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Morpho-Anatomical Plasticity of Scots Pine (*Pinus Sylvestris* L.) Needles in Natural and Urbanized Areas of the Karaganda Region

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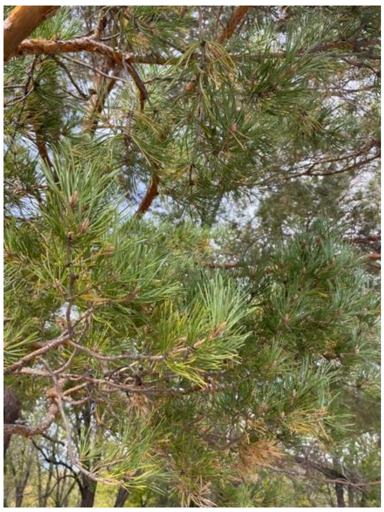
ABSTRACT

This study evaluates the morpho-anatomical variability of Scots pine (*Pinus sylvestris* L.) needles in contrasting environments of the Karaganda region, Central Kazakhstan. Samples were obtained from four populations representing natural forests (Karkaraly) and urbanized areas (Temirtau, Balkhash, Karaganda). Morphological indicators (needle length, width, and thickness) and anatomical traits (epidermis, hypodermis, mesophyll, vascular bundles, and resin canals) were assessed under a light microscope (200×) using ImageJ software. Ten trees were analyzed per site, with 20 needles per tree, and ten cross-sections per needle measured. One-way ANOVA followed by Tukey's HSD and principal component analysis (PCA) were applied. Needles in urban populations averaged 37.7 mm in length, significantly shorter than those from natural sites (61.3 mm, $p < 0.05$). Resin canal diameters were reduced in Temirtau and Karaganda, while Balkhash showed enlarged canals and conducting tissues, suggesting a compensatory adjustment. ANOVA confirmed significant site-level differences ($F(3, 36) = 25.4$, $p < 0.001$). PCA distinguished natural and urban groups, with morphological traits strongly contributing to PC1 (56.9% variance) and anatomical variables to PC2 (29.2%). The findings demonstrate that Scots pine exhibits high structural plasticity under urban stress. Reduced needle dimensions and resin canals in industrial zones indicate stress responses, while the enlarged conducting elements in Balkhash reflect adaptive defense. Resin canal size emerges as a sensitive anatomical marker of air pollution, supporting the use of morpho-anatomical traits for ecological monitoring and sustainable management of P. sylvestris populations in Central Kazakhstan.

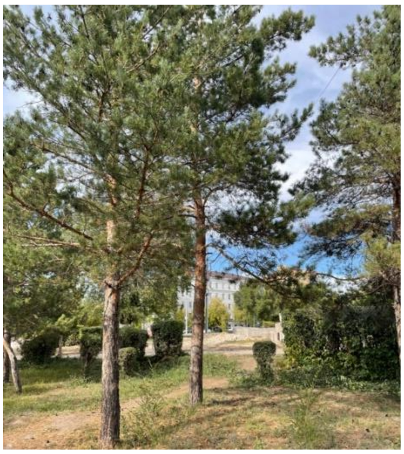
1. INTRODUCTION

Scots pine (*Pinus sylvestris* L.) is among the most widely distributed coniferous species in Eurasia, growing across diverse climatic zones and soil types. It plays a crucial role in maintaining the stability of forest ecosystems. In Kazakhstan, including the forest-steppe zone of the Karaganda region, this species represents a dominant component of both natural and artificial stands.

