RESEARCH ARTICLE

Suitability of *Mentha arvensis* L. for Phyto-stabilization and Remediation of Heavy Metals/Metalloid Rich Fly-ash Contaminated Soil

Vippan Kaur[1,2], Subodh Kumar[1], Akanksha Singh[2], Poornima Vajpayee[1*]

DOI: 10.18811/ijpen.v12i01.08

Abstract

Agricultural fields nearby thermal power plant receives air blown fly ash in huge amounts. The fly ash contains heavy metals/metalloids along with essential elements for plant growth and productivity. The conventional crops growing in the fly ash-contaminated agrifields accumulate toxic metals in different tissues. Thus, there is a need to explore the crop plant of economic importance for Phyto-stabilization of fly ash contaminated agrifields and to slow down the rate of metals/metalloids entering the food chain. In the present study, *Mentha arvensis* (var. Kosi) was used to evaluate the physiological and biochemical responses in six concentrations (w/w) of fly ash (0, 15, 30, 45, 60, and 100%). The morphometric traits, growth, and photosynthetic pigments were analysed in *M. arvensis* and found maximum shoot & root length, biomass, number of leaves, and primary branches at 15% fly ash mixed soil. Chlorophyll a, b, total chlorophyll, and carotenoids were elevated with 15% fly ash exposure, while reduced at 100% fly ash. Oxidative stress markers (malondialdehyde & hydrogen peroxide) were also significantly (Tukey-HSD post-hoc, $p < 0.05$) upregulated with rising levels of fly ash. Further, the level of Cellular antioxidants (cysteine, TPC, NP-SH) was increased up to 60% fly ash. The study also documented the differential accumulation of Iron, Copper, Chromium, Manganese, and Mercury in leaves and stems of M. *arvensis* at different fly ash concentrations, while reduced level of Potassium was observed with increasing fly ash levels. The antioxidative responses and potential of test plant to accumulate metal make it suitable for eco-stabilization in fly ash contaminated agrifields.

Keywords: Fly-ash; Phyto-stabilization, *Mentha arvensis*; Metals/metalloid; Oxidative stress.

Highlights:

- FA amendment increased metals (Fe, Cu, Cr and Hg) accumulation in leaves and stems of *M. arvensis.*
- Concentration-dependent upregulation of oxidative stress markers was observed in FA-treated test plants.
- High FA amendment also increased cysteine, total phenolic contents and non-protein thiols in leaves of M. arvensis.
- Maximum growth of *M. arvensis* was observed at 15% FA, however, a significant decrease was also observed at higher FA (45- 100%) amendments.

International Journal of Plant and Environment (2026); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

An ever-increasing population has increased the urbanization and industrialization for a luxurious livelihood. The surge in industrial activities has significantly risen the electricity demand. Coal sources have been serving a major role in electricity generation across the world. Combustion of coal in the thermal power plants (TPP) leads to the generation of huge amount of fly-ash (FA) along with electricity. As stated by the Central Electricity Authority (CEA), the fly-ash generation in India was 226.95 mt in 2019-20, which increased to 270.82 mt in the year 2021-2022 (CEA, 2022). Fly ash is a fine powder exhibiting essential and non-essential, toxic elements. Thermal power plants collect the fly-ash using an electrostatic precipitator to avoid fly-ash particles entering the atmosphere and dump the excess fly-ash at dumping sites. The contamination of agricultural fields by air blown fly-ash may impose harmful impacts on crop growth, yield, and productivity (Pandey and Singh, 2020). Recently, Goswami *et al.,* (2023) documented the accumulation of Nickel (Ni), Cobalt (Co), Chromium (Cr) and Lead (Pb) in root, shoot and grains of barley on the application of fly-ash (0, 15, 30 and 50%). Singh *et al.,* (2023) also documented the accumulation of Cadmium (Cd), Lead (Pb), Chromium (Cr), Arsenic (As), and Mercury (Hg) in vegetable and staple crops (cabbage, Indian spinach, tomato, radish, carrot, onion, wheat and rice) grown near Bokaro TPP, Jharkhand, India. Further, the

[1]Ecotoxicology, Bioremediation and Nanotoxicology Laboratory, Department of Botany, University of Lucknow, Lucknow-226007, U.P., India

[2]Division of Crop Production and Protection, CSIR-(CIMAP) Central Institute of Medicinal and Aromatic plants, Lucknow-226015, U.P., India

***Corresponding author:** Poornima Vajpayee, Ecotoxicology, Bioremediation and Nanotoxicology Laboratory, Department of Botany, University of Lucknow, Lucknow-226007, U.P., India, Email: devpoornima@rediffmail.com; Vajpayee_poornima@lkouniv.ac.in

How to cite this article: Kaur V, Kumar S, Singh A, Vajpayee P. (2026). Suitability of *Mentha arvensis* L. for Phyto-stabilization and Remediation of Heavy Metals/Metalloid Rich Fly-ash Contaminated Soil. International Journal of Plant and Environment. 12(1), 67-76.

Submitted: 07/04/2026 **Accepted:** 23/04/2026 **Published:** 31/05/2026

accumulation of Pb and Cd was highly pronounced in all the crops studied by them and the concentration of both the metals went above the permissible limits suggested by FAO/WHO (2011). The presence of hazardous metals in consumable parts of various staple and vegetable crops may lead to environmental and public health challenges by entering in the food-chain (Upadhyay *et al.,* 2021). Several fuel trees, herbaceous species, natural grasses etc. have been reported for phytoremediation and restoration of FA dumps and agricultural fields in the vicinity of thermal power plants (Abhishek *et al.,* 2025; Yadav *et al.,* 2022; Ram and Masto, 2014). A few studies have recently suggested the use of commercially important essential oil (EO) yielding plants as a viable alternative crop in fly-ash degraded agrifields instead of conventional staple and food crops to enhance the livelihood of marginal farmers in vicinity of coal fired TPP (Kumar *et al.,* 2024; Ultra, 2020; Panda *et al.,* 2018). The EO market size is expected to grow at 7.6% CAGR from 2024 to 2030, which was 23.74 billion USD in 2023 (Baser and Bonello, 2025). Thus, the cultivation of essential oil-yielding crop *Mentha arvensis* in agrifields (highly contaminated by air blown fly-ash) may boost the economic benefits to the farmers.

Mentha arvensis L., Lamiaceae, is an aromatic, perennial, herbaceous plant, widely cultivated globally (Tiwari, 2016). The EO of *M. arvensis* L., a source of natural menthol used in pharmaceuticals, flavouring agent in food and cosmetics (Faisal *et al.,* 2023; Kim *et al.,* 2021; Tiwari, 2016). Further, antioxidant, antifungal and antibacterial properties of *M. arvensis* have also been documented (Alonso Leite Dos Santos *et al.,* 2024; Sharma *et al.,* 2024; Parić *et al.,* 2024). Therefore, *M. arvensis* may serve as an ideal plant for phyto-stabilization and remediation of constituent heavy metal/metalloid in fly ash contaminated areas. Besides, the commercial cultivation of *M. arvensis* instead of conventional crops in fly ash affected areas, may enhance the income and improve the lively hood of marginal farmers.

Hence, the present study was designed to evaluate the physiological and biochemical adaptations of *M. arvensis* L. (var. Kosi) in response to constituent heavy metal/metalloids present in fly-ash contaminated soils prior to provide economically viable, sustainable environmental solution for fly-ash degraded agrifields in vicinity of coal based thermal power plants.

