiol. Biochem;* **58:** 46-53.
512 23. Liu, H., Weisman, D., Yuan-bei, Y., Cui, B., Huang, Y., Colon-Carmona, A. and Wang,
513 Z. 2009. An oxidative stress response to polycyclic aromatic hydrocarbon exposure is rapid and
514 complex in *Arabidopsis thaliana*. *Plant Sci;* **176:** 375- 382.
515 24. Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G. 2005.
516 Determination of the total phenolic, flavonoid and pralin contents in Burkina Fasan honey, as
517 well as their scavenging activity. *Food Chem;* **91:** 571-577.

16

25. Mita, S., Murano, N., Akaike, M. and Nakamura, K. 1997. Mutants of *Arabidopsis thaliana* with pleiotropic effects on the expression of the gen for beta-amylase and on the accumulation of anthocyanin that is inducible by sugars. *Plant J;* **11:** 841-851.

26. Moustakas, M., Moustaka, J. and Sperdouli, I. 2022. Hormesis in photosystem II: a mechanistic understanding. *Curr. Opin. Toxicol;* **29:** 57-64.

27. Obinger, C., Maj, M., Nicholls, P. and Loewen, P. 1997. Activity, peroxide compound formation, and heme d synthesis in *Escherichia coli* HPII catalase. *Arch. Biochem. Biophys;* **342:** 58-67.

28. Patowary, R., Patowary, K., Devi, A., Kalita, M.C. and Deka, S. 2017. Uptake of total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbons (PAHs) by *Oryza sativa* L. grown in soil contaminated with crude oil. *Bull. Environ. Contam. Toxicol;* **98:** 120-126.

29. Rioux, V., Catheline, D., Bouriel, M. and Legrand, P. 1999. High performance liquid chromatography of fatty acids as naphthacyl derivatives. *Anal;* **27:** 186-193.

30. Rolland, F., Baena-Gonzalez, E. and Sheen, J. 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annu. Rev. Plant. Biol;* **57:** 675-709.

31. Salehi-Lisar, S.Y. and Deljoo, S. 2015. Physiological effect of phenanthrene on *Triticum aestivum* L., *Helianthus annus* and *Medicago sativa. Eur. Asia. J. Bio. Sci;* **9:** 29-37.

32. Sharma, A., Kumar, V., Shahzad, B., Ramakrishnan, M., Singh Sidhu, G. P., Bali, A. S. and Zheng, B. 2020. Photosynthetic response of plants under different abiotic stresses: a review. *J. Plant Growth Regul;* **39:** 509-531.

33. Sushkova, S., Minkina, T., Tarigholizadeh, S., Antonenko, E., Konstantinova, E., Gülser, C., Dudnikova, T., Barbashev, A. and Kizilkaya, R. 2020. PAHs accumulation in soil-plant system of *Phragmites australis* Cav. in soil under long-term chemical contamination. *Eurasian J. Soil Sci;* **9:** 242-253.

34. Sushkova, S., Minkina, T., Tarigholizadeh, S., Rajput, V., Fedorenko, A., Antonenko, E., Dudnikova, T., Chernikova, N., Yadav, B.K. and Batukaev, A. 2021. Soil PAHs contamination effect on the cellular and subcellular organelle changes of *Phragmites australis* Cav. *Environ. Geochem. Health;* **43:** 2407–2421.

35. Tarigholizadeh, S., Motafakkera, R., Salehi-Lisar, S.Y. and Kazemi, E.M. 2021. High resistance of *Panicum miliaceum* L. to phenanthrene toxicity based on growth response and antioxidant system assessment. *Acta. Agric. Slov;* **117:**1–13.

36. Tarigholizadeh, S., Motafakkerazad, R., Salehi-Lisar, S.Y., Mohajel Kazemi, E., Sushkova, S. and Minkina, T. 2022. Phenanthrene uptake and translocation by *Panicum miliaceum* L. tissues: an experimental study in an artificial environment. *Environ. Geochem. Health;* **1:** 1-12.

37. Thacharodi, A., Hassan, S., Singh, T., Mandal, R., Khan, H.A., Hussain, M.A. and Pugazhendhi, A. 2023. Bioremediation of polycyclic aromatic hydrocarbons: An updated microbiological review. *Chemosphere;* 138498.

38. Winterbourn, C.C., Mcgrath, B.M. and Carrell, R.W. 1997. Reactions involving superoxide and normal and unstable hemoglobins. *Biochem J;* **155:** 493-502.

39. Wu, X. and Zhu, L. 2019. Prediction of organic contaminant uptake by plants: modified partition-limited model based on a sequential ultrasonic extraction procedure. *Environ.Pollut;* **246:** 124-130.

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565 پاسخ های فیزیولوژیکی و بیوشیمیایی ذرت (*Zea mays*) به سمیت فنانترن

566 مهدیه هوشانی، سید یحیی صالحی لیسار، علی موفقی، و روح الله متفکر زاد

567 هیدروکربن‌های آروماتیک چند حلقه‌ای (PAHs)، دسته‌ای از آلاینده‌های آلی، مشاهده شده‌اند که اثرات مضری بر رشد

568 گیاه و فرآیندهای فیزیولوژیکی مختلف دارند. در این تحقیق اثر غلظت های مختلف فنانترن (0، 25، 50، 75 و 100 پی

569 پی ام) بر پارامترهای رشد، رنگدانه های فتوسنتزی، برخی آنزیم های آنتی اکسیدانی و برخی ترکیبات بیوشیمیایی گیاه

570 ذرت مورد بررسی قرار گرفت. آزمایش ها به صورت کشت گلدانی گیاهان در شرایط کنترل شده در قالب طرح کاملاً

571 تصادفی (CRD) با سه تکرار برای هر تیمار انجام شد. افزایش غلظت فنانترن تمام پارامترهای رشد مورد مطالعه را

572 کاهش داد و محتوای رنگدانه فتوسنتزی را به طور قابل توجهی افزایش داد. فنانترن منجر به افزایش فعالیت کاتالاز،

573 پراکسیداز و سوپراکسید دیسموتاز در ریشه شد اما در اندام هوایی تنها فعالیت پراکسیداز افزایش یافت. علاوه بر این،

574 غلظت‌های بالاتر فنانترن با افزایش سطح مالون دی‌آلدئید و پراکسید هیدروژن همراه با کاهش محسوس در محتوای قند

575 محلول در هر دو بافت اندام هوایی و ریشه همراه بود (p < 0/05). همچنین افزایش غلظت فنانترن در اندام هوایی باعث

576 کاهش محتوای کل فنل و فلاونوئید نسبت به آنتوسیانین شد. تیمار فنانترن منجر به کاهش قابل توجه غلظت اسید لوریک،

577 مریک اسید، اسید پالمیتیک و اسید اولئیک در اندام هوایی ذرت شد. در نتیجه به نظر می رسد که غلظت بالای فنانترن

578 باعث ایجاد استرس اکسیداتیو در ذرت می شود و گیاهان سیستم آنتی اکسیدانی آنزیمی خود را برای تعدیل شرایط تنش

579 بهبود می بخشند. علاوه بر این، آسیب غشای سلولی توسط فنانترن منجر به تضعیف سیستم ریشه گیاهان و اختلال در

580 جذب آب و عناصر غذایی و در نهایت کاهش رشد گیاه می شود.

581