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ORIGINAL ARTICLE

Portulaca oleracea Exposed to Polycyclic Aromatic Hydrocarbon Pollution: Mapping Down Nutraceutical and Histochemical Changes

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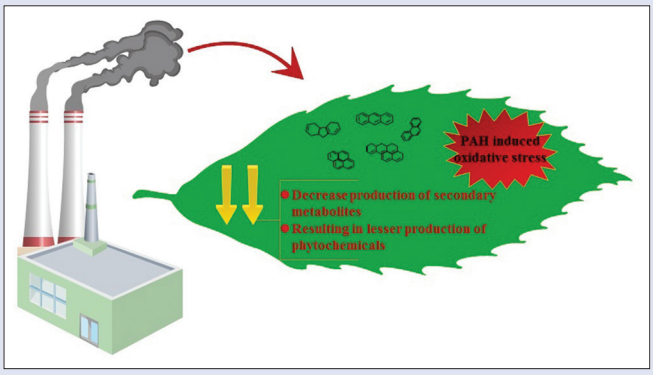
ABSTRACT

Aim: Polycyclic aromatic hydrocarbons (PAHs) are extremely carcinogenic environmental pollutants which have now become a global environmental problem. Such pollutants can accumulate in soil and can adversely affect the growth and physiology of plants. The current study aims to investigate the effect of such pollutants on the plants secondary metabolism with special emphasis on the production of nutraceutical principles. **Materials and Methods:** A well-known dietary plant *Portulaca oleracea* was collected from agricultural land in close vicinity to thermal power units, and results were compared to a background sample. Pigment estimation, evaluation of total phenolics/flavonoids, and Non-enzymatic antioxidant principles, namely ascorbic acid and α-tocopherol were carried out. Primary metabolites, such as total proteins and carbohydrates, were also estimated. The identification of individual phenolic and flavonoid principles were also carried out. Results were supported with real-time histochemical evidence and scanning electron microscopy (SEM) studies. **Results:** Chlorophyll a showed a significant reduction of 40.85% when compared to control and so was the case with β-carotene, which recorded a decline of 33.5% when compared to control. Total phenolics and flavonoids, ascorbic acid, and α-toopherol showed a decline of 42.25%, 28.17%, 45.5%, and 81.9%, respectively, when compared to control. Gallic acid, p-coumaric acid, ferulic acid, naringenin, quercetin, ellagic acid, and rutin were found to be significantly lesser in pollution exposed plants. Significant cell death was evident from histochemical studies as well, along with blockage of stomata as reflected from SEM studies. **Conclusion:** *P. oleracea* exposed to PAH pollution showed a significant decline in the production of phytochemicals with special emphasis on nutraceutical contents.

Key words: Flavonoids, oxidative stress, phenolics, polycyclic aromatic hydrocarbon, *Portulaca oleracea*

SUMMARY

- Nutraceutical profiling of an edible plant *Portulaca oleracea* subjected to polycyclic aromatic hydrocarbon (PAH) pollution grown in agricultural land in close vicinity to thermal power units have been done to understand the impact of PAH pollution on plants secondary metabolism
- Significant reduction of phenolic and flavonoid principles took place for plants exposed to PAH pollution with increased cell death and stomata blockage.



Abbreviations used: PAH: Polycyclic aromatic hydrobarbons; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; DMSO: Dimethyl sulfoxide; HPLC: High-performance liquid chromatography; MAE: Microwave-assisted extraction; TPC: Total phenolic content; TFC: Total flavonoid content; SEM: Scanning electron microscopy; ROS: Reactive oxygen species; PAL: Phenylalanine ammonia-liaza; LC-MS: Liquid chromatography-mass spectrometer; DAD: Diode array detector; GAE: Gallic acid equivalents; QUE: Quercetin equivalents.

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INTRODUCTION

Phenolics are the most liked bioactives by natural product researchers because of their versatility in structure and wide spectrum of bioactivities. Research status of phenolics has been critically reviewed by our research group, which shows that phenolics have a positive intervention in almost all diseases.[1] Phenolics owing to its antioxidant activity, play a critical role in maintaining the redox status of the biological system.[2] Free radicals are known to play a vital role in the pathological progression of many diseases and can very well exacerbate any pathological condition.[3] Phenolics and flavonoid principles which are consumed by human beings through daily intake of leafy vegetables and fruits not only fulfils the nutritional requirements of the body but also helps to maintain the oxidative homeostasis.[4] Leafy vegetables are also part of India's cultural heritage and are often used in various customs, traditions, and food culture of common Indian household. In this regard, *Portulaca oleracea* L.(Purslane) is an annual green herb with edible succulent stems

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and leaves with spinach-like taste and grown wildly with a cosmopolitan distribution.[5] It has a very high nutritional value than when compared to major cultivated vegetables, courtesy to its rich content of phenolics/flavonoids/carotenoids/ascorbic acid/tocopherols/coumarins and fatty acids such as α-linolenic acid making it a rich potential source of all naturally occurring antioxidants.[6] Several biological properties have been attributed to *P. oleracea,* such as antibacterial, wound-healing, analgesic, anti-inflammatory, muscle relaxant, cardioprotective, and many more.[7] The overall pharmacological and phytochemical profile of this plant makes it an important plant for dietary consumption.

The plant is commonly grown anywhere from gardens, farmlands, roadsides, dumps, and any other available space as the plant does not require stringent growing conditions. Such cultivated lands are often chosen without any information on the pollution status of the concerned area.[8] Raipur, the capital city of Chhattisgarh state of India which served as the study site for this research, is the third worst city in India in terms of pollution and has found its way on the list of top twenty polluted cities as well according to 2016 WHO reports, courtesy unplanned industrial invasion.[9] According to earlier reports, plant, soil, and air quality in and around thermal power plants are always kept under scanner. Atmospheric emission of polycyclic aromatic hydrocarbons (PAHs) from thermal power plants and their subsequent deposition on nearby agricultural lands or water bodies and even foliar deposition as well may act as an easy route for translocation of these carcinogenic entities into the plant system.[10,11] Human exposure to PAHs is known to lead to elevated levels of DNA adducts, mutations, reproductive effects, cancers of the lung, respiratory tract, and urinary bladder.[12,13] PAHs on entry into the plant system can easily infringe into the human biological system through the consumption of such contaminated plants.[14] PAHs can induce oxidative stress inside the plant system, which can dysfunction the metabolic machinery leading to a compromised production of secondary metabolites.