Materials and Methods

Collection of Experimental Soil, Fly-ash and its Characterization

The experimental soil was collected from the Department of Botany, University of Lucknow (26.8633° N, 80.9360° E). However, FA was obtained from Feroze Gandhi Unchahar Thermal Power Plant (FGUTPP), Unchahar, Raebareli, Uttar Pradesh, India (25°54′50″N & 81°19′50″E). Both the experimental soil and FA were evaluated for colour, texture, chemical properties and presence of heavy metals. The pH of FA and experimental soil was recorded using Thermo-scientific pH meter (Model: Orion star A214) and EC was determined by using the soil/fly-ash and distilled water in ratios of 1:2.5. Nitrogen, Phosphorus, Potassium, and organic contents in FA and experimental soil were also analysed (Watanabe and Olsen, 1965; Subbiah and Asija, 1956; Merwin and Peech, 1951; Dickman and Bray, 1940; Walkley *et al.,* 1934). Scanning electron microscopy (SEM; for imaging), energy dispersive X-ray analysis (EDX)

Inductively coupled plasma optical emission spectroscopy (ICP-OES, 5110, Agilent, USA) were used for element profiling. Briefly, 0.4 g of soil and fly-ash were digested in nitric acid and hydrogen peroxide (HNO_3:H_2O_2 in 3:1) using microwave assisted acid digestion system (Mars 6™, CEM Corporation, Matthews, NC, US) and diluted to 50 ml. The digestates were used for ICP-OES analyses of metals in both samples.

Raising nursery

The suckers of *M. arvensis* L. (Var. Kosi) were collected from CSIR-CIMAP, Lucknow, Uttar Pradesh, India. The suckers were washed properly with sterilized double-distilled water. The nursery was raised as described by Kumar *et al.,* (2023) in the garden, Department of Botany, University of Lucknow. After 25 days, the plantlets with erratic growth were discarded and the plantlets of equal number of leaves and height were transferred into earthen pots filled with different treatment soils prepared for the present study.

Preparation of Fly-ash treatments

The present study was conducted in six kg earthen pots (12inch diameter) in a complete randomised block design (CRBD). Initially, the experimental fly-ash and soil was sun dried and impurities (pebbles) were removed through sieving. A total of six fly-ash and soil mixtures, w/w (M_1: Control (soil 100%), M_2: Fly-ash 15% & Soil 85%, M_3: Fly-ash 30% & Soil 70%, M_4: Fly-ash 45% & Soil 55%, M_5: Fly-ash 60% & Soil 40% and M_6: Fly-ash 100%) were prepared and filled in earthen pots (six for each treatment). All the treatment pots were prepared a day before initiation of the experiment and left overnight to set the mixtures after irrigation. Twenty-five-day old plantlets were transplanted in the treatment pots and irrigated using tap water every day. The plastic trays were placed below every experimental pot and any leachate released from the pots was poured back to prevent metal/metalloid leakage. The plants were harvested on 60th day of the experimental period and stored at -20°C for analysing physiological parameters.

Growth, morphometric traits and phytopigments

The morphometric traits of test plants were recorded on the 60th day of the experiment. The number of primary branches and leaves was counted before harvesting the plants from the treatment pots. Whole plant biomass, shoot height and root length were recorded after uprooting the plants from all the treatments. Further, photosynthetic pigments (total chlorophyll (Tchl, chlorophyll a (chl a), chlorophyll b (chl b) and carotenoids) were also analysed using 80% acetone (Arnon, 1949; Duxbury and Yentsch, 1956). The quantified results of pigments have been reported in mg g^{-1} FW.

Elemental analysis

The leaf and stem samples from all the treatments were dried in hot air oven at 70±2°C for seven days and ground to prepare a powder form. The fine powder (0.4 g) was acid digested in a microwave digester (Mars 6™, CEM Corporation, Matthews, NC, US) as per Kumar *et al.,* (2025). Thereafter, the digestates were diluted to 50 ml and used for elemental analysis using ICP-OES

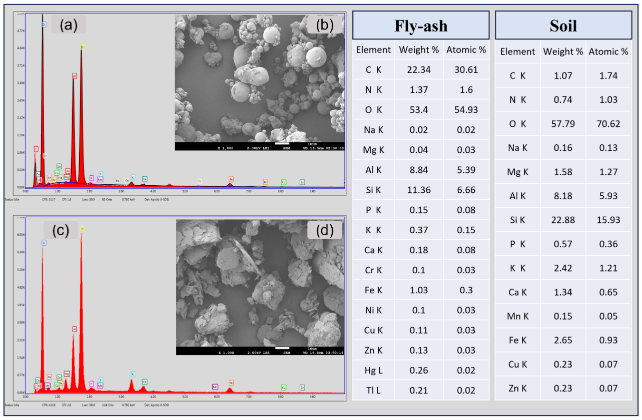

Fig 1: Scanning electron microscopy of fly-ash and Soil. (a) SEM-EDAX spectra of fly-ash, (b) Scanning electron micrograph of fly-ash particles, (c) SEM-EDAX spectra of soil, (d) Scanning electron micrograph of soil particles

(5110; Agilent, USA). Metal (Fe, Cu, Mn, K, Cr and Hg) in the leaves and stems were analysed and reported as µg g^{-1} DW. The detection limits for Fe, Cu, Mn, K, Cr and Hg were 0.05-20, 0.03-10, 0.02-5.0, 0.5-500.0, 0.03-5.0 and 0.5-1000 mg/L, respectively.

Malondialdehyde, Hydrogen Peroxide and Relative Water Analysis

Malondialdehyde (MDA) level in the leaves of *M. arvensis* was analysed as described by Heath and Packer, (1968) and reported as µM g^{-1} FW. Hydrogen Peroxide (H_2O_2) was also analysed in the leaves of experimental plants from all the treatments as per Velikova *et al.,* (2000). Relative Water Content (RWC) in leaves was also analysed after recording fresh, dry and turgid weights

Table:1 Physicochemical specification of experimental FA and soil

Physico-chemical properties	*Soil*	*Fly-ash*
Texture	Sandy loam	Silt
Colour	Yellowish brown	Light grey
pH	7.06±0.04	8.67±0.09
Electrical conductivity (dS/m)	0.412±0.017	0.545±0.027
Organic content (%)	0.75±0.04	0.15±0.005
Available Nitrogen (Kg/ha)	458.2±18.23	38.91±1.73
Available Phosphorus (Kg/ha)	69.27±3.43	28.11±1.20
Available Potassium (Kg/ha)	226.6±22.13	125.43±4.26
Iron (µg g^{-1} DW)	997.67±34.49	5143.43±216.90
Manganese (µg g^{-1} DW)	29.90±1.28	164.9±6.06
Copper (µg g^{-1} DW)	6.13±0.31	13.50±0.60
Chromium (µg g^{-1} DW)	3.93±0.15	11.83±0.29
Mercury (µg g^{-1} DW)	0.52±0.02	3.966667±0.15

Values represented as mean±SD.

of *M. arvensis* leaves from all the treatments (Kumar *et al.,* 2023).

Cellular antioxidants

Free proline, cysteine, non-protein-thiols (NP-SH) and total phenolic content (TPC) was determined in leaves of *M. arvensis* from all the treatments as described by Bates *et al.,* (1973), Gaitonde (1967), Ellman (1959) and Ainsworth and Gillespie (2007), respectively. All the studied cellular antioxidants were quantified using a standard curve. Free proline and cysteine have been reported in µmol g^{-1} FW, while NP-SH and TPC has been reported as µg g^{-1} FW and mg GAE g^{-1} FW, respectively.

Statistical analysis

All the results reported in the present study as mean ± standard deviation (SD). Further, One-way ANOVA and Tukey HSD test ($p < 0.05$) was applied for statistical significance using RStudio version 4.4.1. Correlation analysis was also employed in this study and the correlation coefficient (r) was calculated for all the studied parameters.

