iForest
Biogeosciences and Forestry

Research Article
doi: 10.3832/ifor4574-018
vol. 18, pp. 102-108

Evaluating anatomical and biochemical responses to cement dust pollution in *Quercus castaneifolia* C.A. Mey. and *Zelkova carpinifolia* (Pall.)

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Understanding how plants respond to industrial pollutants is crucial for assessing environmental impacts and developing effective management strategies. This study focused on the structural and biochemical adaptations of *Quercus castaneifolia* and *Zelkova carpinifolia* in response to cement dust pollution, which poses a significant environmental risk and adversely affects plant growth. We investigated structural changes in leaves and analyzed key biochemical markers in trees growing near a cement factory in Neka city, Mazandaran province, Iran, to examine the defense mechanisms these species use against dust pollution. Our results demonstrated notable anatomical adaptations in both species, including increased druse crystals and sclerified parenchyma cells. Additionally, *Q. castaneifolia* leaves showed wall collapse of xylary vessel elements under dust exposure. Biochemically, we observed elevated phenylalanine ammonia-lyase (PAL) activity and higher concentrations of phenolic compounds and lignin in leaves from polluted sites. While *Q. castaneifolia* exhibited higher PAL activity, *Z. carpinifolia* showed greater levels of phenolics and lignin, alongside more pronounced anatomical changes. These findings highlight species-specific defense strategies and resilience to cement dust pollution. The study underscores the importance of integrating both anatomical and biochemical parameters in ecological assessments and suggests that environmental management strategies should account for species-specific responses to pollution.

Keywords: Cement Dust Pollution, Leaf Anatomy, Phenylalanine Ammonia-Lyase, Lignin, *Quercus castaniefolia*, *Zelkova carpinifolia*

Introduction

Cement factories have long been recognized as significant contributors to environmental pollution, releasing hazardous substances that threaten human health and the ecosystem (El-Sherbeny & Najm 2016, Shah et al. 2019). The dust emitted by these industries contains elevated levels of heavy metals, including arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), thallium (Tl), aluminum (Al), beryllium (Be), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), and zinc (Zn), all of which have been established as toxic by various environmental studies (Bermudez et al. 2010, Darivasi et al. 2016). Beyond environmental contamination, the impact of cement dust extends to plant morphology and physiology, causing alterations in crucial parameters such as plant height, leaf number, area, and the fresh and dry weight of shoots and roots. Moreover, reductions in chlorophyll and carotenoid content and overall levels of plant sugars, proteins, and fats have been observed (Shah et al. 2020).

Despite the detrimental effects, particular plant species exhibit remarkable tolerance to cement dust contamination, attributed to genetic predisposition and concurrent biochemical and structural adaptations (Veselkin 2004, Erdal & Demirtas 2010). In response to pollutant toxicity, plants activate various enzymatic systems to mitigate or eliminate the damaging effects of pollutants (Emamverdian et al. 2015). Phenylpropanoid metabolism is implicated in this response, particularly the role of the primary enzyme phenylalanine ammonia lyase (PAL) in the plant phenylpropanoid pathway. Elevated PAL enzyme activity and the production of phenolic compounds, such as lignin, are typical plant reactions to adverse environmental conditions, with phenol content as an indicator of plant stress severity (Chen et al. 2020).

With their expanded surface area and stomata, leaves are particularly susceptible to pollution. Stomata serve as key pathways for the penetration of pollutants into the intercellular and cellular spaces of leaves (Gao et al. 2022). Consequently, leaf anatomy is critical in discerning the effects of different contamination levels. Recent studies have emphasized the importance of anatomical modifications, mesophyll changes, and phenolic compound deposits in response to air pollution in various plant species (Gostin 2010, El-Sherbeny & Najm 2016). The biochemical features of leaves, especially phenolic content, change in response to the level of air pollutants. Plants exposed to high pollution exhibit resistance and are effective bioindicators of air

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Received: Feb 03, 2024 - Accepted: Jan 25, 2025

Citation: Kelij S, Hosseinzadeh Z, Rezapour A (2025). Evaluating anatomical and biochemical responses to cement dust pollution in *Quercus castaneifolia* C.A. Mey. and *Zelkova carpinifolia* (Pall.). iForest 18: 102-108. - doi: 10.3832/ifor4574-018 [online 2025-05-10]

Communicated by: Michele Colangelo

pollution (Dineva 2004, Thawale et al. 2011).

In light of previous investigations, which revealed elevated concentrations of heavy metals, such as Co, Cr, Cu, Cd, Mn, Ni, and Zn, in the vicinity of the Neka cement factory surpassing standard levels (Darivasi et al. 2016), the present study aims to unveil the structural and biochemical responses of two tree species, *Zelkova carpinifolia* (Pall.) and *Quercus castaneifolia* C.A. Mey., situated in proximity to the factory. This investigation was conducted in contrast to trees from a control site unaffected by the factory's influence. The ecological and economic significance of *Q. castaneifolia*, a pivotal species within the Hyrcanian forests, and *Z. carpinifolia*, known for its high-quality wood and captivating canopy, underscores the critical need to comprehend their adaptive responses to cement dust pollution. These insights are not only vital for the preservation of these species but also have broader implications for biodiversity and ecological stability in Iranian forests (Sheikh Hasan et al. 2013, Ahmadi et al. 2014).

Materials and methods

Study area and sampling

The study focused on two distinct areas, classified based on pollution levels, as per the data provided by the Environmental Protection Organization of Mazandaran province. The polluted site, situated 2.5 km south of Neka, Iran (36° 39′ 5″ N, 53° 17′ 49″ E), was close to the Neka Cement Factory, while the control area was the Zarrin Abad Forest, located 11 km southeast of Sari, and 25 km away from the cement factory. The polluted site was approximately 500 m from the factory. Sampling was done in October 2021 from the upper half of the canopy in a southern direction. Three individual trees of each species were selected from the control and the polluted sites, and mature and uniform leaves were collected from each tree.

