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# EFFECT OF SALINITY ON THE GROWTH AND DEVELOPMENT OF ORNAMENTAL EVERGREENS

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## ABSTRACT

Salt stress is the main problem facing evergreen plants in cities. To a large extent, these plants have stunted growth, lose their ornamental qualities and finally die. The aim of this study was to investigate the response of a selected three ornamental evergreen plants: *Pachysandra terminalis*, *Buxus sempervirens* and *Hedera helix*, to the effects of three different concentrations of sodium chloride (NaCl) – 100, 200 and 300 mM. As a result of a number of experiments, it was found that increased NaCl concentrations resulted in inhibition of plant growth – even more than 90% shorter growth, as in the case of ivy. In addition, the analyses made it possible to conclude that NaCl influences biochemical changes in plant tissues, in particular chlorophyll, soluble proteins or stress parameters such as MDA or free proline. The results obtained allow the validity of the use of selected species in urban greenery in temperate climates to be established.

**Keywords**: salt stress, sodium chloride, ornamental plants, morphological changes, biochemical changes

## INTRODUCTION

Salinity is a significant global issue, with saline soils covering approximately 400 million hectares (mln ha), or nearly 3% of the Earth's total land area, as of 2011. The fraction of land lost to cultivation due to soil salinity, according to researchers, will increase in the coming years as a result of the effects of global warming or inadequate irrigation [Janz et al. 2012]. Taking into account new reports in the literature, the degree of salinity has already reached approximately 950 mln ha [Dustnazarova et al. 2021, Thaker et al. 2021]. According to the FAO (Food and Agriculture Organisation of the United Nations), 3% of the world's soils are salinised in the upper layers, while 6% are in the lower layers [FAO 2023]. In December 2024, the same organisation issued a report on the problem of soil salinisation. According to analysis by FAO experts, as much as 1.4 billion hectares (bn ha) of land in the world is salinised, representing 10.7% of the global land area. In addition, a further 1 bn ha are believed to be at risk. According to the FAO, if current trends continue, saline soils could account for 24 to 32% of the Earth's land surface by the end of the 21st century [FAO 2024].

Plants are the organisms most exposed to stress factors, including salinity. High salt concentrations cause stunted growth and development in the first stage, yellowing of individual organs in the later stage and finally even death of the whole plant [Gupta and Huang 2014, Safdar et al. 2019, Toscano et al. 2020]. The occurrence of long-term substrate salinisation leads to a reduction in the size of the root system in plants, due to partial dieback. With regard to the aboveground part of the plant, a strong shortening of the shoots can be observed. Salinity also affects the reduction of leaf blades in plants [Jameel et al. 2024]. In the case of flowering plants, there may be a reduction in

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inflorescences/flowers, but instead there will be significantly more of them than in plants not exposed to salt stress [Li and Li 2017, Cerrato et al. 2024]. A key factor influencing plant growth and appearance is the EC (electrical conductivity) value. An increase in EC in both the soil and irrigation water alters the plant's water potential, leading to reduced water absorption or even complete inhibition [Ahmadi and Souri 2020, Corwin and Yemoto 2020].

Pivotal to proper plant growth and development is what happens in their tissues. This relates primarily to the effect of high concentrations of salt on biochemical changes. One of the most important changes that occur is a reduction in the content of the plant pigment – chlorophyll. A reduction in its content in plant cells leads to a disruption in the proper functioning of photosynthesis in plants [Kibria and Hoque 2019, Jameel et al. 2024, Boorboori and Li 2025]. Furthermore, rising salinity levels lead to a reduction in the concentration of soluble proteins in certain plants [Hakim et al. 2014]. The malondialdehyde (MDA) level in plant tissues is a key indicator of oxidative stress and can also reflect the extent of cellular damage [Biczak et al. 2016]. Elevated salt levels lead to a significant accumulation of free proline, which helps alleviate the effects of osmotic stress [Cirillo et al. 2016, Rahneshan et al. 2018]. Additionally, the production of hydrogen peroxide ($H_2O_2$) in plant tissues increases, which is highly toxic and can cause chlorophyll degradation, often resulting in dieback [Shahid et al. 2020, Lu et al. 2021]. The strongly increasing $H_2O_2$ content is correlated with the activity of antioxidant enzymes, primarily catalases and peroxidases, whose function is to neutralise the threat of increased $H_2O_2$ by degrading it [Kim et al. 2018, Lu et al. 2021, Cerrato et al. 2024].

The highest levels of salinity are observed during the winter period along traffic routes. This problem is due to the use of salt by road services to reduce icing on roads. However, what is beneficial for humans is not necessarily so for plants [Devecchi and Remotti 2004, Marosz 2004, Marosz 2011]. Evergreen plants, including deciduous ornamental shrubs as well as climbers and perennials, are largely exposed. In addition to being characteristically evergreen, these plants are characterised by higher resistance to weather conditions such as low temperatures. However, they cannot always cope with high salt concentrations [Sedaghathoor and Zare 2019]. Common evergreen species along pathways include common ivy (*Hedera helix* L.) [Roeder and Meyer 2022], European boxwood (*Buxus sempervirens* L.) [De Jong et al. 2012] or Japanese spurge (*Pachysandra terminalis* Siebold & Zucc) [Ju et al. 2016].