Henceforth, this study was undertaken to investigate the nutraceutical integrity of the edible plant *P. oleracea* grown in such agricultural lands, which are in close vicinity of thermal power units. Nutraceutical profiling in terms of total phenolics, flavonoids, carotenoids, ascorbic acid, and tocopherol content and other primary metabolites were carried out and compared with a background sample (control sample collected from a site which is deemed to be free from industrial pollution) to develop an understanding on the issue that plants grown at industrially polluted area could fall prey to environmental insults in terms of induced oxidative stress, leading to depleted production of nutritional principles apart from being serving as the storehouse of such pollutants.

MATERIALS AND METHODS

Description of the study area

Siltara is an industrial area situated 20 km from the capital city of Raipur and has several major coal-based thermal power units. Cultivated lands in the vicinity as close to 0.5 km from one of these industrial thermal power unit was chosen as the target collection site (sample group). Other coal-based units were also located in close vicinity (within 2–4 km radius) to the target collection site.

Sample collection and processing

P. oleracea leaves was collected from the above mentioned site during winter season in downwind direction (December). It has been reported that PAH accumulation in soil and plant foliar parts occurs more in winter owing to lesser volatility of PAH due to lower temperatures in winter.[15,16] Since the collection site is in close vicinity to thermal power units so it was assumed that PAHs would probably be the major pollutant affecting the plants metabolism. Fully grown and matured leaves were collected, washed thoroughly with distilled water, air-dried, powdered, and homogenized by using mortar pestle. Samples were stored in zip lock bags for further extraction and analysis. A background site which served as the control was selected at Takhatpur village with the nearest highway located 10 km from the collection site. The village cultivation land served as the control due to non-existence of any potential source of pollution. Most of the lands within the village are used for cultivation and rural settlement.

Standards and reagents

A standard mixture consisting of sixteen PAH's (Acenaphthene, Acenapthylene, Anthracene, Benz[*a*] anthracene, Benzo[*a*]pyrene, Benzo[*b*]fluoranthene, Benzo[*ghi*]perylene, Benzo[*k*]fluoranthene, Chrysene, Dibenz[*a, h*]anthracene, Fluoranthene, Fluorene, Indeno[1,2,3-*cd*]pyrene, Naphthalene, Phenanthrene, Pyrene) 1 × 1 mL, 10 µg/mL each component in acetonitrile (part number: CRM47940) was purchased from TraceCERT (Supelco). Gallic acid (certified reference material, TraceCERT), 2-aminoethyl diphenylborinate (Neu's reagent), 2,2-diphenyl-1-picrylhydrazyl (DPPH), α-tocopherol, β-carotene (≥97% purity) and Folin-denis reagent was purchased from Sigma Aldrich (St. Louis, MO, USA). Ascorbic acid, anthrone, commasine blue G, quercetin and Evan's blue reagent were purchased from HIMEDIA Co. Ltd., (India). All solvents were procured from Thermo Fisher Scientific (India).

Pigment concentration (chlorophyll/β-carotene)

Content of chlorophyll a and b were analyzed spectrophotometrically according to lichtenthaler[17] by using fresh leaf samples which were homogenized using 80% dimethyle sulphoxide. The homogenate was centrifuged at 10000 rpm for 5 min and supernatant was collected and measured spectrophotometrically at 645 nm and 663 nm, respectively, using ultraviolet (UV)-spectrophotometer (Shimadzu, Japan) and calculated according to the procedure given by Lichtenthaler.[17] Results were expressed as µg/g fresh weight. Extraction of β-carotene from dried plant matrix was carried out through Microwave-Assisted Extraction (MAE) as per the method of Kala *et al*.[18] Briefly, hexane: Acetone: Ethanol mixture (50: 25: 25 v/v) was used as the extraction solvent and high-performance liquid chromatography (HPLC) analysis was performed using acetonitrile: methanol: ethyl acetate (80:10:10 v/v) as the mobile phase and UV detection was carried out at 450 nm. An external standard was used for the identification and quantification of β-carotene.

Nutraceutical profiling

Plant extract for determining the nutraceutical profile which is the main component under investigation was prepared by MAE method as developed by Kala *et al*.[18] In this regard, a commercially available microwave extractor CATA-R (Catalyst Systems, Pune, India) equipped with power levels (multiple), time controller, temperature controller, powerful exhaust system, and stirring device was used. MAE extraction mode offers a rapid exhaustive extraction of bioactive principles from plant matrix in an ecofriendly way. Briefly, 1 g plant sample was extracted in 20 mL methanol using optimized MAE operational conditions as developed by Kala *et al*.[18]

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method by reconstituting the dried extract residue in methanol.[19] Absorbance at 765 nm was recorded spectrophotometrically using gallic acid as the standard. Results were expressed as mg gallic acid equivalents per g of dried extract.

The total flavonoid content (TFC) of crude extract was determined by the aluminium chloride colorimetric method.[20] Quercetin was used as the standard in this case. The absorbance was measured at 510 nm and the results were expressed as mg/gm of dried extract quercetin equivalents.

The quantification of ascorbic acid was done by HPLC. The mobile phase composition of 0.2% metaphosphoric acid in water: Methanol (90: 10), with UV detection at 254 nm was used.[19] The identification of peak was carried out using authentic external standard.

Carbohydrate estimation was carried out using the anthrone method using glucose as the standard and absorbance was recorded spectrophotometrically at 620 nm.[19]

α-tocopherol was determined by HPLC using mobile phase methanol: Water (98:2) and detection was carried out at 292 nm.[20]

Protein extraction was carried out using fresh leaf samples (1 g), homogenized in 2 mL of 50 mM Tris-HCl buffer, pH 7.2, containing 0.1 mM ethylenediamine-tetra acetic acid along with 1% w/v polyvinyl-pyrrolidone at 4°C. Homogenate was centrifuged 10,000 g for 15 min, and the resultant crude supernatant was used for protein estimation.[21] The concentration of proteins was determined by the Bradford method.[22] The absorbance was measured at 595 nm spectrophotometrically. Calibration curve was constructed using bovine serum albumin.