Results and Discussion

Characterization of soil and fly ash

Experimental soil used in the present study exhibited low pH and EC (pH:7.06, EC:0.412) as compared to fly-ash (pH:8.67, EC:0.545). The texture of the soil was sandy loam, while fly-ash was silty powder. The organic content (%), available N, K and P was higher in soil than fly-ash (Table 1). The availability of N, K and P has been represented in kg ha^{-1} in the experimental soil and fly-ash. Further, the results of ICP-OES revealed the Metal (Fe, Mn, Cu, Cr and Hg) rich composition of fly-ash in contrast to experimental soil (Table 1). The amount of heavy metal (Cu, Cr, Hg) was higher in fly-ash than in experimental soil. The SEM and SEM-EDAX also confirmed the elemental profile and texture of fly ash and soil surface. The SEM micrographs showed the rough surface of soil, while fly-ash particles were tiny and smooth at 1000X magnification (Fig. 1b, d). The peaks of different elements (Na, Mg, Al, Si, P, K, Ca, Fe, Cu, and Zn) have been observed in the spectra of both soil and fly-ash (Fig. 1a, c). Few peaks for metal (Cr, Ni, Hg and Tl) were only visible in fly-ash spectra (Fig. 1).

Elemental analysis

The metal (Fe, Cu, K, Mn, Cr and Hg) accumulation was analysed in leaves and stems of *M. arvensis*. The Fe level in the leaves of *M. arvensis* significantly (Tukey-HSD post-hoc, $p < 0.05$) increased by 1.61, 2.31, 2.88, 3.22 and 3.61-fold at treatment M_2, M_3, M_4, M_5 and M_6, respectively, compared to control. However, the increment observed in Fe level was 1.52, 1.89, 2.31, 3.51 and 4.72fold in stems of test plant at treatments M_2, M_3, M_4, M_5 and M_6, respectively (Fig. 2a). Cu accumulation in leaves of *M. arvensis* was also increased in concentration regulated manner (1.24, 1.45, 1.53 and 1.64fold at treatment M_3, M_4, M_5 and M_6, respectively) but no significant difference (Tukey-HSD post-hoc, $p > 0.05$) in Cu level was observed at treatments M_1 (100% soil) and M_2 (15% FA). However, the levels of Cu significantly increased (Tukey-HSD post-hoc, $p < 0.05$) by 1.24, 1.49, 1.63, 1.80 and 2.12fold at treatment M_2, M_3, M_4, M_5 and M_6, respectively with respect to control (Fig. 2b). The levels of Mn in leaves of *M.*

arvensis increased significantly (Tukey-HSD post-hoc, $p < 0.05$) at >15% fly-ash and found maximum (1.70fold to control) at treatment M_6 (100% fly-ash) while significant rise (1.33fold) was observed in stems of test plant at treatment M_2 (15% fly-ash). The leaves and stems of *M. arvensis* exhibit no significant difference (Tukey-HSD post-hoc, $p > 0.05$) in Mn levels at treatment M_3, M_4 and M_5 (Fig. 2c). The increased Fe, Cu and Mn levels in the leaves of *M. arvensis* at treatments with higher FA proportion may be due to higher levels of these metals in fly-ash than soil as evident in this study (Table 1). The accumulation of transition metals viz., Fe, Cu and Mn may enhance oxidative stress in plants by generating free radicals. The increased Fe and Cu may catalyse the Fenton's reaction, generating hydroxyl radicals by mono-electron transfer using hydrogen peroxide as a substrate, resulting in physiological abnormalities and ceasing the growth of the plant (Michalak, 2006). The reduced growth of the test plant with higher fly-ash concentrations (>30%) confirms the heavy metal-induced growth reduction in *M. arvensis*. The results of the current study are in agreement with a study reporting the accumulation of Fe and Mn and reduced growth in *Brassica juncea* and *Spinacea oleracea* L. grown at 50 and 100% fly-ash (Mashau *et al.,* 2018). Another report also documented the more uptake and accumulation of heavy metals (Fe, Cu, Mn) in *Docus carota* with increasing fly-ash concentration (Shakeel *et al.,* 2021). In contrast to transition metals, the K levels were downregulated in higher fly-ash concentrations. Maximum decrease of 1.52 and 1.36fold was observed at treatment M_6 in stems and leaves, respectively (Fig. 2d). Since the present study also reports the oxidative stress induced membrane damage at high FA concentrations, thus the phenomenon of ion leakage may support the low K with increasing FA concentrations. Similarly, Ansari *et al.,* (2022) also reported the downregulation of K levels in radish grown in 25, 30 and 35% fly-ash. Further, a significant increase in Cr levels was observed in leaves and stems of *M. arvensis* at all the tested concentrations of fly-ash. The maximum accumulation of Cr in leaves and stems (2.14 and 2.56, respectively) of *M. arvensis* was found at treatment M_6 (Fig. 2e). Cr accumulation in leaf accumulates ROS, inhibits pigment synthesis, PSI & PSII and affects metabolic processes (Patra *et al.,* 2024). It is evident in this study that increased Cr accumulation in treatments with high fly-ash concentrations (>30%) resulted in reduced photosynthetic pigments and elevated antioxidants. Moreover, the levels of Hg increased by 2.0fold in leaves and 2.23fold in stems at treatment M_6 as compared M_1 (Fig. 2f). İşkil *et al.,* (2022) reported that Hg induced oxidative damage in *Triticum aestivum* L. and alters the expression of genes for abiotic stress response. Thus, the reduced growth of the test plant (>30%) in the present study may be a collective response of metals accumulation and induced oxidative stress. In contrast, no reduction in growth pattern of *M. arvensis* was observed up to 30% FA (Table 2, Fig. 3), instead of accumulated heavy metals in leaves and stem tissues. Recently, it has been reported that *Mentha spp.* have high feasibility to grow in metal rich environment (Mahdavian, 2026; Dinu *et al.,* 2021). Our results in this study also suggest the high potential of *M. arvensis* to grow in metals/metalloid-rich fly-ash contaminated soils. Further, a positive correlation between fly-ash levels and all studied metals (Fe, Cu, Mn, Cr and Hg) except K was observed (Fig. 6a).

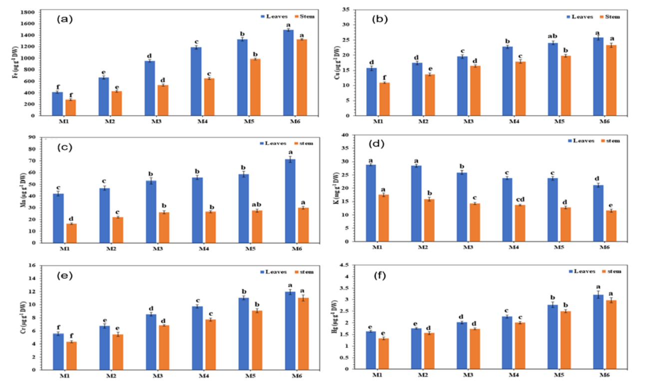

Fig 2: Accumulation of metals in leaves and stems of *Mentha arvensis* (var. Kosi) grown in different fly-ash concentrations (M1: Control, M2:15% FA, M3:30% FA, M4:45% FA, M5:60% FA, M6:100% FA). Values represented as mean±SD (n=6). One-way ANOVA with Tukey-HSD post-hoc ($p < 0.05$), Different letters on bars represent significance at $p < 0.05$ and vice versa.

A negative correlation (Correlation coefficient, r:-0.95 for leaves and r:-0.97 for stems at df=4, p<0.05) between K and fly-ash concentrations in soil supports our observation that fly-ash induce oxidative stress and membrane damage resulting into reduced K in *M. arvensis*.