The aim of the experiments was to investigate the changes resulting from the effects of salinity on three selected species of ornamental evergreen plants: Japanese spurge, European boxwood and common ivy, using three different concentrations of sodium chloride (NaCl) (100, 200 and 300 mM).

## MATERIAL AND METHODS

### Plants material and growing condition

The plant material consisted of three species of ornamental evergreen plants: Japanese spurge, a perennial, European boxwood, a shrub, and common ivy, a climber. All three species came from the resources of the Department of Ornamental Plants, the Warsaw University of Life Sciences. They were biennial plants planted in P9 pots in peat substrate with pH of 6.5–7. The whole experiment was conducted under greenhouse conditions, where the plants had the same temperature conditions: 15 °C during the day and 6–8 °C at night. In addition, all plants had equal access to natural sunlight. Watering was carried out at a three-day interval.

### Experiment design

The experiment was set up on 1 December 2022. 120 plants were used for each of the selected species, resulting in 30 plants per treatment (3 replicates of 10 plants each). In order to introduce the plants to salt stress, watering with an aqueous NaCl solution was applied at three concentrations, chosen on the basis of preliminary test studies and available literature, with 100 mL applied to each pot (Fig. 1). Watering with the salt solution was carried out cyclically every fortnight, giving a total of four treatments. The experiment was completed on 19 January 2023.

### Measurements of electrical conductivity (EC)

During the experiment, EC measurements were taken using a METER ProCheck handheld reader to which a TEROS 12 soil moisture, temperature, and

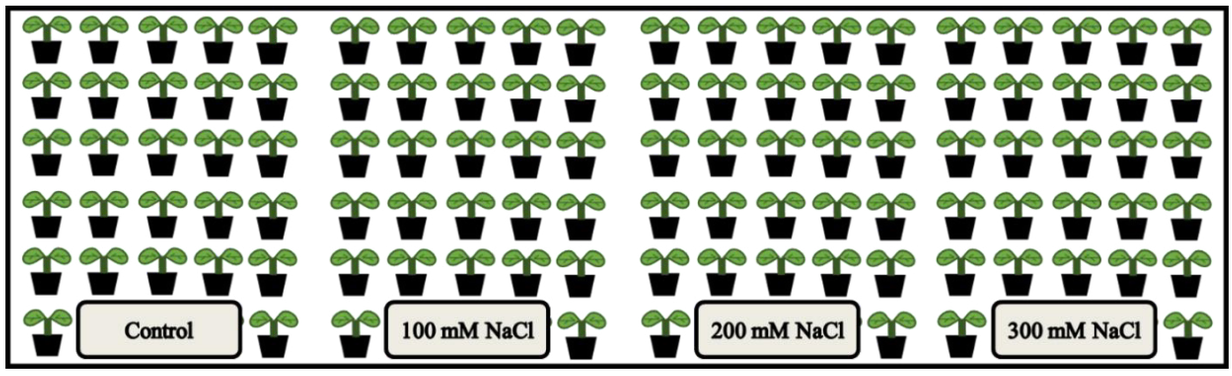


**Fig. 1.** Scheme of applied concentrations of NaCl

electrical conductivity sensor was connected. These measurements were taken twice, in the middle of the experiment (21.12.2022) and at the end (19.01.2023).

### Measurements of growth

Approximately two months after the start of the experiment, shoot length measurements were taken to record differences in the length of new growth in the different combinations. In addition, the necessary photographic documentation was taken during the measurements.

### Biochemical analyses

At the end of the experiment, plant material in the form of leaves − four well-developed and free of necrotic and chlorotic damage from each plant − was collected to understand the biochemical changes occurring in plants as a result of salt stress. Until the analyses, the plant material was stored in a deep freezer at –86 °C.

To determine the effect of a high NaCl concentration on the content of the basic plant pigments, chlorophyll and carotenoids, the method of Lichtenthaler and Wellburn [1983] was used. Plant material (0.5 g) was ground in a mortar in the presence of a little quartz sand and 5 mL of cold acetone at a percentage concentration of 80%. The extracts obtained were then filtered through filter paper into 50 mL volumetric flasks and made up to the mark with acetone. After obtaining clear filtrates, the absorbance was measured at four wavelengths − 470 nm, 646 nm, 652 nm and 663 nm.

Soluble proteins were analysed according to the method of Bradford [1976]. A 0.5 g of sample was ground with a mortar in hot 80% ethanol. This was then centrifuged for 20 minutes (min) at 20,000 rpm. After centrifugation, the clear solution had to be transferred into resealable test tubes and made up to 25 mL with ethanol (80%). A 0.1 mL each of the supernatant was taken into the tubes and 5 mL of Bradford reagent was added to it. After thorough mixing, samples were incubated for 5 minutes. After the time had elapsed, the absorbance was measured at 595 nm.