Soil physicochemical properties and heavy metal analysis

Soil samples from control and polluted sites were collected at 0-20 cm depth with four replicates each. The samples were dried, mixed thoroughly, and sieved before examination. Soil composition was assessed using the hydrometer technique. EC and pH were measured in soil-water mixtures. Samples were digested with a 2:1 mixture of nitric and perchloric acid. Heavy metalswere analyzed using ICP-MS, with calibration standards for accuracy. Results were reported in µg g^{-1} of dry soil weight.

Anatomical study

Transverse sections were prepared from the central portion of the mature leaves by free-hand sectioning (the section thickness typically ranged from 10 to 50 micrometers). Light microscopy was employed, and the photographs were taken using a Canon digital camera. Anatomical traits were measured by Digimizer software version 5.3.5 (MedCalc Software Ltd, Ostend, Belgium).

PAL activity assay

Leaf samples (300 mg) were homogenized in 6.5 mL Tris-HCl buffer at pH 8.8, supplemented with 15 mM of β-mercaptoethanol. The resulting extract underwent centrifugation at 12,000 rpm at 4 °C for 30 min, and the obtained supernatant was utilized for the enzymatic assay. A mixture containing 1 ml of the extraction buffer, 0.5 ml of 10 mM L-phenylalanine, 0.15 ml of supernatant, and 0.35 ml of double-distilled water was placed at 37 °C for 1 h. The reaction was terminated by adding 0.5 ml of 6 M HCl. The amount of trans-cinnamic acid formed was measured spectrophotometrically (Biochrom WPA Biowave II) at 290 nm. The enzyme activity was expressed in µmoles of trans-cinnamic acid per mg protein per hour (Ziaei et al. 2012).

Total phenolic content

The total phenolic content was determined using the Folin assay described by Singleton et al. (1999). A reaction mixture comprising 1 ml of plant extracts, 0.5 ml of 50% FCR dissolved in 3 ml of deionized water, and 1 ml of 5% Na_2CO_3 solution was stored in the dark at room temperature for 2 h. The absorbance at 765 nm was measured spectrophotometrically (Biochrom WPA Biowave II), and the total phenolic content was expressed as mg of gallic acid equivalents per g fresh weight.

Total lignin content

The isolated cell wall material (5 mg) was subjected to a treatment involving a combination of 2.5 ml of 25% (w/w) acetyl bromide in HOAc and 0.1 ml of 70% $HClO_4$ at 70 °C for one hour with gentle swirling every 10 min. After ice cooling, the mixture was transferred to a 25 ml volumetric flask containing 5 ml of 2M NaOH and 6 ml HOAc and filled up to 25 ml with acetic acid. Acetyl bromide soluble lignin was quantified using a known absorption coefficient (20.0 g^{-1} L cm^{-1}) and absorbance at 280 nm (Ghanati et al. 2002).

Statistical analysis

Biochemical data were analyzed using one-way ANOVA, followed by the Duncan multiple range test, and anatomical data were analyzed using a *t*-test. The mean values, derived from nine replicates, were considered statistically significant at $p<0.05$. Statistical software SPSS®, version 21 (IBM Corporation, Armonk, NY, USA). was applied for statistical analysis, and Excel software version 2021 (Redmond, WA, USA) was used for diagrams.

Results

Soil physicochemical properties and heavy metal concentrations

Soil texture analysis revealed higher silt and clay content in the polluted area compared to the control area, with no significant pH difference. The electrical conductivity (EC) was higher in the control area than the contaminated area, but neither region exhibited saline soil conditions overall (Tab. 1). The contaminated soils exhibited elevated concentrations of cadmium (Cd), lead (Pb), zinc (Zn), and chromium (Cr) in relation to the unpolluted samples, indicating significant changes in soil characteristics and the accumulation of heavy metals attributed to pollution. (Tab. 1).

Tab. 1 - Physicochemical properties and heavy metal concentrations of the studied soils. Values are presented as mean ± standard deviation, with 4 replicates. Asterisks (*) indicate significant differences at the 5% level.

Category	Parameters	Control	Polluted
Physicochemical properties	Sand %	29.00 ± 1.41	30.75 ± 1.70
	Silt %	49.00 ± 1.80	55.00 ± 2.06*
	Clay %	22.00 ± 2.00*	13.75 ± 1.00
	EC	0.85 ± 0.03	1.05 ± 0.02*
	pH	7.50 ± 0.01	7.53 ± 0.02
Heavy metal concentrations (µg g^{-1})	Cd	1.05 ± 0.05	2.25 ± 0.23*
	Pb	35.20 ± 1.83	38.50 ± 1.20*
	Cu	28.22 ± 2.71	24.50 ± 1.58
	Zn	50.25 ± 3.75	69.60 ± 1.91*
	Ni	42.35 ± 3.96	40.62 ± 2.86
	Cr	74.62 ± 1.97	131.25 ± 2.79*

Leaf anatomy and morphological changes

Quercus castaneifolia

In describing the leaf structure of *Q. castaneifolia*, it is important to note the presence of a bifacial mesophyll structure, with trichomes found on both the adaxial and abaxial surfaces of the leaf. Notably, the frequency of trichomes was higher on the lower leaf surface. The midrib exhibited a large stele resulting from merging vascular bundles, surrounded by a continuous ring

