

## INFLUENCE OF SODIUM CHLORIDE AND SODIUM SULFATE ON ZONAL PELARGONIUM AND MICROORGANISMS COLONIZING ROOT ENVIRONMENT

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### Abstract

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Increasingly often we may observe soil and substrate contamination with sodium. Excess of sodium in root environment causes physiological disorders in plants affecting their quality. To assess the effect of sodium salts on the quality of zonal pelargonium two-year experiments were established. Increasing doses of NaCl or Na<sub>2</sub>SO<sub>4</sub> were applied, raising sodium contents in the substrate to 100, 200, 300, 400 and 500 mg Na·dm<sup>-3</sup>. Substrate with no sodium salt added comprised the control. An increase in sodium contents had a negative effect on all biomorphological features of pelargonium. However, low content of sodium in the peat substrate may be beneficial for zonal pelargonium. Sodium chloride has a stronger negative effect on zonal pelargonium than sodium sulfate. Up to 200 mg Na·dm<sup>-3</sup> in the substrate pelargonium retains its high decorative value. Salinity also affects microorganisms colonizing the peat substrate. Both bacteria and actinomycetes are sensitive to sodicity. Increased sodium salts contents in the peat substrate caused only a slight change in CFU of fungi in the substrate.

**Key words:** ornamental plants, salinity, biomorphological features, bacteria, fungi

**Abbreviations:** CFU – colony-forming units; DM – dry matter

### Introduction

Increasingly often we may observe soil and substrate contamination with sodium. Urban soils are often contaminated by sodium salts used in winter for de-icing of roads (Devecchi and Remotti, 2004). High amounts of this element may be found in composts, particularly those produced with shrimp waste amendment (Fortin and Karam, 1998; Heu et al., 2003). Moreover, in some areas the availability of good quality water is limited and therefore for plant watering semi-saline underground water is utilized. It causes a gradual build-up of Na in the root zone to levels (Sonneveld, 2000). Excess of sodium in soil or substrate causes physiological disorders in plants affecting their quality. The importance of this problem for urban landscape architecture is shown by studies conducted in many cities (Czerniawska-Kusza et

al., 2004; Cunningham et al., 2008; Green et al., 2008). For example in Poznan (Poland) soil contained 11 – 340 mg Na dm<sup>-3</sup> (Wilkaniec et al., 2012). It results in dieback of many species of ornamental plants. There is an extensive body of scientific literature on plant responses to elevated or high Na content in the root environment. Such research typically concerns the effect of total salt concentration or the effect of sodium chloride. For this reason some authors specifically distinguish salinity from sodicity (Rietz and Haynes, 2003), with salinity being a broader term. Relatively few studies have focused on the effects of Na<sub>2</sub>SO<sub>4</sub> on plant growth and physiology, even if Na<sub>2</sub>SO<sub>4</sub> is present at higher concentrations than NaCl in the soils and groundwater in many areas (Banuelos et al., 1993). Moreover, information concerning plant response to salinity is not always conclusive, thus it is very difficult to interpret results of experiments conducted

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using different research methods. For example, in the case of pelargonium it may be assumed that it is a plant of low tolerance (Kotuby-Amacher et al., 2000; Nowosielski, 1988) or moderately tolerant (Miyamoto, 2008) to salinity. In view of the above, two-year experiments were established in order to assess usefulness of *Pelargonium zonale* to cultivation in urban area polluted by sodium salts. Root environment is also a habitat of microorganisms, and therefore the effect of sodium salts on bacteria, actinomycetes and fungi was also evaluated.