In addition, analyses were carried out to determine the degree of plant stress, i.e. the free proline content was examined according to the method of Bates et al. [1973]. A 0.5 g of plant material was ground with a mortar in 10 mL of a 0.3% aqueous sulphosalicylic acid solution, centrifuged for 20 min at 4 °C at 18,000 rpm. The supernatant was then used in a volume of 2 mL for further analysis. To the supernatant was added 2 mL of reagent A (ninhydrin acid; dissolve 1.25 g of ninhydrin in 30 mL of glacial acetic acid, then add 20 mL of 6 M phosphoric acid − 150 mL of $H_3PO_4$ per 500 mL of $H_2O$; this reagent should be kept at 4 °C, its shelf life is 24 h) and 2 mL of glacial acetic acid and incubated for one hour in a water bath at 100 °C. After this time, a cold bath was used to cool the analysed samples. Then 4 mL of toluene was added and mixed to obtain 2 phases. For measurement with a spectrophotometer, the top layer (toluene) was extracted and measured against the standard curve at 520 nm. Pure toluene was used as a blank test.

Malondialdehyde (MDA) content was tested according to the method of Hodges et al. [1999]. A 0.5 g of plant material was ground in 3 mL of 0.1% TCA and then centrifuged for 15 min at 20,000 rpm at 4 °C. After

centrifugation, the supernatant was collected in empty glass tubes. Sample mixtures consisted of 1.5 mL of 0.5% TBA dissolved in 20% TCA, 0.75 mL of supernatant and 0.75 mL of 0.1 M phosphate buffer, pH 7.6. For the blank, 0.1% TCA in a volume of 0.75 mL was used instead of supernatant. The prepared samples were incubated in a water bath for 30 min at 95 °C, with the samples covered with aluminium foil. After the time had elapsed, the samples were centrifuged for 15 min at 20,000 rpm at 4 °C to obtain the clear supernatant needed for spectrophotometric determination at two wavelengths: 532 and 600 nm. The MDA content was calculated according to the formula:

$$\text{MDA content (nmol·mL}^{-1}) = \\ =[(A_{532} - A_{600}) / 155000]\cdot 10^6$$

The hydrogen peroxide content of the plant material was analysed using the method of Siedlecka [2010]. Plant material samples (0.5 g) were ground in K-phosphate buffer using a mortar. After grinding and transferring the samples to plastic tubes, they were centrifuged for 20 min at 20,000 rpm. The obtained supernatants were decanted into glass tubes used for further analysis. A 0.1 mL of extract was transferred to the glass tubes and then made up to 0.5 mL with K-phosphate buffer. To the resulting solution, 0.5 mL of 0.1 M K-phosphate buffer and 1 mL of 1 M potassium iodide (KI) were added. The mixture was mixed and incubated for one hour in the dark. After incubation, the absorbance was measured at 390 nm.

In addition, the activities of the basic oxidative stress enzymes, i.e. catalases [Goth 1991] and peroxidases [Toczko and Grzelińska 2001], were determined.

**Catalase activity:** The same supernatant used for the hydrogen peroxide assay was employed to determine catalase activity. The samples were divided into two groups. A volume of 0.05 mL of plant extract was transferred into each test tube, followed by the addition of 0.45 mL of potassium phosphate buffer (0.1 M, pH 6.8). Group A samples were supplemented with 1.0 mL of potassium phosphate buffer (0.1 M), while group B samples received 1.0 mL of hydrogen peroxide ($H_2O_2$) solution (65 µM) prepared in the same buffer. To establish appropriate reaction backgrounds, two control samples were also prepared: sample K (buffer control), containing 1.5 mL of potassium phosphate buffer (0.1 M), and sample C ($H_2O_2$ control), consisting of 0.5 mL of potassium phosphate buffer (0.1 M) and 1.0 mL of $H_2O_2$ solution (65 µM), also prepared in the buffer. All tubes were incubated in the dark for 10 min. After this period, 1 mL of 32.5 mM ammonium molybdate was added to each tube and mixed thoroughly. Absorbance was then measured at 405 nm [Goth 1991].