Urban environments expose trees to a wide range of stress factors, including air and soil contamination, fluctuations in temperature, and periodic water shortages. In response, plants develop structural adjustments at both morphological and anatomical levels. The needles of *P. sylvestris* are particularly effective bioindicators due to their high sensitivity to external conditions [1, 2]. Previous studies have demonstrated that long-term exposure to pollutants leads to distinct changes in pine needle anatomy, providing valuable information on adaptive strategies in disturbed habitats [3]. In addition, needle asymmetry and variations in morphological traits have been successfully applied as early indicators of atmospheric pollution and its temporal dynamics (Figure 1) [4].



a) Needles from a natural area (Karkaraly)



b) Needles from an urbanized area (Karaganda)

Figure 1. *Pinus sylvestris* L.

The ecological plasticity of Scots pine, supported by its wide genetic diversity, enables it to maintain productivity under unfavorable environmental conditions [5, 6]. However, structural responses vary depending on habitat quality. Research has shown that changes in mesophyll proportion, needle cross-sectional area, and vascular elements are closely linked to soil fertility, water supply, and pollution intensity [7-9]. These modifications are not random but reflect adaptive processes at the population level, influencing overall vitality and resilience [10-13]. In industrially impacted areas, reductions in central cylinder size and resin canal diameter have been reported, underscoring the sensitivity of these traits to pollution [14-16].

Thus, morpho-anatomical features of pine needles can serve as reliable markers of ecological stress and long-term climatic influences [17-24]. Plant plasticity - the ability to adjust structural traits in response to changing environments - remains one of the central concepts in modern ecological research. Despite extensive studies across Eurasia, the morpho-anatomical plasticity of *P. sylvestris* needles has not been sufficiently explored in Kazakhstan, particularly in the Karaganda region.

Hypothesis: Urbanization significantly modifies the morpho-anatomical structure of Scots pine needles.

Objective: To analyze adaptive structural features of *P. sylvestris* needles in natural and urbanized sites of the Karaganda region.

Tasks:

- To collect and map needle samples from natural and urban populations;
- To describe and compare morpho-anatomical traits under different ecological conditions;
- To statistically assess variation using ANOVA and PCA.

2. MATERIALS AND METHODS

2.1 Study sites and sampling points

The study was conducted in natural and urbanized areas of the Karaganda region, Central Kazakhstan. Four sampling sites were selected to represent contrasting ecological conditions and varying levels of anthropogenic impact:

P1 - Temirtau (50.062989° N; 72.951103° E) and P4 - Karaganda (49.806224° N; 73.080321° E): Urban-industrial areas characterized by high technogenic stress due to factories, transport networks, and urban infrastructure.

P2 - Balkhash (46.851308° N; 74.975437° E): A mixed site with moderate anthropogenic influence, including city-adjacent green belts and park areas.

P3 - Karkaraly (49.411991° N; 75.467682° E): A natural forest belt with minimal human disturbance, serving as a reference site.

Environmental descriptions (air quality data, distance to industrial facilities, traffic intensity, and proximity to urban infrastructure) were recorded for each site to assess the degree of anthropogenic pressure. The geographic coordinates of all sampling points were measured using a GPS device and mapped (Figure 2).