## Material and Methods

Investigations on *Pelargonium zonale* 'Samba' were conducted in 2008 and 2009 from April to July. Prior to planting the substrate (highmoor peat) was limed to pH 6.0. After liming the content of calcium and magnesium increased to 1245 mg Ca and 160 mg Mg per dm<sup>-3</sup> of substrate. For fertilization (including NPK side dressing) 300 mg N, 220 mg P, 350 mg K, 75 mg Fe, 35 mg Mn, 30 mg Zn, 10 mg Cu, 2 mg B and 2 mg Mo per dm<sup>-3</sup> of peat were applied. Using NaCl or Na<sub>2</sub>SO<sub>4</sub> the content of Na was increased to 100, 200, 300, 400 and 500 mg Na·dm<sup>-3</sup>. The basis for sodium doses determination were earlier performed studies on chemical properties of urban soils (Wilkaniec et al., 2012). Substrate with no sodium salt added comprised the control. Mean content of Na<sup>+</sup>, Cl<sup>-</sup>, S-SO<sub>4</sub><sup>-2</sup> in the peat substrate and EC of peat substrate on the beginning of experiments are presented in Table 1. Pelargoniums were planted to containers filled with 7 dm<sup>3</sup> of substrate. In each container 3 plants were grown, with each treatment composed of 10 containers. Four weeks after planting (the stage of emergence of flowering shoots) leaf blade area was measured (the first leaf located above the second inflorescence). Measurements were taken using a scanner and the SKWER software. At the stage of flowering fresh weight of aboveground part of plants was determined,

plant height and length of the main shoot were measured, number of lateral shoots, leaves and inflorescences were counted. Moreover, the total length of lateral shoots was calculated. The results were processed statistically using analysis of variance for a two-factor experiment with an aid of the software package Statistica. The anthocyanin content was measured in flower petals. Pigments were measured colorimetrically, with absorbance (in petals previously ground in 0.5 M HCl) at a wavelength of 530 nm. Findings were read from the curve for cyanidine chloride (C<sub>15</sub>H<sub>11</sub>O<sub>6</sub>Cl) following the method developed by Wang et al. (2000). Results are presented in the form of a graph and polynomial regression equations. The effect of salinity on microbial populations in the peat substrate was also evaluated. Bacterial counts were determined on nutrient agar by incubating plates at the temperature of 26°C for 48 hours (Kańska et al., 2001). Mould fungi were determined on the Martin nutrient medium following 5-day incubation (Martin, 1950) at the temperature of 24°C. Numbers of actinomycetes were determined on the Pochon medium by carrying out 5-day culturing at the temperature of 28°C (Kańska et al., 2001). The obtained results were converted into 1 g dry matter of the substrate.

## Results and Discussion

An increase in sodium levels had a negative effect on all biomorphological features of pelargonium (Table 2). It was particularly evident in case of fresh weight (with the exception of 100 mg Na treatment) and total length of lateral shoots in plants grown in the substrate with the greatest sodium content. The results of measurements were lower by approx. 45% in relation to plants from the control. Very similar trends were observed in experiments on many species, e.g. winter wheat (Zheng, 2008) and soybean plants (Müller Queiroz et al., 2012). Munns (2002) stated that salinity reduces the ability of plants to take up water, and this quickly causes reductions in their growth rate along with a suite of metabolic changes identical to those caused by water stress.

Another symptom of the adverse action of increasing sodium salt rates was a reduction in the number of leaves (with the exception of 100 mg Na treatment) and leaf blade area, i.e. the assimilating area of plants. However, no leaf necrosis was found. Reduction of assimilating organ area was also observed in *Asteriscus maritimus* (Rodriguez et al., 2005) and *Salvinia natans* (Jampeetong and Brix, 2009) *Paulownia* clones (Miladinova et al., 2013) grown at saline stress. The reduction of leaf area can be considered as an avoidance mechanism, minimizing water losses when the stomata are closed, which happens to many species under osmotic stress

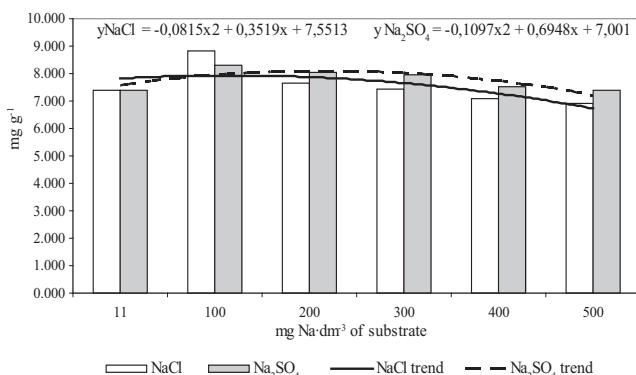
**Table 1**  
**Mean content of Na<sup>+</sup>, Cl<sup>-</sup>, S-SO<sub>4</sub><sup>-2</sup> in the peat substrate and EC of peat substrate on the beginning of experiments**

