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Grass vegetation dynamics in soil contaminated with salt

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To maintain safety during winter, de-icing salt (sodium chloride) is sprinkled over roads. Snow mixed with highway salt later is cleared by snowploughs and usually left on roadsides where trees are planted. On conifers, adversely affected by de-icing salt, needle shedding is accelerated.

Chloride and calcium are essential elements for plants. Conifers also use sodium in their metabolism. In greater amounts, however, especially sodium and chloride are toxic to plants. The aim of investigation is to determine which of the analyzed herbaceous vegetation species (perennial ryegrass, fescue grass, meadow-grass) shows the highest degree of resistance to toxic impact of salts (stress), to set the toxic limits of salt concentrations, and to analyze how salts impact on the core parameters of herbaceous vegetation.

Key words: herbaceous vegetation species, road maintenance salt, zeolite

INTRODUCTION

Chemical substances often used for pavement maintenance are salts (chlorides). Usage of chlorides is direct and indirect pollution of environment and has negative influence on ecosystems – soil, groundwater, surface water, vegetation and fauna (Backstrom et al., 2004; Oškinis, Kasperovičius, 2005; Storpirštytė et al., 2004; Vosylienė et al., 2006).

Soil salinity is a major abiotic stress in plant agriculture worldwide. This has led to research into salt tolerance with the aim of improving crop plants. However, salt tolerance might have much wider implications because transgenic salt tolerant plants often also tolerate other stresses including chilling, freezing, heat and drought (Zhu, 2001).

Soil salinity existed long before humans and agriculture, however, the problem has been aggravated by agricultural practices such as irrigation. Today, ~20% of the world's cultivated land and nearly half of all the irrigated lands are affected by salinity. High concentrations of salts cause ion imbalance and hyperosmotic stress in plants. As a consequence of these primary effects, secondary stresses such as oxidative damage often occur (Rhoades, Loveday, 1990).

High salt stress disrupts homeostasis in water potential and ion distribution. This disruption of homeostasis occurs at both the cellular and the whole-plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death. To achieve salt tolerance, three interconnected aspects of plant activities are important. First, damage must be prevented or alleviated. Second, homeostatic conditions must be re-established in the new, stressful environment. Third, growth must resume, albeit at a reduced rate (Zhu, 2001).

After almost a century since its first appearance in the scientific literature, Arabidopsis has now been widely adopted as a model plant of choice for biological research (Somerville, Koornneef, 2002). Its many advantages include a small genome, short life cycle, small stature, prolific seed production, and ease of transformation. In addition, a wealth of genomics resources exists, such as a completely sequenced genome, a near saturation insertion mutant collection, a genome array that contains the entire transcriptome, and more than 50,000 molecular markers. Add in a vibrant collaborative community, and it is easy to see why Arabidopsis is often the system of choice for plant research. While the culminating achievement of Arabidopsis research, the release of its complete genome sequence, is still fresh in memory, a new 10-year project of comparable importance has already begun. The ambitious goal of this program is to know the function of all Arabidopsis genes by 2010 (Somerville, Dangl, 2000). This knowledge will facilitate the development of a virtual plant, a computer model, that will use information about each gene product to simulate the growth and development of a plant under many environmental conditions. However, it has not escaped many plant biologists that another goal is to use knowledge gained from research on Arabidopsis to facilitate the understanding of biological phenomena in other plant species. One specific challenge is how to utilize information gained from Arabidopsis research to produce new commercial plant varieties. In this perspective, we reflect on the critical role Arabidopsis is playing in unravelling abiotic stress signal transduction (focusing primarily on salt, cold, and drought stress), and how these new insights on the mechanisms of tolerance towards these stresses are suggesting novel approaches to engineer the next generation of biotech crops (Zhang et al., 2004).

Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed (Flowers, Yeo, 1995). Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. These constraints are most acute in areas of the world where food distribution is problematic because of insufficient infrastructure or political instability. Water and soil management practices have facilitated agricultural production on soils marginalized by salinity, but additional gain by these approaches seems to be problematic. On the horizon are crop improvement strategies that are based on the use of molecular marker conjunction with traditional breeding efforts (Ribaut, Hoisington, 1998; Yokoi et al., 2002).