Growth, morphometric traits and phytopigments

Fig. 3 illustrates the growth pattern in different proportions of fly ash in soil. The highest number of leaves and primary branches were found in treatment M_2, exhibiting 15% FA (Table 2). The increment of fly-ash in the experimental soil resulted in decreased numbers of branches and leaves with respect to the control. The minimum number of branches and leaves was recorded in 100% fly-ash grown plants (Table 2). Whole plant biomass was found elevated by 19.08 and 8.70% in treatments M_2 (15% FA) and M_3 (30% FA), respectively (Table 2). The shoot height (14.27%) and root length (33.03%) of *M. arvensis* were also supported by 15% FA (M_2) compared to plants grown in soil

Fig 3: Growth and morphometric traits of *Mentha arvensis* (var. Kosi) after 60 d exposure of different fly-ash concentrations (a: Control, b:15% FA, c:30% FA, d:45% FA, e:60% FA, f:100% FA)

Table. 2. Growth attributes and morphological traits of *Mentha arvensis* (var. Kosi) at different fly-ash treatments

Treatment	*Whole plant biomass (g)*	*Shoot Height (cm)*	*Root length (cm)*	*No. of primary branches*	*No. of leaves per plant*	*Total Chlorophyll ($mg\ g^{-1}$ FW)*	*Chlorophyll 'a' ($mg\ g^{-1}$ FW)*	*Chlorophyll 'b' ($mg\ g^{-1}$ FW)*	*Carotenoids ($mg\ g^{-1}$ FW)*
M_1	$42.07^c\pm1.10$	$37.13^b\pm0.77$	$24.43^b\pm0.60$	$13.33^b\pm1.16$	$122.00^b\pm5.57$	$1.73^{bc}\pm0.034$	$1.25^b\pm0.020$	$0.474^b\pm0.014$	$0.136^b\pm0.003$
M_2	$50.10^a\pm1.15$	$42.43^a\pm0.60$	$32.50^a\pm0.50$	$16.33^a\pm0.58$	$138.33^a\pm6.51$	$1.97^a\pm0.028$	$1.42^a\pm0.015$	$0.551^a\pm0.014$	$0.165^a\pm0.004$
M_3	$45.73^b\pm0.81$	$40.13^a\pm1.40$	$33.80^a\pm0.26$	$13.67^b\pm0.58$	$124.67^b\pm5.51$	$1.78^b\pm0.082$	$1.26^b\pm0.056$	$0.523^a\pm0.027$	$0.134^b\pm0.006$
M_4	$30.93^d\pm1.14$	$30.57^c\pm1.50$	$25.60^b\pm0.53$	$10.67^c\pm0.58$	$79.33^c\pm3.06$	$1.62^c\pm0.018$	$1.17^c\pm0.015$	$0.450^b\pm0.005$	$0.135^b\pm0.001$
M_5	$25.43^e\pm1.25$	$25.50^d\pm0.78$	$21.83^c\pm0.61$	$9.67^c\pm0.58$	$70.00^c\pm2.00$	$1.43^d\pm0.042$	$1.05^d\pm0.032$	$0.385^c\pm0.011$	$0.111^c\pm0.003$
M_6	$23.57^e\pm0.86$	$21.87^e\pm1.01$	$18.40^d\pm0.87$	$6.30^d\pm0.58$	$50.00^d\pm2.00$	$1.40^d\pm0.013$	$1.08^d\pm0.024$	$0.316^d\pm0.019$	$0.105^c\pm0.001$

Values mean±SD (n=6). One-way ANOVA with Tukey-HSD post-hoc ($p < 0.05$), Different letters on bars represent significance at $p < 0.05$ and vice versa.

(100%). Further, FA alone (treatment M_6) reduced 41.1 and 24.68% shoot height and root length, respectively (Table 2). The negative correlation between fly-ash and morphological traits viz., root length (Correlation coefficient, r: -0.67), shoot height (Correlation coefficient, r: -0.88), whole plant biomass (Correlation coefficient, r: -0.85), no. of leaves (Correlation coefficient, r: -0.91) and primary branches (Correlation coefficient, r: -0.91) were also evident in this study (Fig. 6b). Further, all the studied morphological parameters were significantly positively correlated with each other (Fig. 6b). The enhanced growth of *M. arvensis* at low fly-ash concentrations may be due to available N, P and K in added soil while treatment with higher concentrations of fly-ash might have suffered with N deficiency (Table 1). In a similar vein, few previous studies documented the lower doses of fly-ash supporting growth of *Cymbopogon citratus* and *Brassica juncea* (Shakeel *et al.,* 2019; Panda *et al.,* 2018). Further, Tchl, chl a, chl b and carotenoids were increased by 13.90, 13.60, 16.25 and 21.32% with respect to control at treatment M_2 (15% FA), respectively. However, the plants grown in treatment M_6 (100% FA) showed minimum chlorophylls (Tchl, chl a, chl b) and carotenoid contents (Table 2). The FA concentrations dependent decrease in photosynthetic pigments of *Beta vulgaris* has been reported (Shakeel *et al.,* 2020). The downregulation of photosynthetic pigments in the test plant might be correlated with their lower production or degradation induced by reactive oxygen species (Pandey, 2013). Moreover, the disruption of the chlorophyll structure (replacement of Mg with another heavy metal) might also result in decreased chlorophyll at treatments with higher fly-ash concentrations (Kupper, 1998). Besides, the inhibition of 5-aminolevulinic acid dehydratase (ALA-D), an enzyme for tetrapyrrole precursor porphobilinogen (PBG) biosynthesis, by mercury (Hg) have been reported in maize leaves (Gupta *et al.,* 2013). Chromium (Cr) and Cadmium (Cd) regulated suppression of ALA-D have also been observed in *Nymphaea alba* L. and *Pfaffia glomerata* (Skrebsky *et al.,* 2008; Vajpayee *et al.,* 2000). Hence, the reduced photosynthetic pigments at high level of FA might be due to elevated heavy metals accumulation in *M. arvensis* (Table 2, Fig. 2). Moreover, carotenoids (terpenoids; 40 carbon) perform a protective role by quenching triplet excited chlorophyll and singlet oxygen (Swapnil *et al.,* 2021). In the present study, no downregulation of carotenoids was observed up to treatment $M_{4,}$ suggesting the active participation of carotenoids in their protective role and elimination of reactive oxygen species-regulated stress. The higher accumulation of heavy metals and upregulated oxidative stress markers at treatment M_5 and M_6 suggested the overproduction of ROS, which ultimately leads to cellular oxidative damage and decreases in carotenoid contents. Similar to our study, the heavy metal-induced overproduction of ROS, oxidative damage, and compromised carotenoid content has been documented in Cd-stressed rice plants (Biswas and Pal, 2022). Further, Pearson's correlation analysis suggests a significant ($p<0.05$, df=4) positive correlation between photosynthetic pigments (total chlorophyll, chl a, chlb) and morphological parameters with each other. However, there was significant ($p<0.05$, df=4) negative correlation among fly-ash levels and total chlorophyll (Correlation coefficient, r: -0.82) (Fig. 6b). The negative correlation between total chlorophyll and fly-ash levels suggest the adverse impact of fly-ash on photosynthetic pigment.