**Peroxidase activity:** A 0.5 g portion of plant material was homogenised using a Polytron PT 3000 homogeniser in 10 mL of chilled phosphate buffer (50 µM, pH 7.0). The homogenate was centrifuged at 20,000 rpm for 20 min at 4 °C. The resulting supernatant was collected, and the volume was adjusted to 10 mL with the same buffer. For further analysis, 0.5 mL aliquots of the extracts were transferred to test tubes and brought to a final volume of 1.0 mL with 50 µM phosphate buffer. The samples were then divided into two groups: material samples (group A) and complete reaction samples (group B). To each tube, 0.3 mL of 0.2 M phosphate buffer (pH 7.0) was added. Subsequently, 1.4 mL of distilled water was added to group A samples, and 0.4 mL of distilled water to group B samples. The mixtures were vortexed and incubated in a water bath at 25 °C for 5 min. Following incubation, 0.3 mL of 0.2 M pyrogallol was added to each sample. Additionally, group B samples received 1.0 mL of 0.01 M $H_2O_2$. The reaction mixtures were vortexed again and incubated in a water bath at 25 °C for 10 min. After this step, all tubes were tightly wrapped in black foil. Then, 1.0 mL of 10% $H_2SO_4$ and 1.0 mL of 10% $Na_2SO_3$ were added to each sample. The contents were mixed thoroughly, and absorbance was measured at 430 nm using a spectrophotometer. A control sample was prepared in parallel by combining the following reagents: 0.3 mL of 0.2 M phosphate buffer (pH 7.0), 1.4 mL of distilled water, 1.0 mL of 0.01 M $H_2O_2$, 0.3 mL of 0.2 M pyrogallol, 1.0 mL of 10% $H_2SO_4$, and 1.0 mL of 10% $Na_2SO_3$ [Toczko and Grzelińska 2001].

All analyses were carried out for each species in 5 replicates for each combination. Absorbance was measured using a UV-1601 PC spectrophotometer (Shimadzu, Columbia, MD, USA).

### Statistical analyses

All collected results were analysed using one-way ANOVA in Statistica software (TIBCO Statistica, TIB-

CO Software Inc., Santa Clara, CA, USA). After performing several tests, including the Shapiro-Wilk test and the Tukey test at a significance level of $p \leq 0.05$, specific homogeneous groups were obtained for each parameter tested.

## RESULTS

### Changes in electrical conductivity (EC) in tested plants

On both measurement dates, it was observed in all tested species that the electrical conductivity of the soil was highest in the combination with 300 mM NaCl added to the substrate. In this variant, on the final day of the experiment, the values were 89% higher in spurge and more than 85% higher in both boxwood and ivy compared to the measurements taken from the substrate of the control plants (Table 1).

### Morphological changes in tested plants

The first changes were observed after just three NaCl treatments, when the plants stopped growing uniformly. In the case of spurge, it was observed that the addition of 200 and 300 mM NaCl to the substrate caused the leaves to lose their vivid green colour, showing signs of yellowing or partial necrosis and drooping (Fig. 2).

With regard to the other two species tested, in combinations with increased NaCl content in the substrate, the plants were characterised by a more stocky abo-

**Table 1.** Effect of different concentration of NaCl on EC ($dS \cdot m^{-1}$) in tested species in two terms under salinity stress

| Species | Term | 0 mM NaCl | 100 mM NaCl | 200 mM NaCl | 300 mM NaCl |
|---|---|---|---|---|---|
| *Pachysandra terminalis* | 21.12.2022 | 2.4 ±0.3 a* | 11.6 ±3.1 b | 13.3 ±2.7 b | 25.6 ±1.8 c |
| | 19.01.2023 | 3.1 ±0.3 a | 7.7 ±0.5 b | 11.2 ±0.3 c | 28.3 ±2.4 d |
| *Buxus sempervirens* | 21.12.2022 | 2.1 ±0.4 a | 6.2 ±0.3 b | 16.8 ±6.4 c | 26.1 ±3.0 d |
| | 19.01.2023 | 3.4 ±0.9 a | 9.6 ±1.9 b | 14.9 ±1.9 c | 22.1 ±1.8 d |
| *Hedera helix* | 21.12.2022 | 2.2 ±0.1 a | 8.6 ±0.3 b | 11.9 ±0.6 c | 21.2 ±0.3 d |
| | 19.01.2023 | 2.8 ±0.3 a | 7.8 ±0.5 b | 11.5 ±0.6 c | 19.2 ±0.4 d |

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation


**Fig. 2.** Effect of salinity on the length of growth of *Pachysandra terminalis* plants. From left: 1 – Control, 2 – 100 mM NaCl, 3 – 200 mM NaCl, 4 – 300 mM NaCl


**Fig. 3.** Effect of salinity on the length of growth of *Buxus sempervirens* plants. From left: 1 – Control, 2 – 100 mM NaCl, 3 – 200 mM NaCl, 4 – 300 mM NaCl