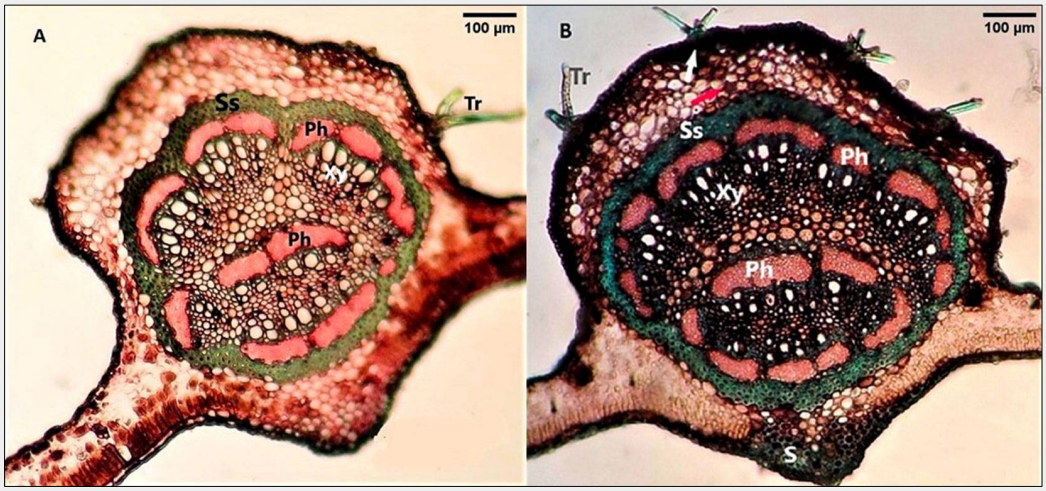

Fig. 1 - Cross sections of the *Quercus castaneifolia* leaf midrib. (A): control site; (B): polluted site; (Tr): Trichome; (Ss): Sclerenchyma sheath; (Ph): Phloem; (Xy): Xylem; (S): Sclerenchyma. The white arrow in (B) indicates dark deposits and red arrow indicates cell with phenolic compound.

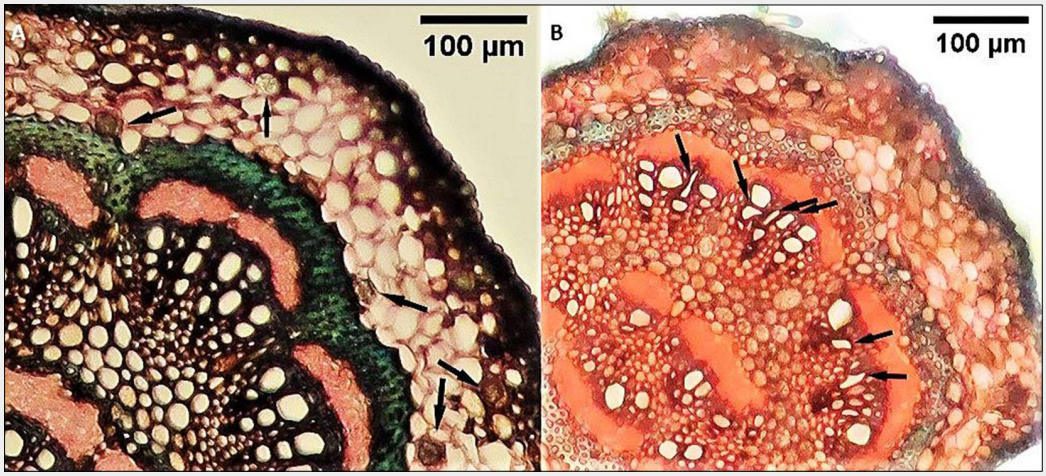

Fig. 2 - Cross sections of the *Quercus castaneifolia* midrib in polluted site. (A): black arrow indicates cell with druse crystal; (B): black arrow indicates deformation of xylary elements.

of phloem fibers forming a sclerenchyma sheath. The amphipheloic Siphonostele nature of the stele was evident due to the presence of phloem both externally and internally to the xylem (Fig. 1).

Comparative anatomy between polluted and control sites showed a significant increase in the upper cuticle thickness in trees from contaminated locations. However, no significant differences were observed in the thickness of the lower cuticle, upper and lower epidermis, or trichome number. Dark deposits and phenolic compound-containing cells were visible in the midrib of *Q. castaneifolia* leaves from polluted areas (Fig. 1B). The thickness of the palisade parenchymic tissue increased significantly in polluted area leaves, while spongy parenchyma and leaf blade thickness showed no significant differences between contaminated and control areas (Tab. 2). No significant differences were noted in the midrib thickness, midrib stele area, or thickness of the sclerenchyma sheath surrounding the midrib stele between control and polluted samples near Neka Cement Factory and Zarrin Abad Forest. However, a significant increase in druse crystals and structural deformities in xylary vessel elements was observed in *Q. castaneifolia* leaves exposed to cement pollution (Fig. 2, Tab. 2).

Zelkova carpinifolia

Leaf anatomy of *Z. carpinifolia* resembled that of *Q. castaneifolia*, the main difference being the absence of inner phloem in the *Z. carpinifolia* leaf midrib, resulting in an ectophloic siphonostele (Fig. 3). In polluted sites, the lower cuticle thickness showed no significant difference, but the lower epi-

Tab. 2 - Leaf anatomical characteristics of *Quercus castaneifolia* from the cement-polluted and control sites. (LCT): Lower Cuticle Thickness; (UCT): Upper Cuticle Thickness; (LET): Lower Epiderm Thickness; (UET): Upper Epiderm Thickness; (SPT): Spongy Parenchyma Thickness; (PPT): Palisade Parenchyma Thickness; (LBT): Leaf Blade Thickness; (MSA): Midrib Stele Area; (MT): Midrib Thickness; (MSST): Midrib Sclerenchyma Sheath Thickness; (DCN): Druse Crystal Number; (TN): Trichome Number. (*): p <0.05.

Parameter	Polluted	Control	t-value	p-value
LCT (µm)	0.055 ± 0.008	0.053 ± 0.005	0.521	0.617
UCT (µm)	0.065 ± 0.007	0.052 ± 0.001	3.707	0.006*
LET (µm)	0.153 ± 0.019	0.172 ± 0.007	-2.046	0.075
UET (µm)	0.173 ± 0.011	0.178 ± 0.024	-0.396	0.703
SPT (µm)	0.644 ± 0.120	0.528 ± 0.064	1.907	0.093
PPT (µm)	0.646 ± 0.151	0.377 ± 0.106	3.242	0.012*
LBT (µm)	1.549 ± 0.343	1.201 ± 0.075	2.214	0.058
MSA (µm)	1.491 ± 0.182	1.295 ± 0.108	2.071	0.072
MT (µm)	8.699 ± 0.688	9.080 ± 2.106	-0.391	0.712
MSST (µm)	0.452 ± 0.078	0.487 ± 0.066	-0.767	0.465
DCN	10.800 ± 1.483	5.600 ± 1.140	6.215	<0.001*
TN	3.600 ± 1.342	3.800 ± 0.837	-0.283	0.784