Content in the peat substrate (mg·dm <sup>-3</sup> )			EC (mS·cm <sup>-1</sup> )	
Na <sup>+</sup>	Cl <sup>-</sup>	S-SO <sub>4</sub> <sup>-2</sup>	NaCl treatment	Na <sub>2</sub> SO <sub>4</sub> treatment
11	51	25	1.27	1.27
100	210	100	1.44	1.34
200	360	160	1.68	1.48
300	512	260	1.72	1.65
400	660	302	2.01	2.07
500	810	374	2.17	2.11

**Table 2****Effect of sodium salts on morphology of pelargonium**

Salt (A)	Dose of sodium ( $\text{mg}\cdot\text{dm}^{-3}$ ) (B)						Mean (A)
	11	100	200	300	400	500	
Fresh weight of plant							
NaCl	45.9	48.8	36.8	34.9	31.1	25.5	37.2
$\text{Na}_2\text{SO}_4$	42.2	46.3	42.6	39.5	37.1	32.3	40.3
Mean (B)	45	47.6	39.7	37.2	34.1	28.9	
LSD <sub>0.05</sub> for: A 0.96; B 2.54; AxB 4.25							
Height of plant							
NaCl	19.7	19.2	17.2	16	15.4	14	16.9
$\text{Na}_2\text{SO}_4$	17.6	19	17.8	16.7	16.4	14.7	17
Mean (B)	18.7	19.1	17.5	16.4	15.9	14.4	
LSD <sub>0.05</sub> for: A ns; B 0.66; AxB 1.15							
Length of main shoot							
NaCl	9.1	8.2	6.9	6.7	5.9	5.5	7.1
$\text{Na}_2\text{SO}_4$	8.1	7.4	7.4	6.6	6.1	5.5	6.9
Mean (B)	8.6	7.8	7.2	6.7	6	5.5	
LSD <sub>0.05</sub> for: A ns; B 0.44; AxB 0.76							
Number of lateral shoots							
NaCl	5.5	4.8	4.1	3.7	3.4	3	4.1
$\text{Na}_2\text{SO}_4$	5.2	4.7	4.5	3.9	3.4	3.1	4.1
Mean (B)	5.2	4.8	4.3	3.8	3.4	3.1	
LSD <sub>0.05</sub> for: A ns; B 0.28; AxB 0.49							
Total length of lateral shoots							
NaCl	16.9	16.1	12.1	10.7	9.9	8	12.3
$\text{Na}_2\text{SO}_4$	13.7	13.5	12.7	12	11.6	9.2	12.1
Mean (B)	15.3	14.8	12.4	11.4	10.8	8.6	
LSD <sub>0.05</sub> for: A ns; B 1.16; AxB 1.86							
Number of inflorescences							
NaCl	5.9	5.9	5.3	4.8	4.1	3.6	4.9
$\text{Na}_2\text{SO}_4$	5.7	5.4	5.2	5	4.8	4.5	5.1
Mean (B)	5.8	5.6	5.2	4.9	4.5	4.1	
LSD <sub>0.05</sub> for: A ns; B 0.42; AxB 0.73							
Number of leaves							
NaCl	33.4	36	28	27.8	26	23.7	29.2
$\text{Na}_2\text{SO}_4$	32.5	33.5	32	30.4	30.1	28.8	31.2
Mean (B)	32.9	34.7	30	29.1	28.1	26.3	
LSD <sub>0.05</sub> for: A ns; B 1.89; AxB 3.33							
Leaf area							
NaCl	30.2	27.1	22.9	21.3	19	16.2	22.8
$\text{Na}_2\text{SO}_4$	32.6	24.4	23.1	22.7	21.7	18.2	23.8
Mean (B)	31.4	25.7	23	22	20.3	17.2	
LSD <sub>0.05</sub> for: A 0.57; B 1.45; AxB 2.50							