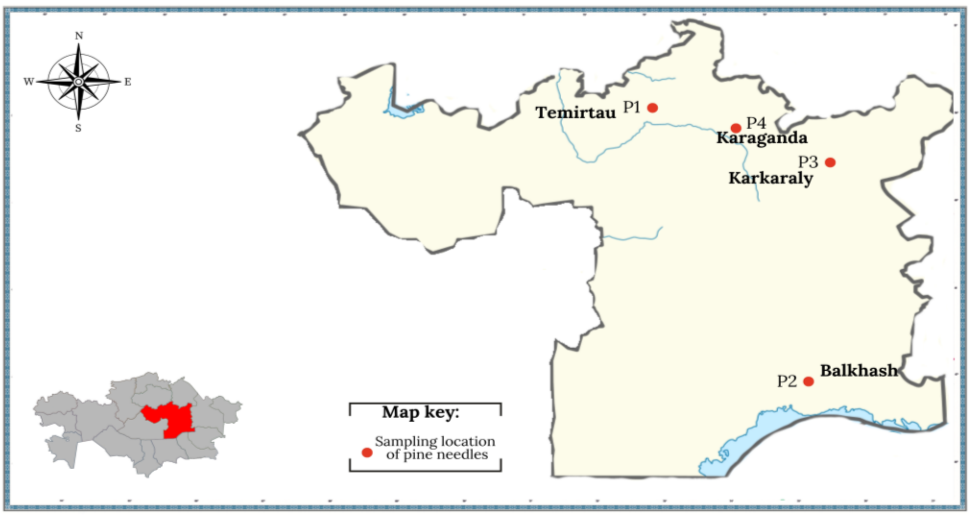


Figure 2. Map of the sampling point of *Pinus sylvestris* L. in the Karaganda region

2.2 Sample collection

Fieldwork was carried out in July-August 2023. At each site, 10 mature trees were randomly selected, located at least 50 m apart to avoid sampling from clones. From each tree, 10 fully developed current-year needles were collected from the middle part of the southern crown exposure, resulting in 100 needles per site and a total of 400 needles. The samples were dried and stored according to standard herbarium procedures, labeled, photographed, and described.

2.3 Morphological measurements

Morphological parameters included needle length (l_n), thickness (t_n), and width (w_n). Measurements were performed using a digital caliper (± 0.01 mm accuracy). For each trait, 100 measurements were made per site (10 needles × 10 trees).

2.4 Anatomical analysis

Needle cross-sections were obtained using a rotary microtome (Leica RM2125 RTS) to produce slices of 15-20 µm thickness. Sections were mounted in glycerin, stained with safranin and fast green, and examined under a light microscope (Leica DM500) at 200× magnification. Images were captured with a digital camera and analyzed using ImageJ 1.53k software (NIH, USA).

The following anatomical traits were measured:

- epidermis thickness (w_e),
- resin canal diameter (d_a),
- conducting beam size (s_b).

For each anatomical parameter, 10 cross-sections per needle were analyzed, giving a total of 1000 measurements per site.

2.5 Statistical analysis

Data analysis was performed using one-way Analysis of Variance (ANOVA). When significant site differences were detected, Tukey's HSD post hoc test was applied. Principal component analysis (PCA) was carried out on standardized data to evaluate the relationships among traits and to visualize clustering of populations. All statistical computations were performed in R software (version 4.3.3). Results are expressed as mean ± standard error, and statistical significance was set at $p < 0.05$.

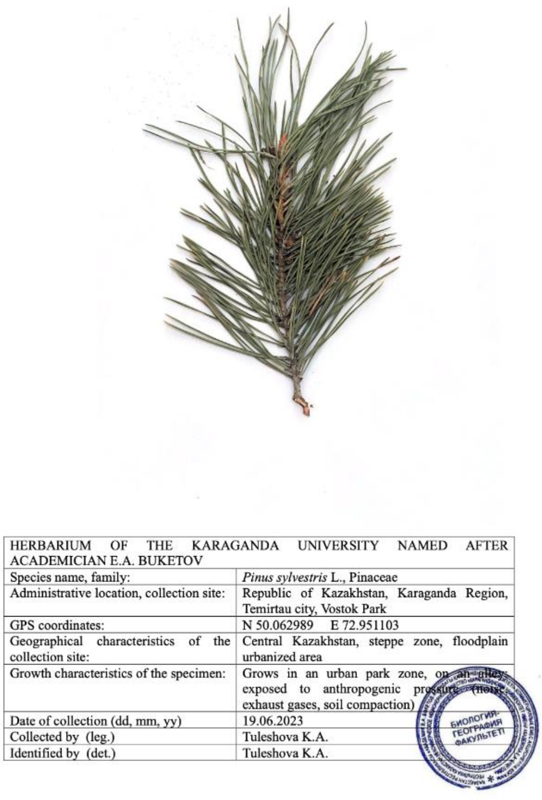
3. RESULTS

3.1 Analysis of herbarium samples

Herbarium specimens of *Pinus sylvestris* L. collected from the study sites allowed for a visual assessment of morphological differences among individual trees in each area. Needle shape, length, color, and density varied according to regional ecological conditions. Samples from urbanized areas exhibited shorter needles with a duller coloration, whereas needles collected from the natural area (Karkaraly) were longer and displayed a more intense green color. Herbarium descriptions and photographic documentation are presented below (Figure 3).