Soil salinity is one of the most significant abiotic stresses for plant agriculture. Apart from the practical goal of genetically improving the salt tolerance of crop plants, salt tolerance research represents an important part of basic plant biology, contributing to our understanding of subjects ranging from gene regulation, signal transduction to ion transport, and mineral nutrition. Research into two other major abiotic stresses, drought and cold, is closely linked with salt stress work. For example, many genes that are regulated by salt stress are also responsive to drought or cold stress. Since salt stress can be applied accurately and reproducibly, many "drought" stress studies in the laboratory use salt stress instead of actual drought. The widely known Hog pathway for osmotic stress perception and signalling in yeast was discovered by using NaCl stress (Zhu, 2000).

The aim of the research is to determine how natural zeolite (of 3–5 mm fraction) inserted in soil contaminated with road maintenance salts (technical NaCl) influences the growth process of grass vegetation (perennial ryegrass, fescue-grass, meadow-grass) when analyzing the length (cm) and phytomass (mg) of the above-ground part.

MATERIALS AND METHODS

Natural zeolite (clinoptylolite) was selected for the research due to a number of its unique structural and functional properties. Zeolites used for lawn preparation add the following characteristics to the soil: reduce soil trample, deeply aerate the soil and plant roots, improve water permeability up to the area of roots, retain the optimum amount of water (up to 35% of the zeolite mass), keep toxic substances preventing them from been discharged into the environment, considerably reduce washing out of fertilizers to the environment, thus reducing the contamination of ground waters, and, based on plants' needs, gradually convey nutritive agrochemically useful substances (Na^+, Ca^{2+}, K^+, Mg^{2+} and others) and water to them, ensure a perfect level of cation (Na^+, Ca^{2+}, K^+) exchange (0.9–1.5 g-eqv./g), and encourage the propagation of useful microorganisms. This results in the growth of stronger plants resistant to adverse environmental factors (stress). Zeolite is used in 3–5 mm fractions since this size is optimum when zeolite is used for environmental purposes (Brannvall, Kazlauskienė, 2005; Pitcher et al., 2004).

Three kinds of grass vegetation, most frequently used for planting of waysides in Lithuania, were selected: perennial ryegrass (*darimo* species) – *Lolium perenne* L.; fescue grass (*prana* species) – *Festuca pratensis* Huds.; meadow-grass (*balin* species) – *Poa pratensis* L.

Compost soil contaminated with crushed technical sodium chloride applied on the Lithuanian roads during wintertime to reduce slipperiness was used for the research. NaCl was treated with potassium ferrocyanide – potassium hexacyanoferrate ($K_4[Fe(CN)_6]$) (Baltrėnas et al., 2006).

The course of research: the seeds of perennial ryegrass, fescue-grass and meadow-grass, each were sown in plastic plant pots containing 1 kg of compost soil. Two NaCl concentrations, 2 g/kg, 5 g/kg, inserted into the soil in the form of solutions during watering, were used in research.

Two quantities of zeolite in the soil were used during this research: (1) 10% of zeolite from the soil volume was inserted in the soil; (2) 20% of zeolite from the soil volume was inserted in the soil.

For the control, each species of herbaceous vegetation was sown in pots in pure soil not contaminated with NaCl but containing 10% and 20% of zeolite from the soil volume (the total of 6 pots for each species) (Sloan, Hegemann, 2003).

The bottom of every plant pot had holes to ensure natural water run-off and prevent excess accumulation of water in soil. The seeds of each species were simultaneously seeded in the medium contaminated with sodium chloride salt and in pure medium (experimental and control plants). In this way the three mentioned species of grass vegetation were seeded into the soil and zeolite mixture in 18 plastic pots with different concentrations of salt.

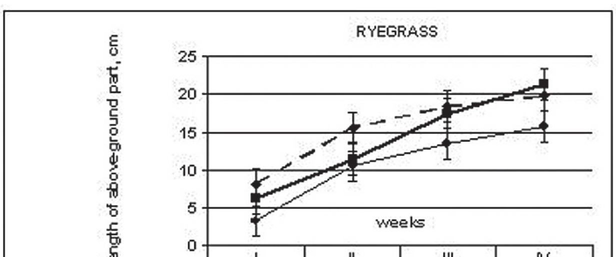
The soil of the following composition was used for the research: nitrogen ($NH_4 + NO_3$) – 100 mg/l; phosphorus (P_2O_5) – 50 mg/l; potassium (K_2O) – 300 mg/l; calcium (CaO) – 300 mg/l; magnesium (MgO) – 80 mg/l; ferrum (Fe_2O_3) – 800 mg/l. Soil for the research was acquired at a company and prepared according to ĮST 2292587–001:1999.