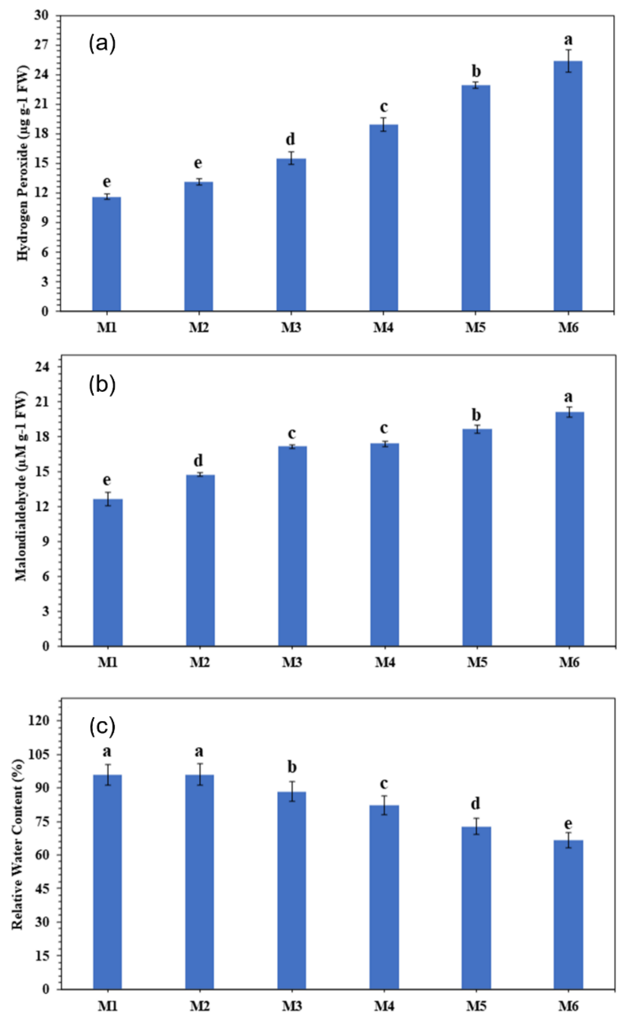

Fig 4: Levels of malondialdehyde, hydrogen peroxide and relative water content in leaves of *Mentha arvensis* (var. Kosi) after 60 days of exposure of different fly-ash concentrations (M1: Control, M2:15% FA, M3:30% FA, M4:45% FA, M5:60% FA, M6:100% FA). Values represented as mean±SD (n=6). One-way ANOVA with Tukey-HSD post-hoc ($p < 0.05$), Different letters on bars represent significance at $p < 0.05$ and vice versa.

Malondialdehyde, Hydrogen Peroxide and Relative Water Content

The enhanced levels of MDA and H_2O_2 in plant tissues reflect the presence of oxidative stress in *M. arvensis* leaves grown in FA-contaminated soils. The MDA contents in treatments M_2 (15% FA), M_3 (30% FA), M_4 (45% FA), M_5 (60% FA) and M_6 (100% FA) were significantly (Tukey-HSD post-hoc, $p < 0.05$) elevated by 1.16, 1.36, 1.38, 1.48, 1.59fold in comparison to control (Fig. 4b). In this study, no significant difference (Tukey-HSD post-hoc, $p > 0.05$) was observed in H_2O_2 level at treatment M_2 (15% FA) with respect to control plants (M_1: 100% soil) while significant increments (Tukey-HSD post-hoc, $p < 0.05$)of 1.34, 1.63, 1.97 and 2.18fold were observed at treatments M_3 (30% FA), M_4 (45% FA), M_5 (60% FA) and M_6 (100% FA), respectively (Fig. 4a). The significant (Tukey-HSD post-hoc, $p < 0.05$) reduction of 3.04, 8.05, 14.33, 24.30 and 30.65% in RWC were also observed at treatments M_2, M_3, M_4, M_5 and M_6 respectively, compared to M_1 (Fig. 4c). The results of the present study indicate the enhanced oxidative stress and membrane damage with increasing fly-ash concentrations. The metals/metalloids present in fly ash may have induced the ROS generation, leading to increasing MDA and H_2O_2 in *M. arvensis* leaves. A study reported the increased levels of MDA in *Ricinus cummunis*, collected from fly-ash contaminated sites (Boda *et al.,* 2017). Few studies on oil-yielding and medicinal plants (*Cymbopogon citratus, Bacopa monnieri* and *Brassica juncea*) also reported the induced MDA with high fly-ash doses (Panda *et al.,* 2020; Ashfaque *et al.,* 2019; Panda *et al.,* 2018). Ansari *et al.,* (2024) also reported increased MDA and H_2O_2 contents at the highest tested fly-ash concentration (25%) in the Kushal variety of *M. arvensis*. Additionally, we observed the decreased RWC at M_3, M_4, M_5 and M_6, might also be due to increased membrane damage. RWC (drought stress indicator) is also linked with the protoplasmic permeability and maintains physiological homeostasis under abiotic stresses (Qadir *et al.,* 2019). In our study, the decrease in RWC at a higher level of fly-ash (≥30%) suggested the fly-ash induced drought stress-like conditions to the exposed plants, which ultimately leads to a decrease in plant growth. Notably, MDA, H_2O_2 and RWC levels in FA-treated plants were significantly correlated with the concentration of FA in treatments (correlation coefficient, r:0.94 and r:0.97, r:-0.97 $p<0.05$, df=4, for MDA, H_2O_2 and RWC, respectively), suggesting the direct relation of fly-ash with oxidative markers (MDA and H_2O_2) while inverse relation with RWC (Fig. 6).

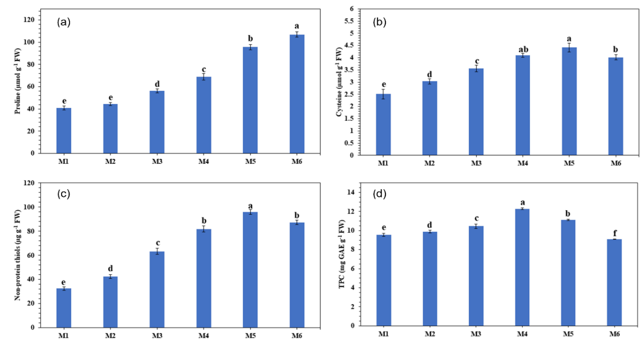

Fig 5: Cellular antioxidants (cysteine, proline, total phenolic content and non-protein thiols) in leaves of Mentha arvensis (var. Kosi) after 60 d exposure of different fly-ash concentrations (M1: Control, M2:15% FA, M3:30% FA, M4:45% FA, M5:60% FA, M6:100% FA). Values represented as mean±SD (n=6). One-way ANOVA with Tukey-HSD post-hoc ($p < 0.05$), Different letters on bars represents significance at $p < 0.05$ and vice versa

Cellular antioxidants

The level of cysteine was upregulated in the leaves of all the treatments as compared to the control. Maximum increment in cysteine content was observed at treatment M_5 by 76.10%, while the lowest increase was recorded at treatment M_2 (20.72%) with respect to the plants grown in 100% soil. Treatment M_3 and M_4 showed 41.83% and 62.94% increase in cysteine contents (Fig. 5b). Notably, the positive correlation (r: 0.793) was observed in between fly-ash and cysteine. Non-protein-thiol (NP-SH) was

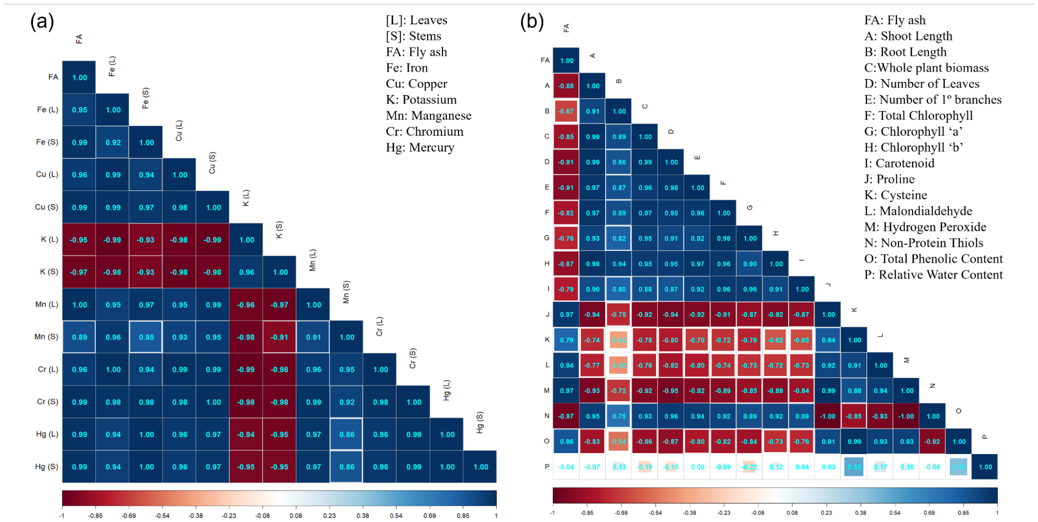

Fig 6: Pearson correlation coefficient 'r'; [a] between accumulated heavy metals in leaves and stem of *Mentha arvensis* (var. Kosi) at different fly-ash exposure and fly-ash, [b] between morphological, physiological traits of fly-ash exposed *Mentha arvensis* (var. Kosi) leaves and fly-ash; r value =0.81(5%) at df (n-2) =4