Antioxidant analysis

The DPPH radical scavenging assay was performed to determine the antioxidant activity of the extract at 100 µg/mL.[20] The absorbance of sample and control was measured at 517 nm with methanol as the blank. Curcumin was used as standard for comparison.

Equation 1: $\text{Scavenging activity}\,(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the test sample.

Liquid chromatography–mass spectrometry analysis

Liquid chromatography–mass spectrometry (LC-MS) analysis of the extract was performed for identification of individual phenolic and flavonoid principles. In this regard, a HPLC-diode-array detection (DAD)-MS analyses was performed using an Agilent 1100 Series LC/mass selective detection system with a DAD coupled to a MS (quadrupole analyser) equipped with an electrospray ionization interface (ESI, Agilent) as per the method described by Salem *et al.*[23] The separation was carried out on a 250 mm × 4.6 mm, 4 µm Hypersil ODS C18 reversed-phase column. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulfuric acid (solvent B).

Histochemical studies

Cell death in leaf tissue (fresh leaves) was visualized by using Evan's blue staining method by immersing fresh leaves in 0.25% (w/v) aqueous solution of Evans blue followed by vacuum infiltration for 6 h.[24] Leaves were then bleached with 95% v/v ethanol. Cell death could be visualized as blue patches on the leaf surface and macro-photographed with a digital camera (Sony DSC-W380, Japan). Cell death zone determination was carried out using WINDias Delta-T leaf area meter (Version 3.3). The colored zone on the leaf surface was mapped down by WINDias and expressed as % area relative to the total surface area of the leaf sample.[25] For visualization of phenolic principles, Neu's reagent (2-amino ethyldiphenyl borinate) was used as per the method described by Chouhan *et al.*[19] Briefly, a thin section of leaf samples was dipped in 1% Neu's reagent for 2–5 min and visualized by using UV-fluorescence microscopy for the localization of phenols. Phenolic principles if present would appear as bright green fluorescence.

Field emission scanning electron microscopy

All the specimens were examined with a JEOL JSM-7610F (Tokyo, Japan) scanning electron microscope under high vacuum condition and at an accelerating voltage of 5.0 kV.

Polycyclic aromatic hydrocarbon extraction and enrichment

Extraction of PAH from powdered leaves (1 g) was performed using automated Soxhlet Extraction Unit, B811 (BUCHI, Switzerland) under warm mode for 120 min with rinsing mode activated for 30 min. Dichloromethane was used as the extraction solvent.[26] Effective condensation was performed with a recirculation chiller (Lab-X, Kolkata, India) maintained at 15°C. Post extraction, the extract was dried and reconstituted in n-hexane for clean by column chromatography using 30 mL mobile phase dichloromethane: Hexane (1:1) for PAH enrichment.[27] A volume of 10 mL of collected eluent was dried and reconstituted by using acetonitrile.

Polycyclic aromatic hydrocarbon analysis

Analysis was carried out using GC-MS according to the method reported and described by Meudec *et al.*[28] Briefly, the initial oven temperature was 50°C, on analysis, it was then held for 5 min, and then raised steadily to 300°C at 12°C min^{-1} with a final holding time of 20 min. The injector, transfer lines and ion source temperatures were set at 260, 305 and 186°C, respectively, as reported by Meudec *et al.*[28] Injection volume of the PAH enriched sample was 1 µL (injected through autosampler using splitless mode). Helium was used as carrier gas. Individual PAH's were identified using external standards (PAH Calibration Mix which consisted of a mixture of sixteen PAH's) and subsequent calibration curve was also prepared for quantification. The final results of PAH quantification was expressed in ng/g.

Statistical analysis

The Duncan's test at the 0.05 probability level and the paired *t*-test at 95% confidence level were performed for comparison of sample with control (for all evaluated parameters) using Graph Pad Prism version 7.0. (GraphPad Software, San Diego, California). Experiments were performed in triplicate and the results were exhibited as means followed by the corresponding standard deviations.

RESULTS AND DISCUSSION

Pigment concentration

The current findings clearly states decreased content of chlorophyll pigments. Chlorophyll a showed a maximum reduction of 40.58% and chlorophyll b exhibited a reduction of 31.25% when compared to control [Figure 1]. The above fact definitely hints toward a compromised photosynthetic apparatus. It may also be noted that chlorophyll a plays a more crucial role towards photosynthesis than chlorophyll b and as reported above maximum reduction of chlorophyll a content for pollution exposed plant is likely to lead toward a dysfunctional photosynthesis.[29] Decline in chlorophyll content for pollution exposed plant may be further be attributed to:

a. Development of alkaline environment within the cell sap due to solubilization of various pollutants[16]
b. Inhibition of essential enzymes involved in biosynthesis of chlorophyll[30]
c. PAH induced generation of reactive oxygen species (ROS) might cause oxidative stress within the plant thus severely jeopardizing the photosynthetic electron transport chain leading to disruption of pigment synthesis.[31]

Similar reports for decline in chlorophyll pigment for pollution exposed plants have been reported by Qadir *et al.*[16] in the past. The results of carotenoid (with respect to β carotene) depletion in pollution exposed plants were in tandem with the reports of chlorophyll. Carotenoids recorded a decline of 33.5% for pollution exposed plant when compared to control [Figure 2]. Results were in agreement with some earlier published reports were significant decline in carotenoid pigments due to the effect of various pollutants have been reported. As reported by Qadir *et al.*,[16] depletion in carotenoid pigment may have serious implications on chlorophyll as well.[32] Carotenoids acts as photoprotective agent and any depletion in carotenoid content is likely to photodegrade chlorophyll pigment. On the other hand, carotenoids are a group of vital lipophilic antioxidants and plays a key role in scavenging ROS.[32] Henceforth, reduction in chlorophyll content in pollution exposed plant is likely to cause redox imbalance thus greatly dysfunctioning the plants homeostasis and metabolism as well.