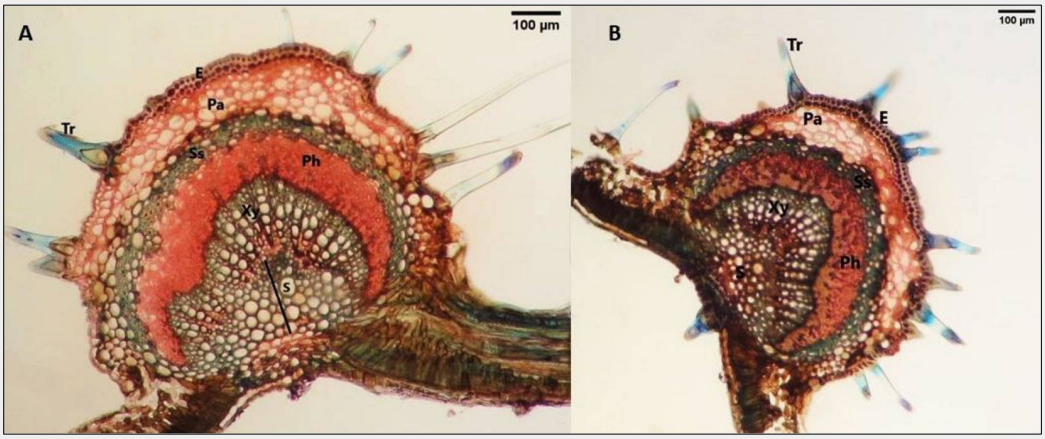

Fig. 3 - Cross sections of the *Zelkova carpinifolia* leaf midrib. (A): control site; (B): polluted site; (Tr): Trichome; (Pa): parenchyma; (Ss): Sclerenchyma sheath; (Ph): Phloem; (Xy): Xylem; (S): Sclerenchyma.

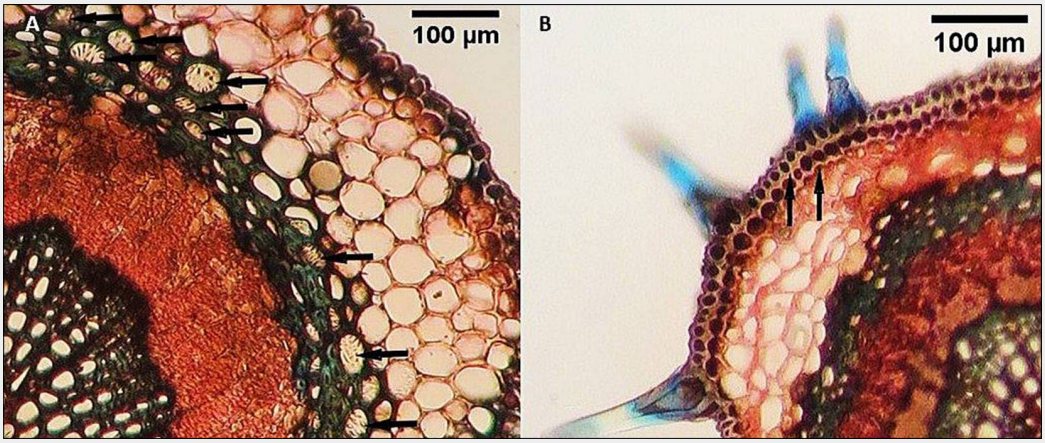

Fig. 4 - Cross sections of the *Zelkova carpinifolia* midrib in the polluted site. (A): black arrow indicates sclerified parenchyma; (B): black arrow indicates thickening of periclinal walls of epidermal cells.

dermis thickness increased significantly, along with thickening of inner periclinal walls of lower epidermal cells (Fig. 4B). The upper cuticle thickness significantly increased in leaves exposed to cement contamination, while the upper epidermis thickness showed no significant difference (Tab. 3). Trichome number exhibited no significant difference between polluted and control areas in *Z. carpinifolia* leaves. Notably, an increase was observed in palisade and spongy parenchyma thickness, leaf blade thickness, and leaf midrib thickness of leaves from polluted areas. The midrib stele area increased significantly in leaves from contaminated sites, while the thickness of the sclerenchyma sheath around the stele remained unchanged (Tab. 3). An increase in the sclerified parenchyma in the leaf midrib was observed near the Neka cement factory compared to Zarrin Abad Forest (Fig. 4A).

Tab. 3 - Leaf anatomical characteristics of *Zelkova carpinifolia* from cement-polluted site and the control site. (LCT): Lower Cuticle Thickness; (UCT): Upper Cuticle Thickness; (LET): Lower Epiderm Thickness; (UET): Upper Epiderm Thickness; (SPT): Spongy Parenchyma Thickness; (PPT): Palisade Parenchyma Thickness; (LBT): Leaf Blade Thickness; (MSA): Midrib Stele Area; (MT): Midrib Thickness; (MSST): Midrib Sclerenchyma Sheath Thickness; (SPN): Sclerified parenchyma Number; (TN): Trichome Number. (*): p <0.05.

Parameter	Polluted	Control	*t*-value	p-value
LCT (µm)	0.068 ± 0.014	0.068 ± 0.005	0.030	0.978
UCT (µm)	0.081 ± 0.0057	0.060 ± 0.005	4.980	0.001*
LET (µm)	0.224 ± 0.027	0.179 ± 0.009	3.403	0.019*
UET (µm)	0.253 ± 0.028	0.235 ± 0.011	1.290	0.233
SPT (µm)	0.688 ± 0.085	0.559 ± 0.050	2.918	0.019*
PPT (µm)	1.041 ± 0.118	0.726 ± 0.139	3.850	0.005*
LBT (µm)	2.353 ± 0.162	1.577 ± 0.080	9.539	<0.001*
MSA (µm)	2.679 ± 0.572	1.953 ± 0.173	2.714	0.045*
MT (µm)	7.370 ± 1.286	5.622 ± 0.774	2.603	0.031*
MSST (µm)	0.519 ± 0.097	0.519 ± 0.054	0.004	0.997
SPN	33.400 ± 4.219	7.400 ± 2.302	12.096	<0.001*
TN	6.200 ± 1.789	4.800 ± 0.837	1.585	0.152

Biochemical responses to pollution

PAL activity significantly increased in the leaves of both species, *Q. castaneifolia* and *Z. carpinifolia*, from polluted sites, with higher levels in *Q. castaneifolia* leaves (Fig. 5). The total phenolic content was remarkably higher in leaves collected from contaminated sites for both species (Fig. 5). Conversely, lignin content increased only in *Z. carpinifolia* leaves from contaminated sites, with no significant difference in *Q. castaneifolia* leaves between polluted and control sites (Fig. 5).