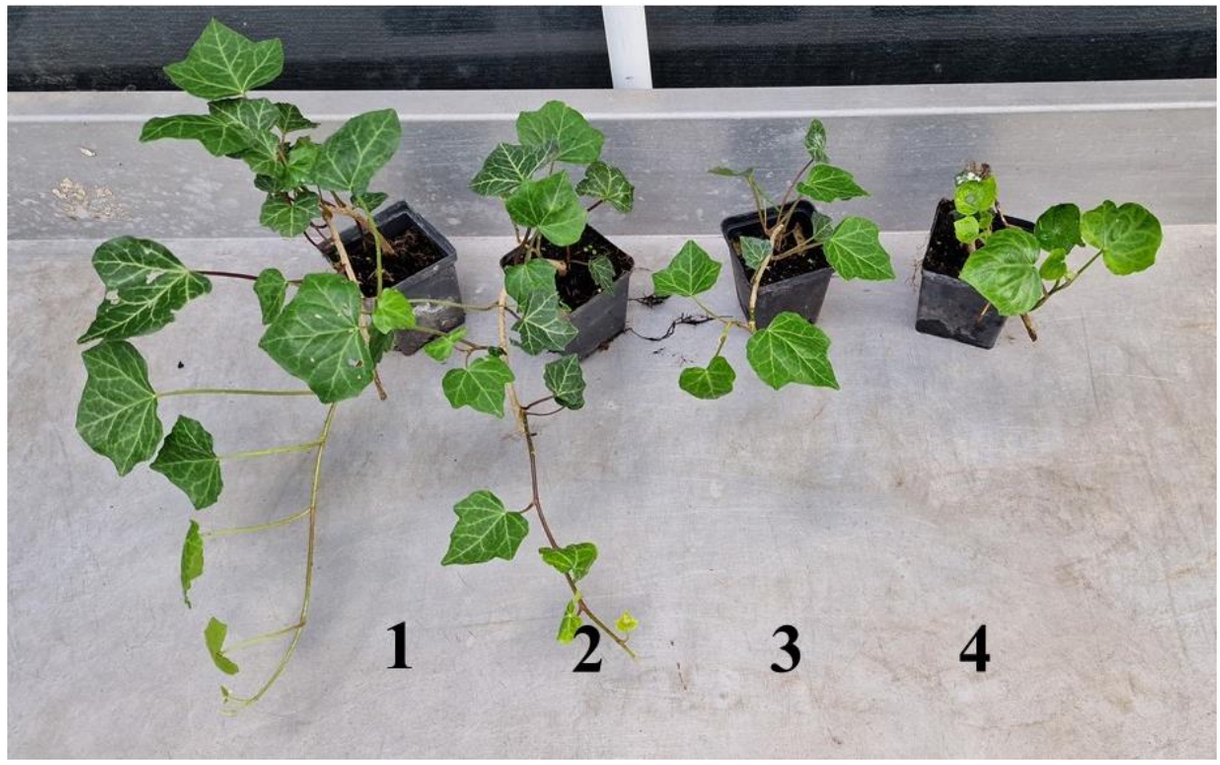


**Fig. 4.** Effect of salinity on the length of growth of *Hedera helix* plants. From left: 1 – Control, 2 – 100 mM NaCl, 3 – 200 mM NaCl, 4 – 300 mM NaCl

veground structure in boxwood and an inhibition of shoot growth in ivy (Figs 3 and 4).

Measurements taken on the final day of the experiment, after the 4-fold NaCl treatment, showed that in the combination with 300 mM NaCl added to the substrate plant growth was reduced compared to the control − approx. 77% in spurge, approx. 86% in boxwood and even by 94% in ivy.

**Biochemical changes**

An important aspect is the changes occurring in the plant itself, i.e. biochemical changes, including changes in the content of basic plant compounds, such as chlorophyll, carotenoids or soluble proteins during salinity stress.

In tested plants, it was observed that a high salt concentration in the substrate (300 mM NaCl) resulted in significantly lower chlorophyll content in the plant tissues, by up to 66% compared to the control in spurge, 41% in boxwood and 17% in ivy. During the analysis performed, the changes in carotenoid content were not recorded for all tested species; however, a slight effect of salinity on carotenoid content was observed only in *Buxus sempervirens* (Table 3).

The results of the analysis of soluble protein content show that the effect of a given stressor depends on the species in which it occurs. In spurge, lower protein content was observed in plants from the combination where NaCl was added to the substrate compared to the control plants, while the opposite trend was noted in the other two species. In both boxwood and ivy, the highest protein content was recorded in the plant material from the combination watered with 300 mM NaCl – in boxwood, it was about 28% higher than in the control, and in ivy about 32% higher (Table 3).

A second important aspect of the biochemical changes occurring in the plant as a result of salt stress is the increase in MDA, free proline, and hydrogen peroxide content. In the plants tested, it was observed that the highest MDA content was in the plant material taken from the combination where 300 mM NaCl was added to the substrate, differing from the control by 5.36 μmol $kg^{-1}$ in spurge, 1.91 μmol $kg^{-1}$ in boxwood, and 3.38 μmol $kg^{-1}$ in ivy (Table 4).

In the case of the second parameter indicating the occurrence of stress, i.e. the content of free proline, the highest values were recorded in the plants grown in the substrate treated with 300 mM NaCl. Compared to the control, the levels were approximately 60% higher in spurge and boxwood, and approximately 67% higher in ivy (Table 4).

Regarding the hydrogen peroxide parameter, an increase in its concentration was observed in plant material from all tested species. In common ivy, the value was three times higher than in the control in the combination where 300 mM NaCl was added to the substrate (Table 4).