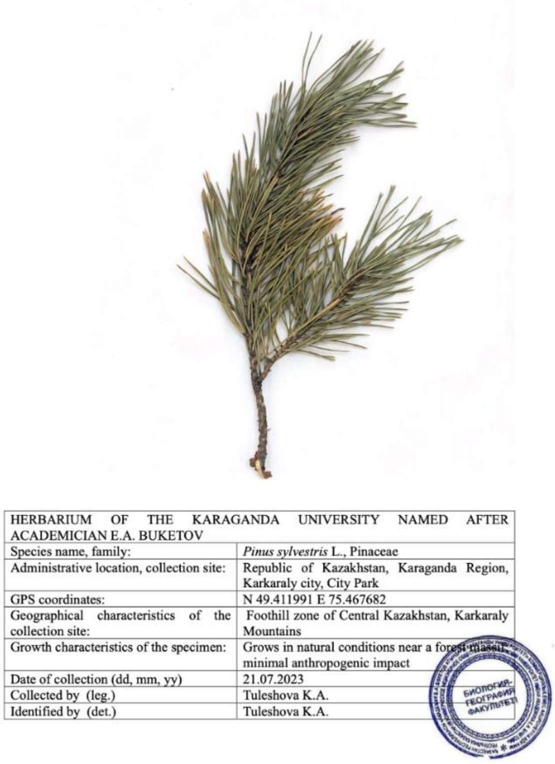
3.2 Morphological parameters

Analysis of morphological traits revealed significant variation in needle length, thickness, and width among the four sampling sites (Table 1). The longest needles were observed in the natural forest of Karkaraly (61.3 ± 3.3 mm), followed by Balkhash (59.4 ± 0.8 mm). In contrast, significantly shorter needles were found in Temirtau (37.7 ± 1.0 mm) and Karaganda (42.7 ± 1.4 mm). One-way ANOVA confirmed highly significant differences among sites ($F(3, 36) = 25.4$, $p < 0.001$). Post-hoc Tukey's HSD showed that needles from Karkaraly and Balkhash differed significantly from those in Temirtau and Karaganda ($p < 0.05$).



(a) P1 - Temirtau

(b) P2 - Balkhash



(c) P3 - Karkaraly

(d) P4 - Karaganda

Figure 3. Herbarium samples of various *Pinus sylvestris* L. species

Table 1. Morphological parameters of *Pinus sylvestris* L. needles

Sampling Points	l_n (mm)	t_n (mm)	w_n (mm)
Temirtau	37.7 ± 1.0	1.13 ± 0.02	0.45 ± 0.02
Balkhash	59.4 ± 0.8	1.42 ± 0.04	0.48 ± 0.02
Karkaraly	61.3 ± 3.3	1.07 ± 0.02	0.93 ± 0.01
Karaganda	42.7 ± 1.4	0.92 ± 0.03	0.57 ± 0.02

Notes: t_n: needles thickness, mm; l_n: needles length, mm; w_n: needles width, mm.

Needle thickness varied significantly among the sampling sites, ranging from 0.92 ± 0.03 mm in Karaganda to 1.42 ± 0.04 mm in Balkhash ($F(3, 36) = 6.9$, $p < 0.01$). Intermediate thickness values were recorded in Temirtau (1.13 ± 0.02 mm) and Karkaraly (1.07 ± 0.02 mm). Needle width also showed significant differences among sites ($F(3, 36) = 12.3$, $p < 0.01$). The highest needle width was observed in Karkaraly (0.93 ± 0.01 mm), while the lowest value was recorded in Temirtau (0.45 ± 0.02 mm). Balkhash (0.48 ± 0.02 mm) and Karaganda (0.57 ± 0.02 mm) exhibited intermediate values.