Effect on nutraceutical principles

TPC and TFC showed a decline of 42.25% and 28.17%, respectively, for the pollution exposed leaves when compared to control [Figure 3]. Phenolics and flavonoids are prominent free radical scavengers and play a pivotal role in maintaining the oxidative balance.[33] Such depletions may likely to disturb the redox homeostasis which can serve as a breeding ground for the onset of several other diseases or may worsen the progression of other existing diseases.[3] Depletion in phenolic resources may possibly be due to the following reasons,

a. Consumption or inhibition associated with the biosynthetic route of phenolics[34]
b. ROS-induced inhibition of phenylalanine ammonia-Liaza enzyme which is the main key enzyme involved in the biosynthetic pathway of phenolic compounds.[35]

Fate of identified individual phenolics and flavonoids has been discussed in the latter section. Ascorbic acid content for pollution exposed plant showed a significant depletion of 45.5% [Figure 4]. Similar decreasing trend was also recorded for α-tocopherol which showed a massive

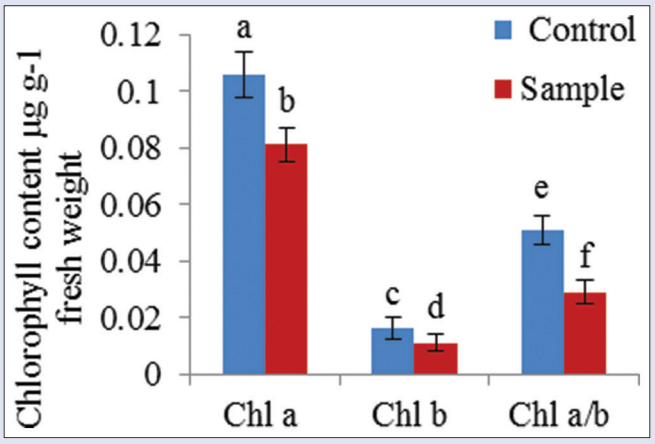

Figure 1: Effect of industrial pollutants on chlorophyll content. Data marked with different letters are significantly different at $P < 0.05$. Results are expressed as mean ± standard deviation ($n = 3$). Control: Control sample collected from a site which is deemed to be free from industrial pollution (background sample), Sample: Plant sample collected from agricultural land in close vicinity to a thermal power unit

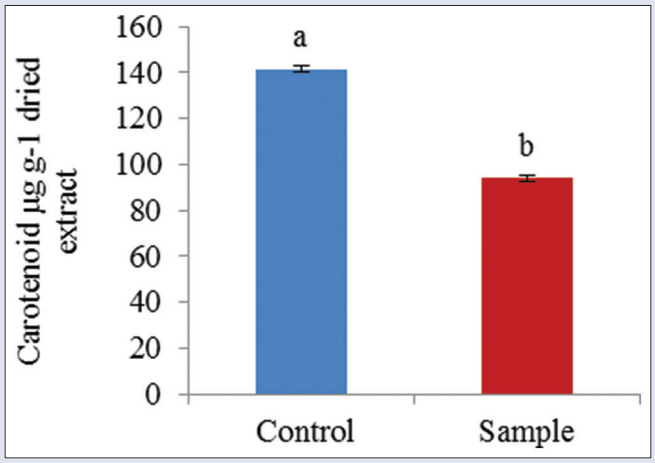

Figure 2: Effect of industrial pollutants on carotenoids (β-carotene) content. Data marked with different letters are significantly different at $P < 0.05$. Results are expressed as mean ± standard deviation ($n = 3$)

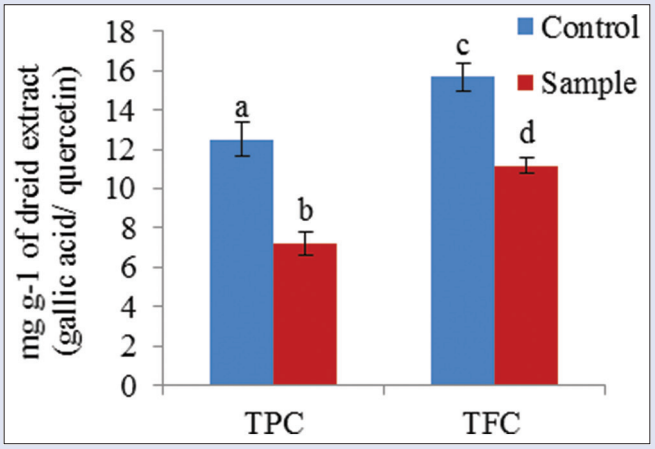

Figure 3: Effect of industrial pollutants on the production of phenolic and flavonoid principles in *Portulaca oleracea*. Data marked with different letters are significantly different at $P < 0.05$. Results are expressed as mean ± standard deviation ($n = 3$)

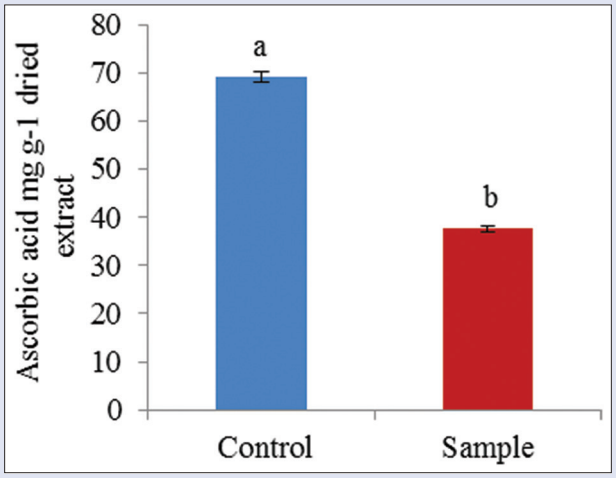

Figure 4: Effect of industrial pollutants on the production of non-enzymatic antioxidant principle ascorbic acid in *Portulaca oleracea*. Data marked with different letters are significantly different at $P < 0.05$. Results are expressed as mean ± standard deviation ($n = 3$)

depletion of 81.9% [Figure 5]. Above results provide conclusive evidence of severe depletion of nutraceutical resources for pollution exposed plant. Since the plant under investigation is a dietary plant with rich nutraceutical content, consumption of such plant grown in industrially polluted areas may not deliver required nutraceutical principles as necessary for the body. Ascorbic acid and tocopherol are the most common non-enzymatic antioxidants responsible for neutralizing excess ROS generation in plants.[36,37] Apart from this, tocopherol also plays a vital role in photosynthesis, regulation of cell division and protection of membrane components which ultimately maintains the integrity of plant cells.[38] Plant has their own internal mechanism to deal with oxidative stress arising due to exposure to various types of environmental pollutants.[29] Results indicate that environmental pollutants due to industrial pollution with special reference to PAHs were so high that the internal defense mechanism was overpowered which indicates the high magnitude of stress being generated within the plants. Results are conclusive enough to state existence of extreme redox imbalance and metabolic dysfunction within plants exposed to industrial pollution. In such a situation it is obvious that the plant's production of secondary and primary metabolites which is responsible for its nutraceutical value shall be compromised. The fact is further supported by depletion of its two major primary metabolite, namely protein and carbohydrate content to the extent of 33.9% and 33.4%, respectively [Figures 6 and 7].