The natural responses of plants to an increase in hydrogen peroxide are changes in the activities of oxidative stress enzymes, i.e. catalases and peroxidases. In all tested species, catalase activity was highest in plant material taken from combinations where 300 mM NaCl was added to the substrate. In spurge, the value was more than 3-fold higher than in the control, in boxwood more than 4-fold, and in ivy even 5-fold. Turning to the second enzyme analysed, peroxidase, its activity was highly species-dependent. In both spurge and boxwood, the lowest values were obtained for the combinations where 200 and 300 mM NaCl were added to the substrate, where in the case

**Table 2.** Effect of different concentration of NaCl on the length of growth of *Pachysandra terminalis*, *Buxus sempervirens* and *Hedera helix* (cm)

| Species | 0 mM NaCl | 100 mM NaCl | 200 mM NaCl | 300 mM NaCl |
|---|---|---|---|---|
| *Pachysandra terminalis* | 11.0 ±1.8 c* | 5.6 ±1.5 b | 2.9 ±1.1 a | 2.5 ±0.9 a |
| *Buxus sempervirens* | 13.3 ±1.4 c | 13.0 ±1.6 c | 3.4 ±1.0 b | 1.8 ±0.8 a |
| *Hedera helix* | 36.5 ±1.9 d | 21.1 ±1.8 c | 14.0 ±1.3 b | 2.2 ±1.3 a |

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation

**Table 3.** Changes in the content of basic compounds in plant material from tested species under salinity stress

| Species | NaCl [mM] | Chlorophyll [mg $g^{-1}$ DW] | Carotenoids [mg $g^{-1}$ DW] | Soluble protein [mg $g^{-1}$ DW] |
|---|---|---|---|---|
| *Pachysandra terminalis* | 0 | 6.57 ±0.06 d* | 0.83 ±0.02 a | 4.25 ±0.01 d |
| | 100 | 5.34 ±0.04 c | 0.78 ±0.11 a | 3.86 ±0.09 c |
| | 200 | 5.04 ±0.03 b | 0.68 ±0.03 a | 3.25 ±0.04 a |
| | 300 | 2.23 ±0.02 a | 0.77 ±0.01 a | 3.56 ±0.02 b |
| *Buxus sempervirens* | 0 | 8.14 ±0.03 d | 0.98 ±0.02 ab | 4.54 ±0.03 a |
| | 100 | 6.63 ±0.02 c | 0.94 ±0.05 a | 5.23 ±0.18 b |
| | 200 | 5.86 ±0.06 b | 0.91 ±0.03 a | 5.23 ±0.04 b |
| | 300 | 4.80 ±0.03 a | 1.04 ±0.02 b | 6.27 ±0.02 c |
| *Hedera helix* | 0 | 8.85 ±0.01 c | 1.42 ±0.04 a | 4.87 ±0.04 a |
| | 100 | 8.26 ±0.05 b | 1.44 ±0.07 a | 5.87 ±0.08 b |
| | 200 | 8.34 ±0.05 b | 1.43 ±0.03 a | 6.09 ±0.03 c |
| | 300 | 7.33 ±0.05 a | 1.34 ±0.05 a | 7.15 ±0.03 d |

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation
DW – dry weight

**Table 4.** Changes in the content and activity of important stress parameters in tested species under salinity stress

| Species | NaCl [mM] | MDA [µmol $kg^{-1}$] | Free proline [µmol $g^{-1}$ DW] | $H_2O_2$ [µg $g^{-1}$ DW] | catalase [mcat $g^{-1}$ DW] | peroxidase [µmol $min^{-1}$ $g^{-1}$ DW] |
|---|---|---|---|---|---|---|
| *Pachysandra terminalis* | 0 | 2.03 ±0.02 a* | 3.32 ±0.04 a | 15.41 ±1.05 a | 180.99 ±3.69 a | 0.084 ±0.003 d |
| | 100 | 2.14 ±0.11 a | 3.94 ±0.11 ab | 22.27 ±0.84 b | 294.11 ±3.69 b | 0.064 ±0,003 c |
| | 200 | 4.59 ±0.03 b | 4.12 ±0.05 b | 24.23 ±0.21 b | 371.08 ±3.57 c | 0.023 ±0.003 b |
| | 300 | 7.39 ±0.04 c | 8.34 ±0.03 c | 23.59 ±0.29 b | 551.52 ±7.97 d | 0.012 ±0.001 a |
| *Buxus sempervirens* | 0 | 0.95 ±0.03 a | 0.93 ±0.09 a | 7.21 ±0.56 a | 156.35 ±9.59 a | 0.050 ±0.001 c |
| | 100 | 1.04 ±0.02 a | 1.13 ±0.01 a | 8.32 ±0.10 ab | 298.78 ±18.98 b | 0.036 ±0.003 b |
| | 200 | 2.57 ±0.11 b | 1.23 ±0.02 a | 9.57 ±0.24 bc | 421.11 ±11.11 c | 0.026 ±0.002 a |
| | 300 | 2.86 ±0.31 b | 2.31 ±0.02 b | 11.29 ±1.15 c | 677.02 ±24.96 d | 0.034 ±0.002 b |
| *Hedera helix* | 0 | 1.94 ±0.05 a | 1.42 ±0.02 a | 8.75 ±0.64 a | 117.29 ±10.14 a | 0.037 ±0.001 ab |
| | 100 | 2.11 ±0.01 a | 1.73 ±0.11 ab | 10.78 ±0.17 b | 426.86 ±28.05 b | 0.041 ±0.001 b |
| | 200 | 3.67 ±0.03 b | 1.94 ±0.08 b | 18.37 ±0.52 c | 479.02 ±17.18 b | 0.032 ±0.001 a |
| | 300 | 5.32 ±0.14 c | 4.35 ±0.02 c | 25.08 ±0.18 d | 629.18 ±16.99 c | 0.056 ±0.003 c |