3.3 Anatomical parameters

Table 2. Anatomical parameters of *Pinus sylvestris* L. needles from different sampling sites (n = 10)

Sampling Points	w_e, µm	d_a, µm	s_b, µm
Temirtau	0.15 ± 0.02	15.06 ± 0.10	0.155 ± 0.002
Balkhash	0.16 ± 0.01	21.85 ± 1.10	0.175 ± 0.008
Karkaraly	0.15 ± 0.01	12.71 ± 0.10	0.140 ± 0.007
Karaganda	0.16 ± 0.01	10.98 ± 0.09	0.155 ± 0.002

Notes: w_e: width of the epidermis, d_a: the average diameter of the resin canals, s_b: the size of the conducting beam.

Epidermis thickness (w_e) remained relatively stable across sampling sites (0.15–0.16 ± SD µm, $p > 0.05$) (Table 2), indicating its conservative role. Resin canal diameter (d_a) varied significantly ($F(3, 36) = 14.7$, $p < 0.01$), largest in Balkhash (21.85 ± 1.10 µm) and smallest in Karaganda (10.98 ± 0.09 µm), with intermediate values in Temirtau and Karkaraly. Conducting beam size (s_b) also showed significant differences ($F(3, 36) = 7.8$, $p < 0.01$), highest in Balkhash (0.175 ± 0.008 µm) and lowest in Karkaraly (0.140 ± 0.007 µm), with intermediate values in Temirtau and Karaganda.

3.4 Microscopic images

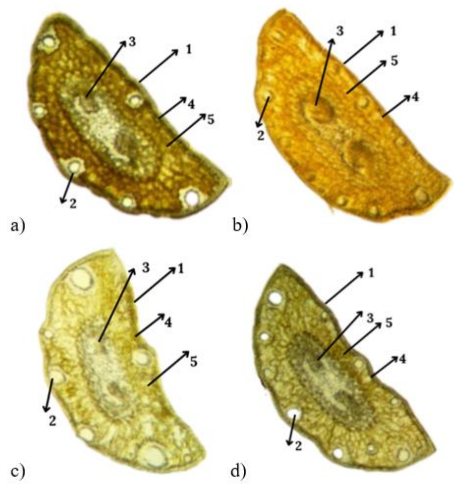


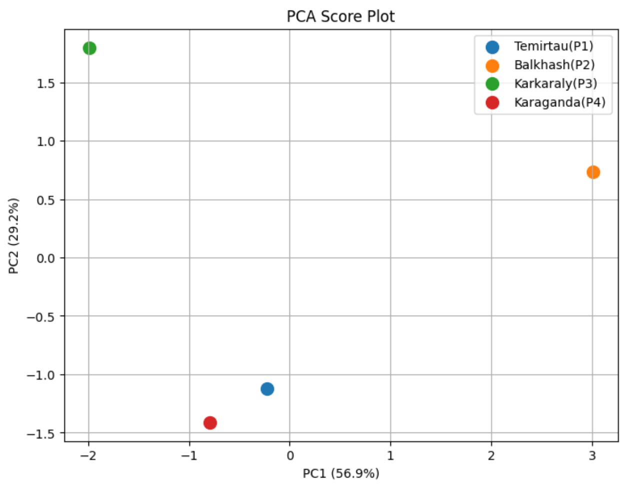
Figure 4. Cross-sections of *Pinus sylvestris* L. needles from different sampling sites in the Karaganda region: a) P1 – Temirtau, b) P2 – Balkhash, c) P3 – Karkaraly, d) P4 - Karaganda

Notes: 1 - epidermis, 2 - resin canals, 3 - conducting beam, 4 - hypodermis, 5 - folded mesophyll.