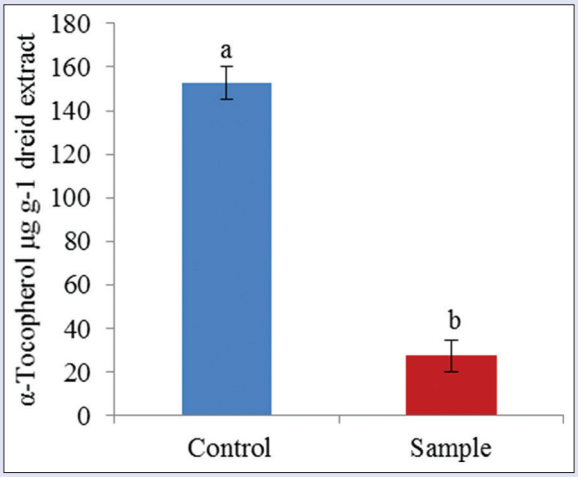

Figure 5: Effect of industrial pollutants on the production of non-enzymatic antioxidant principle α-tocopherol in *Portulaca oleracea*. Data marked with different letters are significantly different at $P < 0.05$. Results are expressed as mean ± standard deviation ($n = 3$). Control: Background sample, Sample: Plant sample collected from agricultural land in close vicinity to a thermal power unit

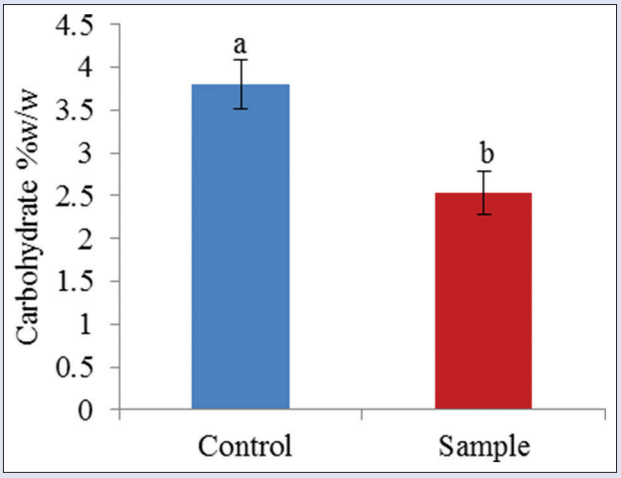

Figure 7: Effect of industrial pollutants on the production of primary metabolite carbohydrate in *Portulaca oleracea*. Data marked with different letters are significantly different at $P < 0.05$. Results are expressed as mean ± standard deviation ($n = 3$). Control: Background sample, Sample: Plant sample collected from agricultural land in close vicinity to a thermal power unit

Antioxidant activity

Having already said about the severe depletion of nutraceutical resources for pollution exposed plant, it becomes obvious that the antioxidant potential of such plant is also at stake. To determine the same, the free radical scavenging activity of the plant extract at 100 µg/mL was determined. Results indicated significant fall in antioxidant activity by

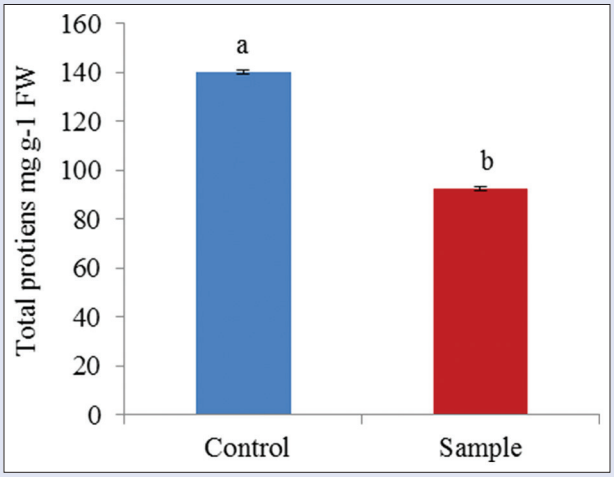

Figure 6: Effect of industrial pollutants on the production of total proteins in *Portulaca oleracea*. Data marked with different letters are significantly different at $P < 0.05$. Results are expressed as mean ± standard deviation ($n = 3$). Control: Background sample, Sample: Plant sample collected from agricultural land in close vicinity to a thermal power unit

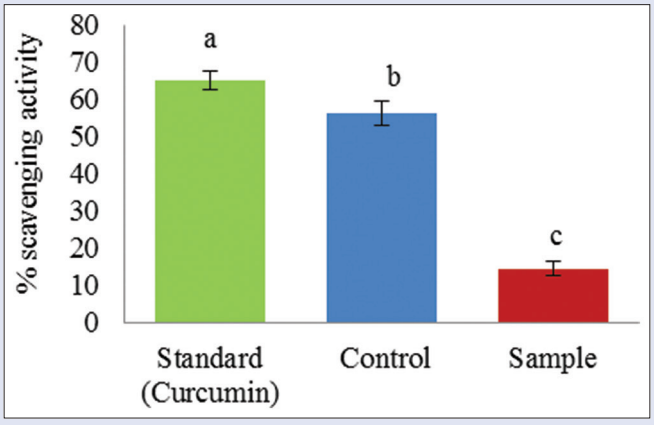

Figure 8: Effect of industrial pollutants on the antioxidant potential of *Portulaca oleracea*. Data marked with different letters are significantly different at $P < 0.05$. Results are expressed as mean ± standard deviation ($n = 3$). Control: Background sample, Sample: Plant sample collected from agricultural land in close vicinity to a thermal power unit

74.2% for pollution exposed plant when compared to control [Figure 8]. In general, edible plants are rich in phenolics and flavonoids and the antioxidant potential of the plant is basically due to the presence of such principles. Drastic fall in antioxidant potential can be directly correlated to the decline of phenolic/flavonoids and other non-enzymatic antioxidant principles (ascorbic acid and tocopherols). Results conclusively states that the plant under investigation is loosing its nutraceutical contents, courtesy to the increasing oxidative stress due to industrial pollution with special emphasis to PAHs.