Discussion

Our investigation at the Neka cement factory revealed clear evidence of environmental pollution induced by cement dust and noxious emissions, corroborating prior research findings (Darivasi et al. 2016). The levels of heavy metals in the soil of the contaminated area were notably higher than in the control region. Furthermore, the contaminated soil exhibited a higher proportion of clay and silt than the control. These differences in soil composition can significantly influence heavy metal concentrations, as soils with higher clay content are

more effective at absorbing and retaining heavy metals (Deng et al. 2018). These findings suggest that cement dust deposition significantly altered soil properties and contributed to heavy metal accumulation, likely affecting plant physiology. Such alterations in soil composition play a critical role in inducing stress responses in *Q. castaneifolia* and *Z. carpinifolia*, which were evident in both leaf anatomical changes and biochemical adaptations.

The deposition of cement dust particles on leaf surfaces, a naturally occurring phenomenon, is influenced by various factors such as phyllotaxy, leaf geometry, structure, and features of the epidermis and cuticle (Sett 2017, Shah et al. 2019). This study specifically focused on assessing the impact of pollution on the cuticle, the primary barrier against toxicant uptake, revealing a significant increase in the thickness of the upper cuticle in both *Q. castaneifolia and Z. carpinifolia* under polluted conditions. Notably, our study found no significant difference in the thickness of the lower cuticle between control and polluted areas for both species. This observation underscores the significance of the upper cuticle as the primary entry point for pollutants, particularly during nighttime when stomata are closed. The variability in the quantity and composition of the leaf cuticle, influenced by factors such as species, age, and environmental conditions, is known to impact contaminant permeability (Agarwal et al. 2019). Our results align with previous studies, such as those on *Olea europaea* leaves, where a thick cuticle acted as a barrier against contaminant penetration (Sawidis et al. 2012), and *Platanus orientalis* leaves, which exhibited a thinner cuticle in urban and polluted areas, potentially contributing to increased transpiration (Pourkhabbaz et al. 2010).

The examination of the epidermal layers indicated an increase in the upper cuticle thickness of both *Q. castaneifolia* and *Z. carpinifolia* in response to cement dust pollution. Additionally, the lower leaf epidermis thickness increased significantly in *Z. carpinifolia*, with thicker periclinal walls of the lower epidermal cells observed in polluted sites acting as an effective barrier to pollutant entry. This aligns with studies on Norway spruce and *Brachiaria decumbens* (Qin et al. 2014, Gomes et al. 2011). Surprisingly, the abundance of trichomes, known for their role in pollution response, did not exhibit significant changes in our study.

Concerning parenchyma thickness, *Z. carpinifolia* demonstrated a significant increase in palisade and spongy parenchyma thickness, while *Q. castaneifolia* exhibited a notable increase exclusively in palisade parenchyma. These observations align with prior studies establishing a positive correlation between palisade parenchyma thickness and resistance to urban environmental pollution, including air pollution (Dineva 2004, Khosropour et al. 2018, Papadopoulou et al. 2023). Furthermore, alterations in midrib thickness and stele area were evident in *Z. carpinifolia*, suggesting an adaptive mechanism to enhance water transfer. The observed augmentation in stele area implies an increase in tracheary elements, facilitating faster water transfer by reducing ion density (Asheqian et al. 2020). In a similar context, Rao et al. (2004) noted an increase in the number of secondary xylem elements in *Dalbergia sissoo* under air pollution stress, accompanied by a reduction in the lumen diameter of xylem. Conversely, *Q. castaneifolia* leaves exhibited collapsed xylary vessel elements, indicating potential tissue damage and susceptibility to pollution-induced stress. This aligns with findings by Krzeslowska et al. (2019) in *Tilia cordata* Miller growing on mining sludge, where internal tissues in the root apex displayed collapse, and abnormal vascular elements were observed. The phenomenon of cell degeneration and deformation is postulated to be affected by the disruptive effects of heavy metals on hormonal balance (Sandalio et al. 2001).