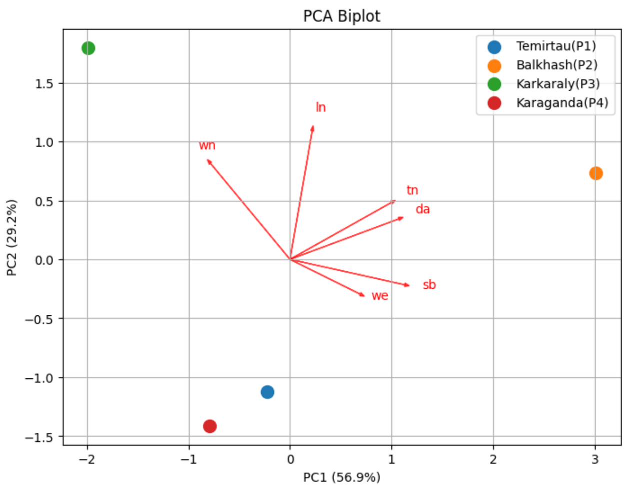
Cross-sections of needles revealed clear structural variation among the four sites (Figure 4). Temirtau (P1): resin canals were visibly reduced ($d_a \approx 15$ μm), and the epidermis was thinner, indicating the effect of technogenic stress. Balkhash (P2): the largest resin canals ($d_a \approx 22$ μm) and well-developed conducting beams were observed, reflecting stronger defense and transport structures. Karkaraly (P3): a balanced and symmetrical structure with medium-sized resin canals ($d_a \approx 13$ μm) was recorded, characteristic of optimal natural conditions. Karaganda (P4): the smallest resin canals ($d_a \approx 11$ μm) were detected, while conducting beams were moderately developed, suggesting adaptation to urban pollution. All images were taken at 200 × magnification with a 50 μm scale bar for reference.

3.5 Principal component analysis

Principal component analysis (PCA) clearly differentiated natural and urbanized populations of Pinus sylvestris (Figure 5). The first principal component (PC1) explained 56.9% of the total variance and was mainly associated with morphological traits, particularly needle length and needle width. The second principal component (PC2) accounted for 29.2% of the variance and was primarily related to anatomical characteristics, including resin canal diameter and conducting beam size.



(a) PCA score plot showing site clustering



b) PCA biplot with variable loadings

Figure 5. Principal component analysis (PCA) of morphological and anatomical parameters of *Pinus sylvestris* L.

The PCA score plot (Figure 5(a)) demonstrated a clear separation of the natural population from the urbanized sites. The natural population from Karkaraly (P3) formed a distinct cluster along the negative side of PC1, whereas the urbanized populations from Temirtau (P1) and Karaganda (P4) clustered separately. The Balkhash population (P2) occupied an intermediate position between natural and urbanized groups, indicating a moderate level of anthropogenic pressure.

The PCA biplot (Figure 5(b)) confirmed that morphological traits showed the highest loadings along PC1, while anatomical traits contributed more strongly to PC2. This pattern indicates that anthropogenic stress primarily affects the anatomical structure of needles, whereas natural environmental conditions promote more stable morphological development.

4. DISCUSSION

The present study shows that the morpho-anatomical traits of *Pinus sylvestris* L. needles are strongly dependent on local environmental conditions. Trees from the natural forest in Karkaraly developed longer and wider needles with well-preserved epidermis and vascular structures, reflecting stable ecological conditions. Conversely, individuals from heavily urbanized areas such as Temirtau and Karaganda produced shorter and thinner needles, with reduced resin canal diameter and less stable anatomical organization. These structural changes can be regarded as adaptive responses to technogenic stress, particularly air pollution and soil contamination.