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation
DW – dry weight

of spurge, the difference compared to the control was up to 7-fold. The only species that stood out in terms of peroxidase activity was ivy, as its highest value was recorded in the combination where the highest concentration of NaCl was used for substrate supplementation − about 34% higher than in the control plants (Table 4).

## DISCUSSION

Strong stress factors are contributing to the degradation of numerous species sensitive to adverse condi-tions, resulting in a decline in the biodiversity of both natural and urbanised habitats [Razzaq et al. 2020]. Researchers are therefore focusing on understanding how plants respond to abiotic stress conditions (i.e. salinity, drought or high concentrations of heavy metals).

The main site of excessive salt ion accumulation, which is harmful to plants, is the soil in which they grow. It is the pillar of good plant growth and development, and any disturbance is due to inappropriate soil physico-chemical parameters, including the electrical conductivity (EC) value [Passioura 1991, Khalil et al. 2015]. n the experiment carried out by the authors of this paper, it was observed that the higher the NaCl concentration in the substrate, the higher the electrical conductivity, reaching around 20 $dS \cdot m^{-1}$ in all the species tested at a concentration of 300 mM NaCl. A similar trend was obtained by Bekmirzaev et al. [2020], who treated *Tetragonia tetragonioides* Pall. plants with three NaCl concentrations (50, 100 and 200 mM NaCl), as the substrate with the highest NaCl concentration exhibited the highest EC value. Also Wu et al. [2001] confirm that in both soil and container cultivation, the application of NaCl increases the electrical conductivity of the substrate, which was also noted by the authors of the present study.

A plant's longevity largely depends on its structural integrity, i.e. shoot growth, absence of damage to leaf blades, and absence of damage caused by external factors [Kumar et al. 2021]. The research conducted in this study showed that plants grown in a substrate with increasing concentrations of NaCl exhibited reduced growth – in some cases, such as ivy, shoot growth was more than 16 times lower compared to the control. In addition, leaf yellowing, chlorotic changes, and, in the case of spurge, complete leaf loss were observed. Similar visual symptoms of growth reduction in two ornamental shrub species (*Hibiscus rosa-sinensis* L. and *Mandevilla splendens* Hook.f.) in response to high salt concentrations in the substrate were observed by Yu et al. [2021]. Studies conducted on *Rosa chinensis* var. *minima* Rouletii confirm that an increase in EC results in reduced plant growth [Asgari and Diyanat 2020].

An important aspect of proper plant growth and development is an adequate content of biologically active compounds, such as plant pigments or proteins. These are responsible for the most important processes in the plant, such as photosynthesis and the stress response [Simkin et al. 2022, Zhang et al. 2022]. In the course of experiments and analyses, it was found that high concentrations of NaCl in the substrate resulted in a decrease in the content of the most important of plant pigments − chlorophyll, with the value in spurge in the 300 mM NaCl combination differing from the control by approximately 66%. A study by Alam et al. [2020] on *Fortunella japonica* Thunb., *Citrus reshni* Hort. ex Tan and *Citrus maxima* Merr., confirms that the higher the EC the lower the chlorophyll content in plant tissues, where the highest difference in concentration of this pigment was 60%. A reduction in chlorophyll content under salinity stress was also observed in two species of beardtongues (*Penstemon barbatus* Cav. and *Penstemon strictus* Benth.), where, in one of them, the value decreased by approximately threefold compared to the control [Paudel and Sun 2024].

A second important parameter in the structure of plant tissues is the concentration of proteins that accumulate when salinity stress occurs, in order to subsequently store nitrogen that could be reused by the plant in the future. In addition, they can play a role in regulating the osmotic potential in plant cells [Parvaiz and Satyawati 2008]. Experiments and analyses performed on three species of ornamental evergreens showed that these values could vary depending on the tested species, as for two of them, boxwood and ivy, the concentration of soluble proteins was higher in plant material from salt-stressed combinations, while for spurge the values were lower than in the control. A study by Xu et al. [2020] showed that the addition of NaCl to the substrate increases soluble protein content, as *Ginkgo biloba* L. seedlings grown in a substrate with 300 mM NaCl resulted in a 3-fold higher protein concentration in plant tissues. Goharrizi et al. [2020a], in their study conducted on pistachio (*Pistacia* L.) plants subjected to salt stress, confirmed that the addition of NaCl to the substrate can result in a reduction of soluble protein content in plant tissues, which in the present study was obtained by the authors for plant material taken from spurge plants grown in substrate with NaCl.