found to increase up to 60% fly-ash exposure and was reduced significantly in plants grown under 100% fly-ash. The rise in NP-SH content was 1.30, 1.95, 2.52, 2.96 and 2.70fold in leaves of *Mentha arvensis* (Fig. 5c). NP-SH and cysteine both were showing significant correlation with each other and fly-ash (Fig. 6b). Low molecular weight thiols play a vital role in heavy metal-induced oxidative stress amelioration. Kumar *et al.,* (2025) reported the increased cysteine and NP-SH in *Ocimum basilicum* with increasing concentrations of Cu nanoparticles and Cu ions exposure (0.2 and 0.4 µg ml^{-1}). Thus, we propose that the increased cellular antioxidants in treatments with heavy metals accumulation suggest the active participation of low molecular weight thiols against abiotic stress. A few studies have suggested the role of thiols in maintaining ROS homeostasis (Kumar *et al.,* 2026; Kumar *et al.,* 2025; Kapoor *et al.,* 2015). Total phenolic contents (TPC) also serve as cellular antioxidants. Results showed no significant (Tukey-HSD post-hoc, $p > 0.05$) change in TPC at treatment M_2, while increased levels were observed at treatments M_3, M_4, and M_5 compared to M_1 (control). The levels of TPC were elevated by 2.02 and 1.16fold at treatment M_4 and M_5, while downregulated by 0.84fold at treatment M_6, compared to control plants (Fig. 5d). Similarly, the elevated phenolics in *Festuca rubra* sown on FA deposits were documented by Gajic *et al.,* (2016). Being a part of lignin biosynthesis in plants, TPC strengthens the cell wall in heavy metal stress conditions and build physical barrier as a protective strategy (Diaz *et al.,* 2001). Hence, the increased phenolics in leaves of *M. arvensis* is suggestive of adaptive responses of the plant for constituent heavy metals under different fly-ash exposure. Further, free proline was also increased ≥ 30% fly-ash grown plants (Fig. 5a). The proline level was maximum at treatment M_6 (2.61fold) followed by 2.33, 1.68, 1.37 and 1.08fold at treatments M_5, M_4, M_3 and M_2 respectively (Fig. 5a). Proline also exhibits the positive correlation (Correlation coefficient, r: 0.96) with fly-ash concentrations (Fig. 6b). The osmo-protectant behaviour of proline improves the osmotic balance of the cell and the potential of proline to trigger metal chelation may be advantageous under constituent heavy metal/metalloid toxicity. In the present study, increased proline at higher fly-ash treatment suggests the adaptive strategy of *M. arvensis* under different fly-ash exposures. This observation is supported by a previous study reporting the highest accumulation of proline in *Azadirachta indica* collected from heavy metal-rich fly-ash dump sites compared to a control site (Qadir *et al.,* 2016). Overall, increased cellular antioxidants in *M. arvensis* in response to fly-ash suggest the active plant defence response against metals/metalloid-induced abiotic stress.

Conclusion

The results observed in the present study indicate the potential of *M. arvensis* (var. Kosi) to grow in fly-ash contaminated soils. The growth (plant height, root length, whole plant biomass, number of leaves and primary branches) and phytopigments of test plant were maximally supported by 15% fly-ash; however, the decreased growth of the test plant was found ≥45% fly-ash in soil. The increased accumulation of metals (Fe, Cu, Mn, Cr and Hg) was observed in leaves and stems of the test plant with increasing fly-ash proportion in soil. The accumulated metals in leaves induced oxidative stress, resulting in membrane damage, evident by enhanced MDA and low K levels in leaves. The increased levels of cellular antioxidants (cysteine, TPC and NP-SH) in leaves are indicative of the adaptive strategy opted by *M. arvensis* (var. Kosi) against stress imposed by constituent heavy metals/metalloids of fly-ash. The present study highlights the dose-dependent response of *M. arvensis* (var. Kosi) in fly-ash mixed soil and the feasibility of its commercial cultivation in mildly degraded agrifields with 15% fly-ash to enhance the livelihood of farmers.

Acknowledgements

We acknowledge the Department of Botany, University of Lucknow-226007, Uttar Pradesh, India, for providing necessary research facilities. Vippan Kaur is highly grateful to the University Grant Commission (UGC) for providing financial support (CSIR-UGC fellowship, Ref. no. 201610170774) for research work. Further, financial assistance in the form of research associateship provided by the Department of Biotechnology (DBT) New Delhi under DBT Builder Programme (BT/INF/22/SP47623/2022) to Subodh Kumar is also acknowledged.

Funding information

There was no funding for this piece of research work.

Conflict of interest

The authors declare that they have no known competing interests.

Author Contribution declaration

Vippan Kaur performed Formal analysis and wrote the original draft of the present manuscript; Vippan Kaur, Subodh Kumar, and Poornima Vajpayee analysed data, performed statistical analysis, reviewed and edited the manuscript; Akanksha Singh and Poornima Vajpayee supervised the experiments of this study; and Poornima Vajpayee provided the necessary lab facilities.

References

Abhishek, S., Ghosh, A., Singh, A.A., Pandey, B. (2025). Identification of metal-tolerant herbaceous species for phytostabilization and ecological restoration of fly-ash dumpsites, *Physiol Mol Biol Plants*, vol. 31 p. 1719–1738. https://doi.org/10.1007/s12298-025-01612-3.

Ainsworth, E.A. & Gillespie, K.M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent, *Nature Protocol*, vol. 2(4), p. 875-877. https://doi.org/10.1038/nprot.2007.102.

Alonso Leite Dos Santos, C., Maria Tavares Moreira, A., Rayanne da Silva Teles, B., (2024). Mentha arvensis oil exhibits repellent acute toxic and antioxidant activities in Nauphoeta cinerea, *Sci Rep*., vol. 14(1), p. 21599. https://doi.org/10.1038/s41598-024-72722-3.

Ansari, M.S., Ahmad, G., Khan, A.A., Heba, M. (2024). Evaluation of Coal Fly-ash for Reinforcing the Plant Growth, Antioxidant Properties, and Essential Oil Content of *Mentha arvensis* L.: A Sustainable Approach to Coal Waste Management, *J. Soil Sci. Plant Nutr*., vol. 24, p. 1369–1393. https://doi.org/10.1007/s42729-024-01642-y.

Ansari, M.S., Ahmad, G., Khan, A.A., Mohamed, H.I., (2022). Coal fly-ash and nitrogen application as eco-friendly approaches for modulating the growth, yield, and biochemical constituents of radish plants, *Saudi J. Biol. Sci*., vol. 29, p. 103306. https://doi.org/ 10.1016/j.sjbs.2022.103306.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidase in Beta vulgaris, *Plant Physiology*, vol. 24, p. 1-15. https://doi.org/10.1104/pp.24.1.1.

Ashfaque, F. &Inam, A. (2019). Interactive effect of potassium and fly-ash, a soil conditioner, on metal accumulation, physiological and biochemical traits of mustard (*Brassica juncea* L.), *Environmental Science & Pollution Research*, vol. 26 (8), p. 7847-7862. https://doi.org/10.1007/s11356-019-04243-w.