Histochemical studies

Two histochemical evidences based on real-time sample assessment was performed to support the claims made above. They are as follows:

a. Evidence for depletion of phenolic principles was provided through real-time *in-situ* detection of plant phenolics using NPR reagent (2-amino ethyldiphenyl borinate). The fluorescence intensity of the leaf tissue is directly proportional to its phenolic/flavonoid contents. Leaf sample of pollution exposed plant showed lesser green fluorescence than when compared to prominent bright green fluorescence of the control [Figure 9]. The observation clearly indicates less abundancy of phenolic principles in the vascular and parenchyma region of the leaf tissue exposed to pollution. The fact validates the above claims of phenolic degradation owing to stress-induced from environmental pollutants[35]

b. Drastic depletion of nutraceutical resources for pollution exposed plant could also indicate ROS impacting on the living status of the plant cells. In this regard, cell death of leaf tissue was quantified by Evans blue staining as evident by blue patches appearing throughout the leaf surface for pollution exposed plant. Results indicated 62% more cell death in pollution exposed plant when compared to control [Figure 10]. It serves as a real-time evidence of compromised cell membrane. As explained in the earlier section, depletion of tocopherols and increased PAH-induced oxidative stress cumulatively have impacted the cell membrane resulting in the loss of its integrity.[21]

Fate of identified phenolics/flavonoids

LC-MS studies revealed the presence of ten phenolic/flavonoid principles in the plant sample under investigation. The identified phenolic/flavonoid principles are p-coumaric acid, gallic acid, ferulic acid, naringenin, luteolin, catechin, quercetin, ellagic acid, chlorogenic acid, and rutin. Observation was recorded under three different categories which are as follows:

- Category I: Consists of those phenolics/flavonoids which showed a significant decline in the plant sample exposed to pollution when compared to control. It includes p-coumaric acid, gallic acid, ferulic acid, naringenin, quercetin, ellagic acid, and rutin which showed a decline (in terms of % area) of 27.7%, 23.6%, 23.6%, 21.8%, 29.4%, 65.2%, and 26.2%, respectively, when compared to control
- Category II: Contains those phenolic/flavonoid principles, which showed an increasing trend in the pollution exposed sample when compared to control. Only chlorogenic acid was included in this group as it showed an increase by 13.99% in pollution exposed plant when compared to control. A similar response was also recorded by Manquian-Cerda *et al.*[39] where increase in chlorogenic acid in leaves of *V. corymbosum* subjected to cadmium stress took place, indicating critical involvement of chlorogenic acid in combating stress induced due to exposure to environmental pollutants[39]
- Category III: Contains those principles which were completely depleted in the sample group but were present in control. Luteolin and catechin were completely depleted in the pollution exposed plant group.

Scanning electron microscopy analysis

Scanning electron microscopy images of the leaf epidermal surface indicated closure of stomata, which may have occurred due to increased accumulation of industrial pollutants or may also be due to a protective

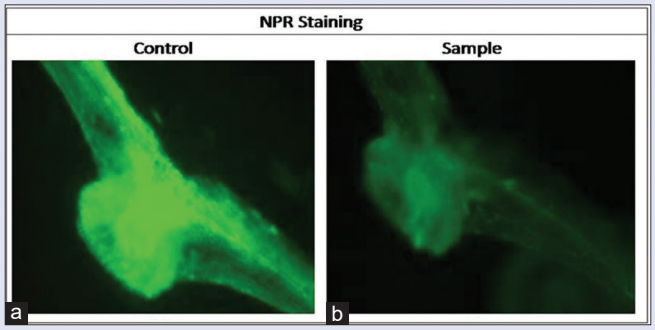

Figure 9: *In situ* detection of phenolic principles in fresh tissue sample of *Portulaca oleracea* using NPR reagent staining method. NPR reagent staining: Fluorescence intensity of the leaf tissue is directly proportional to its phenolic/flavonoid contents. (a) transverse section of control leaf sample exhibiting green fluorescence throughout, (b) transverse section of samples collected from PAH pollution area did not show any comparable green fluorescence

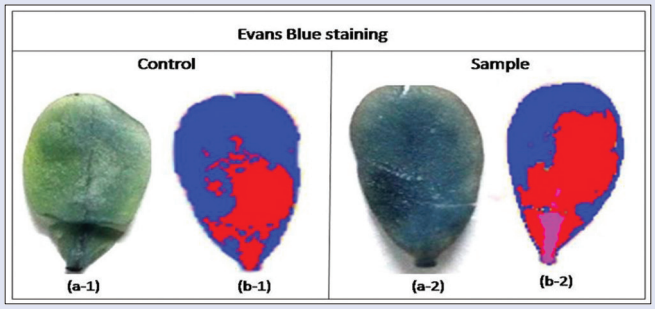

Figure 10: Cell death visualization using Evans blue staining method. (a-1 and a-2) Digital macro photograph of original stained leaf sample. (b-1 and b-2) Colored zone on the leaf surface as mapped down by WINDias. Blue portion (software generated) indicates healthy tissue where no localization/cell death has taken place and red/pink color (software generated) indicates damaged tissue due to localization/cell death

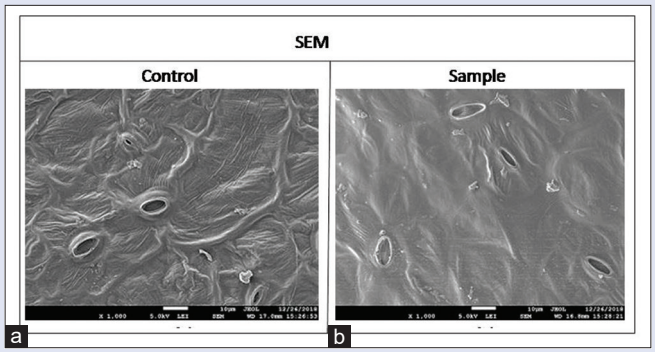

Figure 11: Effect of industrial pollutants on leaf stomata. (a) SEM images indicating open stomata for leaves from control group (not exposed to PAH pollution), (b) SEM images indicating stomatal closure in leaves of sample group (exposed to PAH pollution)