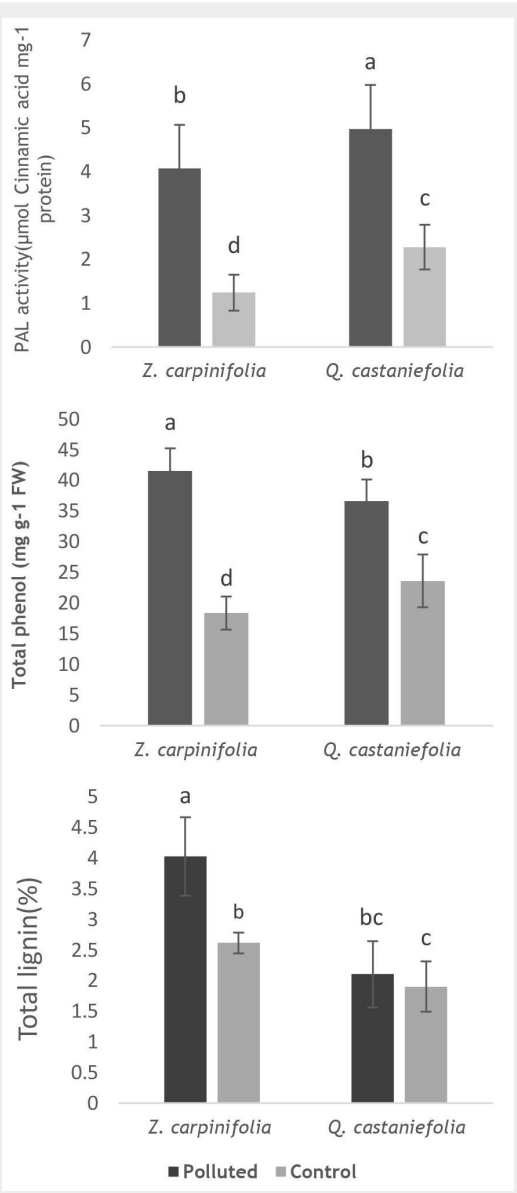

Fig. 5 - PAL activity and total phenolic and lignin content in leaves of *Q. castaneifolia* and *Z. carpinifolia* in the polluted and control areas. Mean value ± standard deviation is shown (n = 6). Different letters indicate significant differences between the means (ANOVA and Duncan test, p< 0.05).

Observations of crystal formation and sclerification in both species underscored the plants' response to severe stress conditions. Calcium oxalate crystal formation and sclerification are recognized mechanisms for regulating calcium levels and detoxifying heavy metals (Evert 2006, Kabata-Pendias 2010). These adaptations were particularly prominent in *Z. carpinifolia*, demonstrating its ability to cope with pollution-induced stress. Sclerification, a recognized plant response to severe stress situations, has been documented in various species. For instance, Batool et al. (2014) reported an association between enhanced sclerification in the root and stem of *Cyperus alopecuroides* and increased tolerance to Cd and Ni in an industrially polluted site.

In comparison, *Q. castaneifolia* exhibited fewer anatomical changes, aligning with the known resilience of *Quercus* species to pollution (Panah et al. 2016, Naji & Taherpour 2019). The observed increase in PAL activity in both species near the cement

factory suggests a non-specific indicator of the plant's adaptive response to pollution, consistent with studies on *Pinus eldarica*, *Prosopis glandulosa*, and others (Oloumi et al. 2018, Gonzalez-Mendoza et al. 2018). PAL is a key enzyme in secondary phenylpropanoid metabolism, and the levels of phenylpropanoids are regulated by environmental factors (Kim & Hwang 2014). PAL activity is positively correlated with various biotic and abiotic stresses. Enhanced activity of PPO (Polyphenol Oxidase), PAL, and CAD (Cinnamyl Alcohol Dehydrogenase) enzymes was reported in needles of *Pinus eldarica* and *Pinus nigra* exposed to heavy metal pollution from the Sarcheshmeh Copper Complex (Oloumi et al. 2018). Also, the highest increment in PAL activity was observed in leaves of *Prosopis glandulosa* treated with cadmium and copper (Gonzalez-Mendoza et al. 2018). This consistency in the modulation of PAL activity levels, reflecting the presence of cement dust pollution, underscores the non-specific nature of this enzymatic response.

The substantial augmentation in total phenolic content in *Q. castaneifolia* and the exceptional accumulation in *Z. carpinifolia* under the influence of cement dust pollution indicate the activation of the phenylpropanoid pathway as a response to environmental stress (Sharma et al. 2019). Phenolic compounds, including phenolic acids, flavonoids, coumarins, tannins, lignans, and lignin, constitute pivotal components of plant secondary metabolites synthesized through the phenylpropanoid pathway (Naikoo et al. 2019). The activation of the phenylpropanoid pathway in plants exposed to diverse stressors, such as salinity, drought, temperature variations, air pollution, and radiation, is well-established in the literature (Sharma et al. 2019). Metal toxicity, as evidenced by increased total phenols and several polyphenols in *Zea mays* under such conditions, further supports the role of phenolic compounds as a response to stress (Kisa et al. 2016). The significant increase in lignin content observed in *Z. carpinifolia*, as opposed to *Q. castaneifolia*, underscores distinct responses to pollution-induced stress within plant species. This finding aligns with the broader consensus established in prior studies on species-specific plant adaptations to varying environmental conditions, as documented by Förster et al. (2021) and Rezapour et al. (2022). While the typical plant response to stress involves enhanced PAL activity accompanied by an increase in both total phenolic compounds and lignin content (Pawlak-Sprada et al. 2011), the reduced lignin accumulation in *Q. castaneifolia* leaves, despite the elevated PAL activity, suggests a deviation in the phenylpropanoid pathway. This implies a shift toward producing different phenolic compounds rather than lignin in response to cement dust pollution.

Conclusions

Our findings indicate substantial quantitative and qualitative alterations in the leaf structures of both *Q. castaneifolia* and *Z. carpinifolia* species when exposed to cement dust pollution. The higher phenylalanine ammonia lyase activity, along with increased concentrations of phenolic compounds and total lignin in both species under such pollution, confirms the key role of phenolic compounds in fostering an adaptive plant response to the adverse impacts of pollution stress. Notably, *Z. carpinifolia* exhibits more pronounced anatomical changes and higher concentrations of total phenolics and total lignin than *Q. castaneifolia*, while *Q. castaneifolia* demonstrates higher PAL activity. These findings highlight the species-specific mechanisms for adapting to environmental stresses and highlight the tolerance of both species to cement pollution stress. Overall, this study contributes to our understanding of plant responses to industrial pollution, emphasizing the need for species-specific considerations in ecological assessments.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

We thank Mr. Abdolreza Yadollahpour for his valuable contribution to the field work, and we would like to express our gratitude to the Narenj Espadana Soil Science Laboratory in Isfahan for their cooperation in analyzing the soil samples.