Baser, K.H. & Bonello, J.M. (2025). Global trade of essential oils. Journal of Essential Oil Research, vol. *37*(2), p. 208–214. https://doi.org/10.1080/10412905.2025.2470791.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies, *Plant Soil*, vol. 39, p. 205-207. https://doi.org/10.1007/BF00018060.

Biswas, A. & Pal, S. (2022). Comparative screening of cadmium stress responses among three rice cultivars, *Environmental and Experimental Biology*, vol. 20 (3), p. 179–192. https://doi.org/10.22364/eeb.20.17.

Boda, R.K., Majeti, N.V.P., Suthari, S. (2017). *Ricinus communis* L. (castor bean) as a potential candidate for revegetating industrial waste contaminated sites in peri-urban Greater Hyderabad: Remarks on seed oil, *Environmental Science & Pollution Research*, vol. 24, p. 19955-19964. https://doi.org/10.1007/s11356-017-9654-5.

CEA, Report on Fly-ash Generation at Coal / Lignite Based Thermal Power Stations and Its Utilization in the Country for the Year 2021–22, Central Electricity Authority, Ministry of Power, Government of India, (2022) https://cea.nic.in/wp-content/uploads/tcd/2022/08/Fly_ash_Generation_and_utilisation_Report_2021_22-1.pdf (accessed on 06 January 2026).

Diaz, J., Bernal, A., Pomar, F., Merino, F. (2001). Induction of shikimate dehydrogenase and peroxidase in pepper (Capsicum annuum L.) seedlings in response to copper stress and its relation to lignification, *Plant Sci*., vol. 161, p. 179–188. https://doi.org/10.1016/S0168-9452(01)00410-1.

Dickman, S.R. & Bray, R.H. (1940). Colorimetric determination of phosphate, *Ind Eng Chem Anal Ed*, vol. 12, p. 665–668.

Dinu, C., Gheorghe, S., Tenea, A.G., (2021). Toxic Metals (As, Cd, Ni, Pb) Impact in the Most Common Medicinal Plant (Mentha piperita), *Int J Environ Res Public Health*, vol. 18(8), p. 3904. https://doi.org/ 10.3390/ijerph18083904.

Duxbury, A.C. & Yentsch, C.S. (1956). Plankton pigment nomograph. *Journal of Marine Research*, vol. 15, p. 92-101.

Ellman, G.L. (1959). Tissue sulphydryl groups, *Arch Biochem Biophys*, vol. 82, p. 70–77. https://doi.org/10.1016/0003-9861(59)90090-6.

Faisal, S., Tariq, M.H., Ullah, R., Zafar, S., (2023). Exploring the antibacterial, antidiabetic, and anticancer potential of Mentha arvensis extract through in-silico and in-vitro analysis, *BMC Complement. Med. Ther*., vol. 23 (1), p. 267. https://doi.org/10.1186/s12906-023-04072-y.

Gaitonde, M.K. (1967). A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids, *Biochemical Journal*, vol. 104 (2), p. 627-633. https://doi.org/10.1042/bj1040627.

Gajic, G., Djurdjevic, L., Kostic, O., (2016). Assessment of the phytoremediation potential and an adaptive response of *Festuca rubra* L. sown on fly-ash deposits, *Ecological Engineering*, vol. 93, p. 250-261. https://doi.org/10.1016/j.ecoleng.2016.05.021.

Goswami, V., Sharma, D., Diwakar, S., Kothamasi, D. (2023). Arbuscular mycorrhizas amplify the risk of heavy metal transfer to human food chain from fly-ash ameliorated agricultural soils, *Environmental Pollution*, vol. 329, p.121733. https://doi.org/10.1016/j.envpol.2023.121733.

Gupta, P., Jain, M., Sarangthem, J., Gadre, R. (2013). Inhibition of 5-aminolevulinic acid dehydratase by mercury in excised greening maize leaf segments, *Plant physiology and biochemistry*, vol. 62, p. 63–69. https://doi.org/10.1016/j.plaphy.2012.10.008.

Heath, R.L. & Packer, L. (1968). Photo peroxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, vol. 125(1), p. 189-198. https://doi.org/10.1016/0003-9861(68)90654-1.

İşkil, R., Surgun-Acar, Y., Catav, Ş.S., (2022). Mercury toxicity affects oxidative metabolism and induces stress responsive mechanisms in wheat (*Triticum aestivum* L.), *Physiol. Mol. Biol. Plants*, vol. 28, p. 911–920. https://doi.org/10.1007/s12298-022-01171-x.

Kapoor, D., Sharma, R., Handa, N., Kaur, H., (2015). Redox homeostasis in plants under abiotic stress: role of electron carriers, energy metabolism mediators and proteinaceous thiols, *Front. Environ. Sci*., vol. 3, p.13 https://doi.org/ 10.3389/fenvs.2015.00013.

Kim, S.Y., Han, S.D., Kim, M., Mony, T.J., (2021). *Mentha arvensis* Essential Oil Exerts Anti-Inflammatory in LPS-Stimulated Inflammatory Responses via Inhibition of ERK/NF-κB Signalling Pathway and Anti-Atopic Dermatitis-like Effects in 2,4-Dinitrochlorobezene-Induced BALB/c Mice, *Antioxidants*, vol. 10, p. 1941. https://doi.org/10.3390/

antiox10121941.

Kumar, A., Jnanesh, A.C., Kumar, D., Lal, R.K. (2024). Response of aromatic Rosa grass to different proportions of fly-ash and vermicompost, Biomass and Bioenergy, p. 107308. https://doi.org/10.1016/j.biombioe.2024.107308.

Kumar, S., Dwivedi, A., Pandey, A.K., Vajpayee, P. (2023). TiO_2 nanoparticles alter nutrients acquisition, growth, biomacromolecules, oil composition, and modulate antioxidant defense system in *Mentha arvensis* L., *Plant Nano Biology*, vol. 3, p. 100029. https://doi.org/10.1016/j.plana.2023.100029.

Kumar, S., Kaur, V., Dwivedi, A., Dubey, A., (2025). Impact of copper oxide nanoparticles on uptake, cellular distribution, growth, ionome, antioxidant defense system and essential oil composition of Ocimum basilicum L. *Environmental Sustainability*, vol. 8, p. 513–531. https://doi.org/10.1007/s42398-025-00366-8.

Kumar, S., Kaur, V., Vajpayee, P. (2026). Unveiling the impact of cerium oxide nanoparticles on bio-macromolecules, antioxidants and bacoside-A of Bacopa monnieri (L.) Pennell, *Plant Nano Biology*, Vol. 15, p. 100247. https://doi.org/10.1016/j.plana.2026.100247.

Kupper, H., Kupper, F., Spiller, M. (1998). In situ detection of heavy metal substituted chlorophylls in water plants, *Photosynthesis Research*, vol. 58(2), p. 123-133. https://doi.org/ 10.1023/A:1006132608181.

Mahdavian, K. (2026). Integrating biotechnology and microbial synergies in Mentha-based phytoremediation for sustainable pollution mitigation, *Ecological Frontiers*, vol. 46(2), p. 450-458. https://doi.org/10.1016/j.ecofro.2025.10.016.

Mashau, A., Gitari, M., Akinyemi, S. (2018). Evaluation of the bioavailability and translocation of selected heavy metals by *Brassica juncea* and *Spinacea oleracea* L. for a South African power utility coal fly-ash, *International Journal of Environmental Research and Public Health*, vol. 15(12), p. 2841. https://doi.org/10.3390/ijerph15122841.

Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress, *Polish Journal of Environmental Studies*, vol. 15 (4), p. 523-530.