n.s. – difference not significant



**Fig. 1. Effect of sodium salts on anthocyanins content in flower petals of zonal pelargonium**

(Ruiz-Sánchez et al., 2000; Jamil et al., 2007). According to Koyro (2006), also gas exchange properties such as net photosynthesis and water use efficiency are affected strongly by NaCl-saline conditions and this is related to stomatal conductance.

In the case of ornamental plants a considerable importance is ascribed to flowering. In this study on pelargonium the greatest salinity caused a reduced number of inflorescences, which depending on the salt decreased by approximately 21–39%. Only small changes of anthocyanin contents in flower petals were observed (Figure 1). The lowest rate of sodium salt caused a slight increase, while successive rates caused a decrease in the pigment concentration. Polynomial regression equations being statistical descriptions of this trend (Figure 1) indicate that a stronger effect was found for NaCl. In the literature, information about the effects of salinity on the pigments content in flower petals is very limited. For example, reduction in the content of pigments (total flavonoids and carotenoids) in *Calendula officinalis* flowers was reported by Khalid and da Silva (2010). According to Reezi et al. (2009), petal color of Na-stressed cut rose had better lightness but worst chroma values. The authors believe that excess concentrations of various ions associated with salinity can cause enzyme inhibition and therefore alter metabolism or physiological function. According to Achard et al. (2006), high salinity can cause a delay in floral transition, which allows the plant time to enhance its resistance responses. In the experiment with pelargonium at 500 mg Na dm⁻³ total salinity amounted only to approx. 2 mS cm⁻¹. For this reason no effect on the beginning of flowering was observed, i.e. the date of flowering in the first inflorescence of pelargonium was not dependent on sodium dose and the type of sodium salts (Table 3).

**Table 3**

**Beginning of flowering in the first inflorescence of pelargonium**

Salt	Dose of sodium (mg·dm⁻³)					
	11	100	200	300	400	500
NaCl	24.06.	23.06.	23.06.	24.06.	25.06.	23.06.
Na₂SO₄	24.06.	24.06.	23.06.	23.06.	23.06.	24.06.

In available literature there is practically no definite evaluation of what salts and particularly at what content of ions in the root medium (sodium, chloride or sulfate) limit growth or markedly deteriorate decorative value of *Pelargonium zonale*. In our study the effect of individual sodium salts was varied. A more negative effect on fresh weight of the aboveground parts and leaf area at comparable sodium contents in the substrate was found for chlorides. A stimulatory effect on the fresh weight of plants and the number of leaves was found only for the smallest NaCl and Na₂SO₄ doses. The other traits, i.e. height of plant, length of the main shoot, number of lateral shoots, total length of lateral shoots, number of inflorescences and leaf area were not dependent on sodium salts. In the literature information concerning sodium salts treatments are contradictory. Sulfate salts were less deleterious than chloride salts to *Cicer arietinum* (Manchanda and Sharma, 1989) and *Capsicum annuum* (Navarro et al., 2002). Also Devecchi and Remotti (2004), when evaluating total damage observed on *Cotoneaster salicifolius* stated that damage on the epigeal part of the plants caused by NaCl is greater than that caused by Na₂SO₄. In contrast, growth and photosynthesis of *Cornus stolonifera* decreased strongly in plants treated with Na₂SO₄ rather than in plants treated with NaCl at the same molar concentration (Renault et al., 2001). Also Amuthavalli and Sivasankaramoorthy (2012) observed that salinity caused by sodium sulfate decreases chlorophyll content in *Cajanus cajan* leaves slightly stronger than sodium chloride.