The contents of malondialdehyde (MDA) [Hamani et al. 2020] and free proline [Hussein and Alshammari 2022] are important indicators of stress occurrence. In a study conducted on three evergreen species, it was found that increasing concentrations of NaCl added to the substrate increased the content of both MDA and free proline. An experiment conducted on little walnut (*Juglans microcarpa* Berlandier) seedlings showed that the concentration of MDA in the plant material was highest at a 300 mM NaCl treatment [Ji et al. 2022]. A more than twofold increase in the content of this parameter was also observed for *Reaumuria songarica* (Pall.) Maxim. seedlings [Yan et al. 2022] and *Sorghum bicolor* (L.) Moench seedlings [Yilmaz et al. 2020]. For the second parameter mentioned above, i.e. the free proline content, the experiment conducted by the authors of this publication showed that higher NaCl concentrations increased the concentration of this compound in the plant material. Similar results were obtained in studies on *Rosmarinus officinalis* Spenn. plants [Hassanpouraghdam et al. 2020], *Portulaca oleracea* L. plants [Hnilickova et al. 2021], and *Linum usitatissimum* L. seedlings [Hussein and Alshammari 2022].

When a stress factor, such as salinity, is intensified, there is a large accumulation of reactive oxygen species (ROS) in plant cells, which are highly toxic to plants [Akyol et al. 2020]. Among the main ones is hydrogen peroxide ($H_2O_2$). Its elevated content in plant tissues can lead to disruption of the plant metabolism through autophagy of chloroplasts and peroxisomes, ultimately leading to activation of the programmed cell death process [Smirnoff and Arnaud 2019]. In the plant material analysed by the authors, it was observed that higher concentrations of NaCl in the substrate resulted in a strong increase in hydrogen peroxide content in plant tissues. A similar result was obtained by Hassanpouraghdam et al. [2019], who used different NaCl concentrations in *Rosmarinus officinalis* L., and at the highest concentration, 225 mM, a fourfold higher concentration of this compound was recorded than in the control. Also, exposure to high salt concentrations in *Lepidium draba* L. plants results in more than a twofold accumulation of the compound $H_2O_2$ in plant tissues [Goharrizi et al. 2020b].

Important parameters in the plant response to stress, including the salinity as studied in the experiment, are the activities of antioxidant enzymes. These enzymes are responsible for the breakdown of harmful $H_2O_2$ into oxygen and water [Berwal et al. 2021], or for reducing its levels through the oxidation of phenolic compounds, during which phenolic polymers are ultimately formed. These polymers contribute to strengthening the cell wall and inhibiting the penetration of harmful compounds into the cells [Kidwai et al. 2020]. Analysis of the plant material collected from the experimental plants revealed that catalase activity also increased with increasing $H_2O_2$ levels. The effect of sodium chloride on pistachio plants confirmed this relationship, as the activity of this enzyme in stressed plants relative to control plants was significantly higher [Goharrizi et al. 2020a]. The study conducted on *Antigonon leptopus* Hook. & Arn. also confirms the results obtained in the authors' experiment, indicating that the increase in catalase activity in the plant is correlated with the rise in NaCl concentration applied to the substrate [El-Zaiat et al. 2020]. For the second oxidative stress enzyme tested, peroxidase, results varied depending on whether the species produced a defence system or not, as the activity of this enzyme was higher only in ivy. A similar relationship to that observed in ivy was found in the study by Jha and Subramanian [2013] on *Oryza sativa* L. or in the study by Cai and Gao [2020] on *Chenopodium quinoa* Willd. The opposite situation was observed by the authors of this paper in spurge and boxwood, where the peroxidase activity in stressed plants was comparable to or lower than in the control. Xu et al. [2020], in an experiment where *Ginkgo biloba* L. plants were subjected to salinity stress, obtained a similar result, as the application of 200 and 300 mM NaCl caused a decrease in the activity of this antioxidant enzyme.

## CONCLUSION

The experiments and analyses carried out suggest that the response of ornamental evergreen plants to salinity is species-specific. The use of Japanese spurge in areas exposed to stress factors such as salinity was found to be inappropriate, as the plants may die back or lose their ornamental value. In contrast, in the other two species tested, the changes were not as drastic, as periodic increases in salt concentrations in the substrate led only to stunted growth, without dieback. As

far as biochemical changes are concerned, the analyses performed show that salt stress can cause severe disturbances in the content of essential compounds in the plant. The analyses revealed that the activation of defence mechanisms, indicated by increased activity of specific oxidative stress enzymes, was species-dependent.

This research provides a foundation for further investigation into the mechanisms by which plants respond to stress conditions. Given the widespread use of evergreen species, it is also important to deepen our understanding of these mechanisms within this group and to develop strategies to mitigate the effects of stress factors.

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