Merwin, H.D., & Peech, M. (1951) Exchangeability of Soil Potassium in the Sand, Silt, and Clay Fractions as Influenced by the Nature of the Complementary Exchangeable Cation. Soil Science Society of America Journal, 15, 125-128. doi:10.2136/SSSAJ1951.036159950015000C0026X.

Panda, D., Barik, J.R., Barik, J., Behera, P.K., (2020). Suitability of Brahmi (*Bacopa monnieri* L.) cultivation on fly-ash-amended soil for better growth and oil content. *International Journal of Phytoremediation*, vol. *23* (1), p. 72–79. https://doi.org/10.1080/15226514.2020.1791052.

Panda, D., Panda, D., Padhan, B., Biswas, M. (2018). Growth and physiological response of lemongrass (*Cymbopogon citratus* (D.C.) Stapf.) under different levels of flyash-amended soil, *International Journal of Phytoremediation*, vol. 20(6), p. 538-544. https://doi.org/10.1080/15226514.2017.1393394.

Pandey, V.C. & Singh, N. (2020). Impact of fly-ash incorporation in soil systems, *Agriculture, Ecosystems & Environment*, vol. 136, p. 16-27. https://doi.org/10.1016/j.agee.2009.11.013.

Pandey, V.C. (2013). Suitability of *Ricinus communis* L. cultivation for phytoremediation of fly-ash disposal sites, *Ecological Engineering*, vol. 57, p. 336–341. https://doi.org/ 10.1016/j.ecoleng.2013.04.054.

Parić, A., Mesic, A., Mahmutović-Dizdarević, I., Jerković-Mujkić, A., (2024). Bioactive potential of *Mentha arvensis* L. essential oil, *Journal of Environmental Science and Health*, *Part B*, vol. 59(9), p. 584–594. https://doi.org/10.1080/03601234.2024.2396730.

Patra, H.K., Patra, D.K., Acharya, S. (2024). Chromium-induced phytotoxicity and its impact on plant metabolism, *Acta. Physiol*. Plant, vol. 4620. https://doi.org/10.1007/s11738-023-03646-0.

Qadir, S.U., Raja, V., Siddiqui, W. (2016). Morphological and biochemical changes in *Azadirachta indica* from coal combustion fly-ash dumping site from a thermal power plant in Delhi, India, *Ecotoxicology and Environmental Safety*, vol. 129, p. 320-328. https://doi.org/ 10.1016/j.ecoenv.2016.03.026.

Qadir, S.U., Raja, V., Siddiqui, W.A., Mahmooduzzafar E.F., (2019). Fly-Ash Pollution Modulates Growth, Biochemical Attributes, Antioxidant Activity and Gene Expression in Pithecellobium Dulce (Roxb) Benth. *Plants (Basel)*, vol. 8 (12), p. 528. https://doi.org/ 10.3390/plants8120528.

Ram, L.C. & Masto, R.E. (2014). Fly-ash for soil amelioration: A review on the influence of ash blending with inorganic and organic amendments, *Earth-Science Reviews*, vol. 128, p. 52-74. https://doi.org/10.1016/j.earscirev.2013.10.003.

Shakeel, A., Khan, A.A., Ahmad, G. (2019). The potential of thermal power plant fly-ash to promote the growth of Indian mustard (*Brassica juncea*) in agricultural soils, *SN Applied Sciences*, vol. 1(4), p. 375. https://doi.org/10.1007/s42452-019-0404-9.

Shakeel, A., Khan, A.A., Alharby, H.F., Bamagoos, A A., (2021). Evaluation of coal fly-ash for modulating plant growth, yield, and antioxidant properties of *Daucus carota* (L.): A sustainable approach to coal waste recycling, *Sustainability*, vol. 13, p. 5116. https://doi.org/10.3390/su13095116.

Shakeel, A., Khan, A.A., Hakeem, K.R. (2020). Growth, biochemical, and antioxidant response of beetroot (*Beta vulgaris* L.) grown in fly-ash-amended soil, *SN Applied Sciences*, vol. 2(8), p. 1378. https://doi.org/10.1007/s42452-020-3191-4.

Sharma, S., Roy, R., Prasad, H., Kumar, B., (2024). Phytochemical analysis and antifungal activity of Mentha against Phytophthora infestans, *South African Journal of Botany*, vol. 172, p. 501-514. https://doi.org/10.1016/j.sajb.2024.07.037.

Singh, P.K., Shikha, D., Saw, S. (2023). Evaluation of potential toxic heavy metal contamination in soil, fly-ash, vegetables and grain crops along with associated ecological and health risk assessment of nearby inhabitants of a thermal power station in Jharkhand (India), *Environ Sci Pollute Res*., vol. 30, p. 7752–7769. https://doi.org/10.1007/s11356-022-22638-0.

Skrebsky, E.C., Tabaldi, L.A., Pereira, L.B., (2008). Effect of cadmium on growth, micronutrient concentration, and δ-aminolaevulinic acid dehydratase and acid phosphatase activities in plants of Pfaffia glomerata, *Brazilian Journal of Plant Physiology*, vol. 20(4), p. 285–294. https://doi.org/10.1590/S1677-04202008000400004.

Subbiah, B.V. & Asija, G.L. (1956). A Rapid Procedure for the Estimation of Available Nitrogen in Soils, *Current Science*, vol. 25, p. 259-260.

Swapnil, P., Meena, M., Singh, S.K., Dhuldhaj, U.P., (2021). Vital roles of carotenoids in plants and humans to alleviate stress with its structure, biosynthesis, metabolic engineering and functional aspects, *Current Plant Biology*, vol. 26, p. 100203. https://doi.org/10.1016/j.cpb.2021.100203.

Tiwari, P. (2016). Recent advances and challenges in trichome research and essential oil biosynthesis in Mentha arvensis L, *Industrial Crops and Products*, vol. 82, p. 141-148. https://doi.org/10.1016/j.indcrop.2015.11.069.

Ultra, V.U. (2020). Growth and yield of lemongrass (*Cymbopogon citratus*) in fly-ash with nutrient amendments and Mycorrhiza for three-ratoon period. *International Journal of Phytoremediation*, vol. 22(4), p. 1-11. https://doi.org/10.1080/15226514.2020.1786005.

Upadhyay, S.K., Ahmad, M., Srivastava, A.K., Abhilash, P.C., Sharma, B. (2021). Optimization of eco-friendly novel amendments for sustainable utilization of Fly-ash based on growth performance, hormones, antioxidant, and heavy metal translocation in chickpea (Cicer arietinum L.) plant, *Chemosphere*, vol. 267, p. 129216. https://doi.org/10.1016/j.chemosphere.2020.129216.

Vajpayee, P., Tripathi, R.D., Rai, U.N., Ali, M.B., (2000). Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in Nymphaea alba L., *Chemosphere*, vol. 41(7), p. 1075-1082. https://doi.org/10.1016/S0045-6535(99)00426-9.

Velikova, V., Yordanov, I., Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science*, vol. 151, p. 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1.

Walkley A. & Black, I.A. (1934). An Examination of the Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method, *Soil Science*, vol. 37, p. 29-38. https://doi.org/ 10.1097/00010694-193401000-00003.

Watanabe, F.S. & Olsen, S.R. (1965). Test of an Ascorbic Acid Method for Determining Phosphorus in Water and $NaHCO_3$ Extracts from the Soil. *Soil Science Society of America Journal*, vol. 29, p. 677-678. https://doi.org/ 10.2136/sssaj1965.03615995002900060025x.

Yadav, S., Pandey, V.C., Kumar, M., Singh, L. (2022). Plant diversity and ecological potential of naturally colonizing vegetation for eco-restoration of fly-ash disposal area, *Ecological Engineering*, vol. 176, p. 106533. https://doi.org/1016/j.ecoleng.2021.106533.