It may be assumed as a general rule formulated on the basis of this study that an increasing content of sodium salts in the substrate resulted in a deteriorated quality of pelargoniums. However, low content of sodium in the peat substrate may be beneficial for zonal pelargonium. In the case of fresh weight of the aboveground parts and number of leaves of pelargoniums – a positive effect was recorded for the lowest sodium rate. A similar response of *Delosperma cooperi* was recorded by Niu and Rodriguez (2006) when investigating tolerance of bed plants to increasing amounts of NaCl in the nutrient solution. Plants watered with a solution at EC 3.2 - 6.4 mS·cm⁻¹ produced greater mass than plants watered with solutions with greater or lower concentrations. Simi-

larly, a slight increment in weight was recorded when growing *Atriplex hortensis* in a substrate with an addition of 4 g NaCl·dm<sup>-3</sup> (Wrochna et al., 2006). A further increase in salinity had a marked negative effect on those plants.

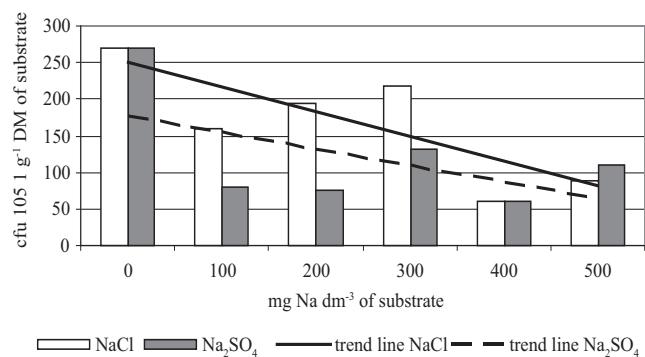
For the gardeners decorative value rating is crucial. Conducting the final evaluation of the effect of sodium salts on quality of pelargonium 'Samba' it was assumed that a 20% reduction of values for measured morphological traits (in relation to control plants) is acceptable. In the presented study it usually refers to the dose of 200 mg Na dm<sup>-3</sup>. The plants from this treatment retain still the high decorative value. The dose 300 mg Na is not toxic, but the quality of plants (especially NaCl treatment) is only satisfactory (Table 4). Reference of these results to literature data is very difficult due to methodological differences. Still we need to cite here a publication by Weinhold and Scharpf (1997). Those authors conducted studies concerning the application of composts from municipal wastes in growing of *Pelargonium zonale* 'Pulsar Red' and its response to sodium and chlorides found in the substrate. The admissible content in substrates was specified by the authors to be 225 mg Na, but they found 780 mg Na·dm<sup>-3</sup> and 880 mg Cl·dm<sup>-3</sup> as the threshold value.

**Table 4**  
**Evaluation of decorative value of pelargonium performed in flowering stage (the highest = 5, the lowest = 1)**

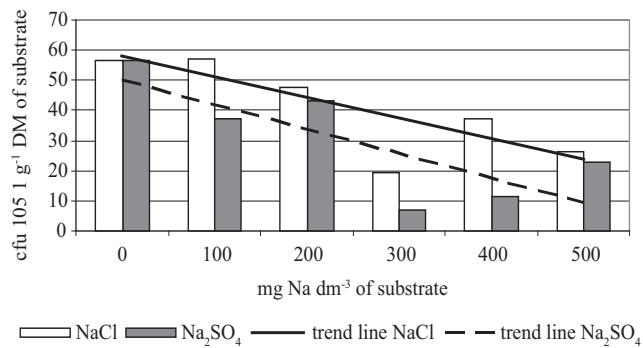
Salt	Dose of sodium (mg·dm <sup>-3</sup> )					
	11	100	200	300	400	500
NaCl	5	5	4	3	2	1
Na <sub>2</sub> SO <sub>4</sub>	5	5	4.5	4	4	3

Salinity may affect not only plants, but also microorganisms colonizing soils and substrates. Changes in the CFU of bacteria and actinomycetes, which were caused by the increase in the amounts of salt in the root environment, varied. In this situation, trend lines were employed for analyses (Figures 2-5). It was found that both bacteria and actinomycetes were sensitive to salinity. Counts of these microorganisms in the media with Na<sub>2</sub>SO<sub>4</sub> were greater than in those containing NaCl. In general, CFU of actinomycetes in the peat substrate were lower than the CFU of bacteria. Also in the case of experiments conducted by Omar et al. (1994), the CFU of bacteria and actinomycetes decreased when NaCl was added.