The statistical analysis confirmed that differences between natural and urbanized sites were not random but significant. ANOVA and subsequent post hoc tests revealed consistent site-level contrasts ($p < 0.05$). PCA further highlighted the separation of populations: morphological parameters largely explained variation along the first component, while anatomical variables differentiated urban from natural stands along the second component. These findings emphasize that anatomical traits, especially resin canal size, are highly responsive to anthropogenic stressors.

Interestingly, the Balkhash population displayed an opposite trend, with enlarged resin canals and vascular bundles. This suggests a compensatory mechanism that may strengthen the defensive potential of trees exposed to moderate environmental pressure. Comparable results have been reported by Jankowski et al. [25], who showed that cold climates lead to shorter needles and thicker epidermis, and by Skripal'shchikova et al. [26], who linked industrial pollution with reductions in conducting elements.

Our observations also align with studies by Yarmishko and Gnatyeva [27] and Mailybaeva [28], which demonstrated that although *P. sylvestris* may initially tolerate disturbed conditions with enhanced juvenile growth, long-term effects include reduced reproductive success and altered seed morphology. This supports the idea that structural plasticity reflects both adaptive strategies and stress-related limitations.

The role of climate is equally important. Konatowska et al. [29] reported that inadequate regeneration capacity limits the persistence of some ecotypes, while Mátyás et al. [30] highlighted the risks associated with warming in southern ranges. Our results from Balkhash are consistent with these findings, suggesting that anatomical enlargement may represent an adaptive buffer against ecosystem instability.

Physiological studies also provide insight into the mechanisms behind structural variation. Chukina et al. [31] and Lukina et al. [32] demonstrated that soil chemical properties affect central cylinder and resin canal development, while Mikhailova et al. [33] and Kalugina et al. [34] reported that pollution reduces photosynthetic pigments but increases protective and antioxidant compounds. Similar patterns were observed in our material, supporting the view that *P. sylvestris* combines anatomical plasticity with biochemical defense strategies under stress.

Overall, our findings highlight that variability in morpho-anatomical features of Scots pine needles is a reliable indicator of environmental quality. Deviations observed in urban areas can serve as early-warning markers for ecological monitoring. From a practical perspective, these results underscore the importance of conserving natural populations and applying adaptive management strategies to ensure the sustainability of *P. sylvestris* under growing urban and climatic pressures [35-39].

5. CONCLUSIONS

This study demonstrated that the morpho-anatomical plasticity of *Pinus sylvestris* needles in Central Kazakhstan is directly shaped by environmental conditions. Natural populations (Karkaraly) exhibited optimal needle morphology and anatomy, while urbanized sites (Temirtau, Karaganda) showed significant reductions in needle size and resin canals, indicating stress-related responses. In contrast, the Balkhash population displayed enlarged resin canals and conducting beams, reflecting compensatory adaptation.

From a theoretical perspective, the findings contribute to the concept of plant plasticity, highlighting that Scots pine anatomy is highly responsive to both climatic and anthropogenic factors. Morphological traits (length, width) primarily reflect general growth conditions, whereas anatomical traits (especially resin canal size) serve as sensitive indicators of urban stress.

From a practical perspective, these results emphasize the value of morpho-anatomical traits as bioindicators in ecological monitoring of polluted environments. They can be applied to track urban air quality and evaluate ecosystem health. For urban forestry, selecting stress-tolerant ecotypes (e.g., populations with stable or enlarged resin canals) may improve the long-term survival and resilience of green plantations in industrial regions.

In conclusion, Scots pine needles represent an effective, low-cost, and reliable tool for monitoring environmental stress. Their variability integrates both adaptive mechanisms and stress responses, offering insights for conservation, restoration, and sustainable management of forest resources in Central Kazakhstan.

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NOMENCLATURE

w_e	width of the epidermis, μm
d_a	average diameter of the resin canals, μm
s_b	size of the conducting beam, μm
n_t	needle thickness, mm
l_n	needle length, mm
w_n	needle width, mm