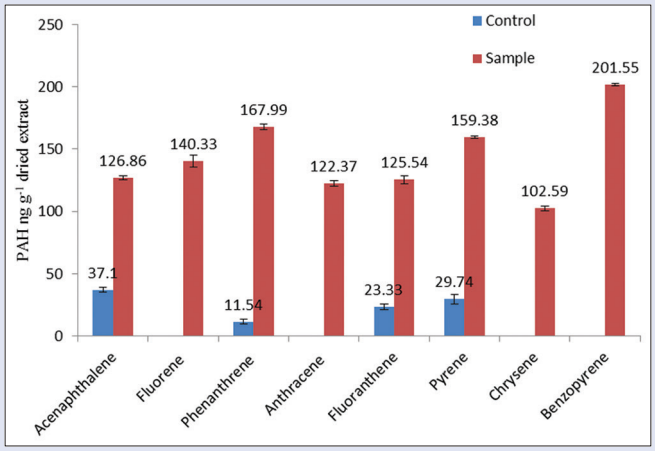

Figure 12: Quantification of identified polycyclic aromatic hydrocarbon's in *Portulaca oleracea*. Formula = (C × *V*/*m*) C = value of "x" which reflects the concentration, to be calculated from calibration curve straight line equation. *V* = final volume including dilution factor (if any) (2 mL). *m* = weight of the main extract in g (value of "m" for control and sample are 0.056 g and 0.067 respectively)

response initiated by the plant.[16,40] Stomatal blockage greatly affects photosynthetic apparatus by denying access to photosynthetically active radiations and also compromises nutrient uptake and internal cooling mechanism of the leaf surface, ultimately leading to troubled homeostasis [Figure 11].

Polycyclic aromatic hydrocarbon analysis

The identification and quantification results of PAH [Figure 12] revealed the presence of eight PAH's in the plant belonging to sample group, which can be divided into two broad groups namely, Group I: Consists of those PAH's which were totally absent in control group but present only in the sample group. It comprised of fluorene, anthracene, chrysene and benzopyrene, out of which benzopyrene is of utmost concern because of its strong carcinogenic and mutagenic properties. Group II: Consists of those PAHs which were present in both control as well as sample group of plants. It comprised acenaphthalene, phenanthrene, fluoranthene, and pyrene. Severe accumulation of high magnitude of various PAH's was evident in the plant collected from agricultural land in close vicinity to thermal power plants. The results [Figure 12] are evident of the fact that accumulated PAH's of such high magnitude have impacted the secondary metabolism of the plant, which led to the depletion of various phytochemicals with special emphasis to nutraceuticals.

CONCLUSION

P. oleracea showed significant depletion in nutraceutical resources when grown in close vicinity to thermal power plants where PAH menace is at the maximum. These reports of nutraceutical depletions in dietary plants grown in close vicinity to thermal power plants are the first reports of its kind. High-intensity metabolic trouble caused by oxidative stress due to exposure to various environmental insults is to be cited as the main reason for such observation. Genetic intervention in developing stress-resistant plants, better phytoremediation of cultivation land close to thermal power plants is the need of the hour.

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Conflicts of interest

There are no conflicts of interest

REFERENCES

1. Kala HK, Mehta R, Sen KK, Tandey R, Mandal V. Critical analysis of research trends and issues in microwave assisted extraction of phenolics: Have we really done enough. Trends Anal Chem 2016;85:140-52.
2. Li S, Chen G, Zhang C, Wu M, Wu S, Liu Q. Research progress of natural antioxidants in foods for the treatment of diseases. Food Sci Hum Wellness 2014;3:110-6.
3. Haida Z, Hakiman M. A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. Food Sci Nutr 2019;7:1555-63.
4. Koch W. Dietary polyphenols-important non-nutrients in the prevention of chronic noncommunicable diseases. A systematic review. Nutrients 2019;11:1-35.
5. Chowdhary CV, Meruva A, Elumalai RK. A review on phytochemical and pharmacological profile of *Portulaca oleracea* Linn. (Purslane). Int J Res Ayurveda Pharm 2013;4:34-7.
6. Uddin MK, Juraimi AS, Hossain MS, Nahar MA, Ali ME, Rahman MM. Purslane weed (*Portulaca oleracea*): A prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. ScientificWorldJournal 2014;2014:951019.
7. Rahimi VB, Ajam F, Rakhshandeh H, Askari VR. A pharmacological review on *Portulaca oleracea* L.: Focusing on anti-inflammatory, anti- oxidant, immuno-modulatory and antitumor activities. J Pharmacopuncture 2019;22:7-15.
8. Mandal V, Chouhan KB, Tandey R, Sen KK, Kala HK, Mehta R. Critical analysis and mapping of research trends and impact assessment of polyaromatic hydrocarbon accumulation in leaves: Let history tell the future. Environ Sci Pollut Res Int 2018;25:22464-74.
9. Drolia R. Chhattisgarh Capital Raipur 7th Most Polluted in world: WHO. Times of India 2016. p. 1-2. Available from: https://timesofindia.indiatimes.com/city/raipur/Chhattisgarh-capital-Raipur-7th-most-polluted-in-world-WHO/articleshow/52252399.cms. [Last accessed on 2016 May 13].
10. Khillare PS, Jyethi DS, Sarkar S. Health risk assessment of polycyclic aromatic hydrocarbons and heavy metals via dietary intake of vegetables grown in the vicinity of thermal power plants. Food Chem Toxicol 2012;50:1642-52.
11. Kipopoulou AM, Manoli E, Samara C. Bioconcentration of polycyclic aromatic hydrocarbons in vegetables grown in an industrial area. Environ Pollut 1999;106:369-80.
12. Rengarajan T, Rajendran P, Nandakumar N, Lokeshkumar B, Rajendran P, Nishigaki I. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pac J Trop Biomed 2015;5:182-9.
13. Kadlubar FF, Badawi AF. Genetic susceptibility and carcinogen-DNA adduct formation in human urinary bladder carcinogenesis. Toxicol Lett 1995;82-83:627-32.
14. Domingo JL, Nadal M. Human dietary exposure to polycyclic aromatic hydrocarbons: A review of the scientific literature. Food Chem Toxicol 2015;86:144-53.
15. Jouraeva VA, Johnson DL, Hassett JP, Nowak DJ. Differences in accumulation of PAHs and metals on the leaves of *Tilia×euchlora* and *Pyrus calleryana*. Environ Pollut 2002;120:331-8.
16. Qadir SU, Raja V, Siddiqui WA. Morphological and biochemical changes in *Azadirachta indica* from coal combustion fly ash dumping site from a thermal power plant in Delhi, India. Ecotoxicol Environ Saf 2016;129:320-8.
17. Lichtenthaler HK. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol 1987;148:350-82.
18. Kala HK, Mehta R, Sen KK, Tandey R, Mandal V. Strategizing method optimization of microwave assisted extraction of plant phenolics by developing standard working principles for universal robust optimization. Anal Methods 2017;9:2089.
19. Chouhan KB, Tandey R, Sen KK, Mehta R, Mandal V. A unique model of gravity assisted solvent free microwave based extraction of essential oil from mentha leaves ensuring