Authors' contribution

SK carried out research, conceptualization, and supervision of the work. SK and ZH were responsible for conducting field and laboratory measurements. SK executed the statistical analysis and wrote the first draft of the manuscript. AR contributed to the conceptualization and performed writing, review, and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

References

Agarwal P, Sarkar M, Chakraborty B, Banerjee T (2019). Phytoremediation of air pollutants: prospects and challenges. Phytomanagement of polluted sites 221-241. - doi: 10.1016/B978-0-12-813912-7.00007-7

Ahmadi A, Kavosi MR, Soltanloo H (2014). *Zelkova carpinifolia* reservoir from Hyrcanian Forests, Northern Iran, a new sacrifice of Ophiostoma novo-ulmi. Biodiversitas Journal of Biological Diversity 15 (1): 48-52. - doi: 10.13057/biodiv/d150107

Asheqian F, Kelij S, Jafari N (2020). The structural adaptations of *Convolvulus persicus*, an endangered plant in the northern coasts of Iran. Nova Biologica Reperta 6 (4): 495-504. - doi: 10.29252/nbr.6.4.495

Batool R, Hameed M, Ashraf M, Fatima S, Nawaz T, Ahmad MSA (2014). Structural and functional response to metal toxicity in aquatic *Cyperus alopecuroides* Rottb. Limnologica 48: 46-56. - doi: 10.1016/j.limno.2014.06.002

Bermudez GM, Moreno M, Invernizzi R, Plá R, Pignata ML (2010). Heavy metal pollution in topsoils near a cement plant: the role of organic matter and distance to the source to predict total and HCl-extracted heavy metal concentrations. Chemosphere 78 (4): 375-381. - doi: 10.1016/j.chemosphere.2009.11.012

Chen Y, Huang L, Liang X, Dai P, Zhang Y, Li B, Lin X, Sun C (2020). Enhancement of polyphenolic metabolism as an adaptive response of lettuce (*Lactuca sativa*) roots to aluminum stress. Environmental Pollution 261: 114230. - doi: 10.1016/j.envpol.2020.114230

Darivasi S, Saeb K, Mollashahi M (2016). Effects of distance from pollutant sources on heavy metal concentrations around Neka cement factory soil. Journal of Environmental Science and Technology 17 (4): 33-44.

Deng A, Wang L, Chen F, Li Z, Liu W, Liu Y (2018). Soil aggregate-associated heavy metals subjected to different types of land use in subtropical China. Global Ecology and Conservation 16 (1): e00465. - doi: 10.1016/j.gecco.2018.e00465

Dineva S (2004). Comparative studies of the leaf morphology and structure of white ash *Fraxinus americana* L. and London plane tree *Platanus acerifolia* Willd growing in polluted area. Dendrobiology 52: 3-8.

El-Sherbeny GAA, Najm AA (2016). Growth, physiological and anatomical behaviour of *Cynanchum acutum* in response to cement dust pollution. Journal of Environmental Science and Technology 9 (4): 345-353. - doi: 10.3923/jest.2016.345.353

Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015). Heavy metal stress and some mechanisms of plant defense response. Scientific World Journal 756120: 1-18. - doi: 10.1155/2015/756120

Erdal S, Demirtas A (2010). Effects of cement flue dust from a cement factory on stress parameters and diversity of aquatic plants. Toxicology and industrial health 26 (6): 339-343. - doi: 10.1177/0748233710369235

Evert RF (2006). Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development. John Wiley and Sons, NJ, USA, pp. 595. - doi: 10.1002/0470047380

Förster A, Hertel D, Werner R, Leuschner C (2021). Belowground consequences of converting broadleaf to conifer forest: Comparing the fine root systems of European beech and Scots pine. Forest Ecology and Management 496: 119457. - doi: 10.1016/j.foreco.2021.119457

Gao P, Zhang X, Xue P, Dong J, Dong Y, Zhao Q, Geng L, Lu Y, Zhao J, Liu W (2022). Mechanism of Pb accumulation in Chinese cabbage leaves: stomata and trichomes regulate foliar uptake of Pb in atmospheric $PM_{2.5}$. Environmental Pollution 293: 118585. - doi: 10.1016/j.envpol.2021.118585

Ghanati F, Morita A, Yokota H (2002). Induction of suberin and increase of lignin content by excess boron in tobacco cells. Soil Science and Plant Nutrition 48 (3): 357-364. - doi: 10.1080/00380768.2002.10409212