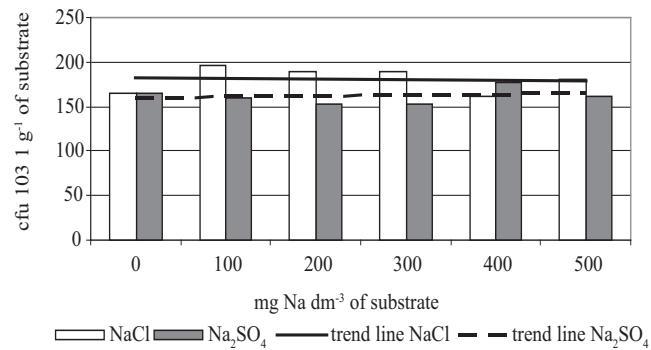
However, not all assessments are so unequivocal. Research results of Černohlávková et al. (2008) demonstrated a stimulation of growth of *Pseudomonas putida* in saline soil samples which were collected in the neighborhood of roads. High bacteria CFU at elevated NaCl doses were also reported by Przybulewska (2006). Increased sodium quantities



**Fig. 2. Influence of sodium salts on number of bacteria in peat substrate**



**Fig. 3. Influence of sodium salts on number of actinomycetes in peat substrate**



**Fig. 4. Influence of sodium salts on number of fungi in peat substrate**

caused only a slight change in CFU of fungi in the substrate. Irrespective of the type of salt, differences did not exceed 18%. However, it was found that, as in the case of bacteria and actinomycetes, also CFU of fungi in the substrates

with  $\text{Na}_2\text{SO}_4$  were greater than in those containing NaCl. Fungi, in comparison with bacteria and actinomycetes, are characterised by the highest resistance to the increase of osmotic pressure in their environment. This can be attributed to a considerably higher osmotic pressure inside their cells, which provides good survivability in the extreme environment for the other groups of microorganisms (Kowal, 2010). According to Sardinha et al. (2003), salinity is one of the most stressing environmental conditions for soil microorganisms. However, at a relatively low salinity in the presented experiment, this failed to affect fungi. Nevertheless, Matsuda et al. (2006) maintained that the problem is more complex. As exemplified by mycorrhizal fungi, the authors demonstrated the existence of fungi both sensitive and resistant to increased quantities of NaCl in the substrate. According to Wielgosz and Szember (2006), the dominance of fungi is not beneficial. These microorganisms are capable of producing many toxic and phyto-pathogenic compounds, which could exert a negative influence on the population sizes of other microbial groups. Usually during decomposition of organic matter bacteria and actinomycetes initially dominate there. Under saline conditions the humus formation process will be disturbed. Despite the unfavorable conditions, certain fungi can fulfill a useful role. Navarro et al. (2011) found that mycorrhizal fungi *Glomus deserticola* and *Glomus intraradices* improved growth rates of pelargonium plants under saline conditions and reduced harmful environmental effects caused by salinity.

## Conclusions

Increasing doses of sodium salts result in a deterioration of quality in *Pelargonium zonale* 'Samba' grown in peat substrate. However, the low content of sodium in the root environment has a beneficial impact on the growth of zonal pelargonium. Up to 200 mg Na·dm<sup>-3</sup> in the substrate the pelargonium retains its high decorative value. High doses of sodium chloride have a stronger negative effect on zonal pelargonium than sodium sulfate. However, no leaf necrosis was found. Both bacteria and actinomycetes are sensitive to sodicity. CFU of these microorganisms in the media with  $\text{Na}_2\text{SO}_4$  were greater than in those containing NaCl. The increased sodium contents in peat substrate causes only a slight change in CFU of fungi in the substrate.

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