biorefinery of leftover waste biomass for extraction of nutraceuticals: Towards cleaner and greener technology. J Clean Prod 2019;225:587-98.

20. Chouhan KB, Tandey R, Sen KK, Mehta R, Mandal V. Extraction of phenolic principles: Value addition through effective sample pretreatment and operational improvement. J Food Meas Charact 2019;13;177.
21. Mohan Murali Achary V, Patnaik AR, Panda BB. Oxidative biomarkers in leaf tissue of barley seedlings in response to aluminum stress. Ecotoxicol Environ Saf 2012;75:16-26.
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
23. Salem N, Msaada K, Dhifi W, Limam F, Marzouk B. Effect of salinity on plant growth and biological activities of *Carthamus tinctorius* L. extracts at two flowering stages. Acta Physiol Plant. 2014;36:433-445.
24. Romero-Puertas MC, Rodriguez-Serrano M, Corpas FJ, Gomez M, Rio LA, Sandalio LM. Cadmium-induced subcellular accumulation of O^{2-} and H_2O_2 in pea leaves. Plant Cell Environ 2004;27:1122-34.
25. Kim KS, Park SH, Jenks MA. Changes in leaf cuticular waxes of sesame (*Sesamum indicum* L.) plants exposed to water deficit. J Plant Physiol 2007;164:1134-43.
26. Frenna S, Mazzola A, Orecchio S, Tuzzolino N. Comparison of different methods for extraction of polycyclic aromatic hydrocarbons (PAHs) from Sicilian (Italy) coastal area sediments. Environ Monit Assess 2013;185:5551-62.
27. Wang W, Meng B, Lu X, Liu Y, Tao S. Extraction of polycyclic aromatic hydrocarbons and organochlorine pesticides from soils: A comparison between Soxhlet extraction, microwave-assisted extraction and accelerated solvent extraction techniques. Anal Chim Acta 2007;602:211-22.
28. Meudec A, Dussauze J, Jourdin M, Deslandes E, Poupart N. Gas chromatographic-mass spectrometric method for polycyclic aromatic hydrocarbon analysis in plant biota. J Chromatogr A 2006;1108:240-7.
29. Ahammed GJ, Wang MM, Zhou YH, Xia XJ, Mao WH, Shi K, *et al.* The growth, photosynthesis and antioxidant defense responses of five vegetable crops to phenanthrene stress. Ecotoxicol Environ Saf 2012;80:132-9.
30. Bashri G, Parihar P, Singh R, Singh S, Singh VP, Prasad SM. Physiological and biochemical characterization of two *Amaranthus* species under Cr(VI) stress differing in Cr(VI) tolerance. Plant Physiol Biochem 2016;108:12-23.
31. Liu D, Zou J, Wang M, Jiang W. Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defence system and photosynthesis in *Amaranthus viridis* L. Bioresour Technol 2008;99:2628-36.
32. Shen Y, Li J, Gu R, Yue L, Wang H, Zhan X, *et al.* Carotenoid and superoxide dismutase are the most effective antioxidants participating in ROS scavenging in phenanthrene accumulated wheat leaf. Chemosphere 2018;197:513-25.
33. Jaskulak M, Rorat A, Grobelak A, Kacprzak M. Antioxidative enzymes and expression of rbcL gene as tools to monitor heavy metal-related stress in plants. J Environ Manage 2018;218:71-8.
34. Jiang S, Weng B, Liu T, Su Y, Liu J, Lu H, *et al.* Response of phenolic metabolism to cadmium and phenanthrene and its influence on pollutant translocations in the mangrove plant *Aegiceras corniculatum* (L.) Blanco (Ac). Ecotoxicol Environ Saf 2017;141:290-7.
35. Zezulka S, Kummerová M, Babula P, Váňová L. Lemna minor exposed to fluoranthene: Growth, biochemical, physiological and histochemical changes. Aquat Toxicol 2013;140-141:37-47.
36. Chao YY, Hong CY, Kao CH. The decline in ascorbic acid content is associated with cadmium toxicity of rice seedlings. Plant Physiol Biochem 2010;48:374-81.
37. Akram NA, Shafiq F, Ashraf M. Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. Front Plant Sci 2017;8:613.
38. Zhu Q, Zhang J, Yu H, Li L, Chen X, Jiang M, *et al.* Maize Cd-tolerant ZmVTE4 encoding γ-tocopherol-methyl-transferase alleviated Cd-toxicity through its product α-tocopherol. Environ Exp Bot 2019;158:171.
39. Manquián-Cerda K, Escudey M, Zúñiga G, Arancibia-Miranda N, Molina M, Cruces E. Effect of cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (*Vaccinium corymbosum* L.) plantlets grown *in vitro*. Ecotoxicol Environ Saf 2016;133:316-26.
40. Per TS, Masood A, Khan NA. Nitric oxide improves S-assimilation and GSH production to prevent inhibitory effects of cadmium stress on photosynthesis in mustard (*Brassica juncea* L.). Nitric Oxide 2017;68:111-24.