Gomes MP, Marques TC, Nogueira MD, Castro

EM, Soares M (2011). Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in *Brachiaria decumbens*. Scientia Agricola 68 (5): 566-573. - doi: 10.1590/S0103-90162011000500009
Gonzalez-Mendoza D, Troncoso-Rojas R, Gonzalez-Soto T, Grimaldo-Juarez O, Cecena-Duran C, Duran-Hernandez D, Gutierrez-Miceli F (2018). Changes in the phenylalanine ammonia lyase activity, total phenolic compounds, and flavonoids in *Prosopis glandulosa* treated with cadmium and copper. Anais da Academia Brasileira de Ciências 90: 1465-1472. - doi: 10.1590/0001-3765201820170622
Gostin I (2010). Structural changes in silver fir needles in response to air pollution. Analele Universitatii din Oradea, Fascicula Biologie 17 (2): 300-305.
Kabata-Pendias A (2010). Trace elements in soils and plants (4th edn). CRC Press, Boca Raton, FL, USA, pp. 548. - doi: 10.1201/b10158
Khosropour E, Attarod P, Shirvany A, Pypker TG, Bayramzadeh V, Hakimi L, Moeinaddini M (2018). Response of *Platanus orientalis* leaves to urban pollution by heavy metals. Journal of Forestry Research 30: 1437-1445. - doi: 10.1007/s11676-018-0692-8
Kim DS, Hwang BK (2014). An important role of the pepper phenylalanine ammonia-lyase gene (PAL1) in salicylic acid-dependent signalling of the defence response to microbial pathogens. Journal of Experimental Botany 65 (9): 2295-2306. - doi: 10.1093/jxb/eru109
Kisa D, Elmastas M, Oztürk L, Kayir O (2016). Responses of the phenolic compounds of *Zea mays* under heavy metal stress. Applied Biological Chemistry 59: 813-820. - doi: 10.1007/s13765-016-0229-9
Krzeslowska M, Timmers AC, Mleczek M, Niedzielski P, Rabeda I, Wozny A, Golinski P (2019). Alterations of root architecture and cell wall modifications in *Tilia cordata* Miller (Linden) growing on mining sludge. Environmental Pollution 248: 247-259. - doi: 10.1016/j.envpol.2019.02.019
Naikoo MI, Dar MI, Raghib F, Jaleel H, Ahmad B, Raina A, Khan FA, Naushin F (2019). Role and regulation of plants phenolics in abiotic stress tolerance: an overview. Plant Signaling Molecules 2019: 157-168. - doi: 10.1016/B978-0-12-816451-8.00009-5
Naji HR, Taherpour M (2019). The effect of simulated dust storm on wood development and leaf stomata in *Quercus brantii* L. Desert 24 (1): 43-49. - doi: 10.22059/JDESERT.2019.72435
Oloumi H, Rezanejad F, Gholipoor Z (2018). Heavy metal accumulation and changes in phenylpropanoid metabolism induced by mining activities in leaves of *Pinus nigra* and *Pinus eldarica*. Acta Biologica Slovenica 61 (1): 25-34. - doi: 10.14720/abs.61.1.15878
Panah A, Karamshahi A, Mirzaei J, Darabi M (2016). Phytoremediation of Cd, Zn, Pb and Mn in leaf of nine trees species around the cement factory (phytoremediation of heavy metals in trees species). Journal of Research in Environmental Health 2 (3): 212-220. - doi: 10.22038/jreh.2016.8076
Papadopoulou S, Stefi AL, Meletiou-Christou MS, Christodoulakis NS, Gkikas D, Rhizopoulou S (2023). Structural and physiological traits of compound leaves of *Ceratonia siliqua* trees grown in urban and suburban ambient conditions. Plants 12 (3) 514: 1-18. - doi: 10.3390/plants12030514
Pawlak-Sprada S, Arasimowicz-Jelonek M, Podgórska M, Deckert J (2011). Activation of phenylpropanoid pathway in legume plants exposed to heavy metals. Part I. Effects of cadmium and lead on phenylalanine ammonia-lyase gene expression, enzyme activity and lignin content. Acta Biochimica Polonica 58 (2): 211-216. - doi: 10.18388/abp.2011_2267
Pourkhabbaz A, Rastin N, Olbrich A, Langenfeld-Heyser R, Polle A (2010). Influence of environmental pollution on leaf properties of urban plane trees, *Platanus orientalis* L. Bulletin of Environmental Contamination and Toxicology 85: 251-255. - doi: 10.1007/s00128-010-0047-4
Qin X, Sun N, Ma L, Chang Y, Mu L (2014). Anatomical and physiological responses of Colorado blue spruce to vehicle exhausts. Environmental Science and Pollution Research 21: 11094-11098. - doi: 10.1007/s11356-014-3015-4
Rao KS, Rajput KS, SrinivasT (2004). Seasonal cambial anatomy and development of xylem in *Dalbergia sissoo* growing under the influence of combined air pollutants. Journal of Sustainable Forestry 18 (1): 73-88. - doi: 10.1300/J091v18n01_05
Rezapour A, Labbafi M, Oja T (2022). The impact of soil warming on fine root trait responses of trees, deciduous *vs.* coniferous: a meta-analysis. Forestry Studies 77 (1): 67-75. - doi: 10.2478/fsmu-2022-0013
Sandalio LM, Dalurzo HC, Gomez M, Romero Puertas MC, Del Rio LA (2001). Cadmium-induced changes in the growth and oxidative metabolism of pea plants. Journal of Experimental Botany 52 (364): 2115-2126. - doi: 10.1093/jexbot/52.364.2115
Sawidis T, Krystallidis P, Veros D, Chettri M (2012). A study of air pollution with heavy metals in Athen's city and Attica basin using evergreen trees as biological indicators. Biological trace element research 148: 396-408. - doi: 10.1007/s12011-012-9378-9
Sett R (2017). Responses in plants exposed to dust pollution. Horticulture International Journal 1 (2): 53-56. - doi: 10.15406/hij.2017.01.00010
Shah K, Amin NU, Ahmad I, Ara G, Rahman MU, Zuo X, Xing L, Ren X (2019). Cement dust induces stress and attenuates photosynthesis in *Arachis hypogaea*. Environmental Science and Pollution Research 26: 19490-19501. - doi: 10.1007/s11356-019-04861-4
Shah K, An N, Ma W, Ara G, Ali K, Kamanova S, Zuo X, Han M, Ren X, Xing L (2020). Chronic cement dust load induces novel damages in foliage and buds of *Malus domestica*. Scientific Reports 10 (1): 12186. - doi: 10.1038/s41598-020-68902-6
Sharma A, Shahzad B, Rehman A, Bhardwaj R, Landi M, Zheng B (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. Molecules 24 (13): 2452. - doi: 10.3390/molecules24132452
Sheikh Hasan F, Saeidi Mehrvarz S, Asri Y (2013). *Quercus castaneifolia* ecotyping on the basis of effective ecological factors on leaf and fruit morphological characteristics in the forests of Guilan province (N Iran). Rostaniha 14 (2): 149-162. - doi: 10.22092/botany.2014.101283
Singleton VL, Orthofer R, Lamuela-Raventós RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology 299: 152-178. - doi: 10.1016/S0076-6879(99)99017-1
Thawale PR, Satheesh Babu S, Wakode RR, Singh SK, Kumar S, Juwarkar AA (2011). Biochemical changes in plant leaves as a biomarker of pollution due to anthropogenic activity. Environmental Monitoring and Assessment 177 (1-4): 527-35. - doi: 10.1007/s10661-010-1653-7
Veselkin DV (2004). Anatomical structure of ectomycorrhiza in *Abies sibirica* Ledeb. and *Picea obovata* Ledeb. under conditions of forest ecosystems polluted with emissions from copper-smelting works. Russian Journal of Ecology 35: 71-78. - doi: 10.1023/B:RUSE.0000018930.17569.09
Ziaei M, Sharifi M, Behmanesh M, Razavi K (2012). Gene expression and activity of phenylalanine amonialyase and essential oil composition of *Ocimum basilicum* L. at different growth stages. Iranian Journal of Biotechnology 10: 32-39. [online] URL: http://www.ijbiotech.com/article_51455_d66d8df400d836aac30d0ea782b40637.pdf