Both the experimental and the control plants were grown under identical conditions, i. e. at the same temperature and lighting, were watered with identical amounts of water (100 ml) and at the same time (every 5 days). The experimental plants were grown in the northern part of the laboratory without being exposed to direct sunlight, i. e. unfavourable ecological conditions were chosen (Baltrėnas et al., 2006).

RESULTS

During the research that extended for 4 weeks the biggest height of the above-ground part was achieved by the ryegrass in both the control (19.8 cm) and the experimental (21.3 cm) plant samples, and the smallest one by the meadow-grass (7.7 cm and 3.2 cm, respectively). During the research room temperature was 20 °C with insignificant changes (1 °C).

As Fig. 1 shows, the ryegrass plants of the control group that were growing in the soil not contaminated with NaCl reached



	I	II	III	IV
control (0 g/kg NaCl in soil)	8.2	15.6	18.3	19.8
2 g/kg NaCl in soil	6.2	11.4	17.5	21.3
5 g/kg NaCl in soil	3.2	10.5	13.4	15.7

Fig. 1. The length of the perennial ryegrass above-ground part in soil contaminated with NaCl and containing *10% of zeolite*

the highest growth during the 1st week. The biggest difference in the heights of the above-ground parts of the plants that were growing in soil contaminated with 2 g/kg and 5 g/kg of NaCl was determined after 4 weeks of growth – 5.6 cm, and the smallest one after 2 weeks – 0.9 cm. The bigger height of the above-ground part of the control group plants is especially obvious in the 1st and 2nd weeks. But in the 3rd week of research a difference between the control and experimental plants that were growing in soil contaminated with 2 g/kg of NaCl was only 0.8 cm. In the 4th week, the height of plants that were growing in soil contaminated with 2 g/kg of NaCl with 10% of zeolite inserted was by 1.5 cm bigger than that of control plants.

As Fig. 2 shows, during the first 2 weeks the plants that grew in soil containing 2 g/kg NaCl were higher than the control plants, and during the 3rd and 4th weeks they were only by 1.0 cm and 1.3 cm lower than the control ones. The biggest difference in above-ground parts heights of the plants that grew in soil contaminated with 2 g/kg and 5 g/kg NaCl was in both the 1st (4.9 cm) and the 2nd (5.6 cm) weeks.

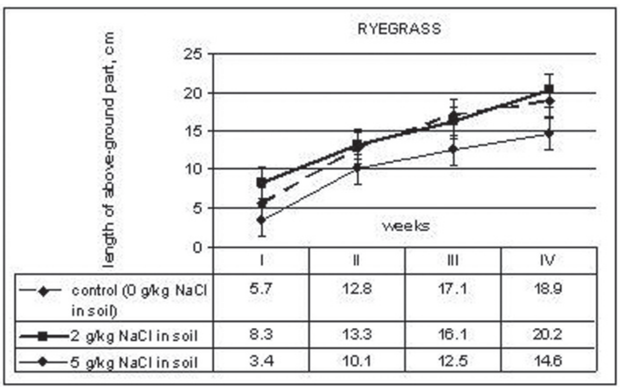


Fig. 2. The length of the perennial ryegrass above-ground part in soil contaminated with NaCl and containing *20% of zeolite*

The obtained findings show that the height of the rye-grass plants was bigger in the experimental plants that grew in soil with 10% zeolite content. Even though in the second case the same concentrations of NaCl in the soil were preserved (2 g/kg and 5 g/kg), and more zeolite, even 20 % from the soil volume, was inserted, this reduced the above-ground height even of the control plants, in particular in the 1st and 2nd weeks. Compared to control plants in soil with 20% zeolite content, the control plants in soil having 10% zeolite content were by 2.5 cm, 2.8 cm, 1.2 cm and 1.9 cm higher according to weeks, respectively.

The meadow-grass showed the worst growth compared to the growth rate of ryegrass and fescue grass. Even the control plants did not germinate during the 1st week (Fig. 3). Nevertheless, due to the properties of zeolite, the height of the above-ground part of the meadow-grass plants in soil contaminated with 2 g/kg NaCl content reached 0.4 cm during the 1st week. During the period of four weeks the difference in heights of the above-ground parts of plants that grew in soil with 2 g/kg and 5 g/kg NaCl content was 0.4 cm, 1.3 cm, 1.3 cm and 0.8 cm, respectively. The control plants, on average, were by around 2.0 cm higher than those that grew in soil containing 2 g/kg of NaCl. The biggest difference in heights of the above-ground parts of control plants and those that grew in soil contaminated with 5 g/kg NaCl was determined in the 3rd week of growth and amounted to 3.5 cm.

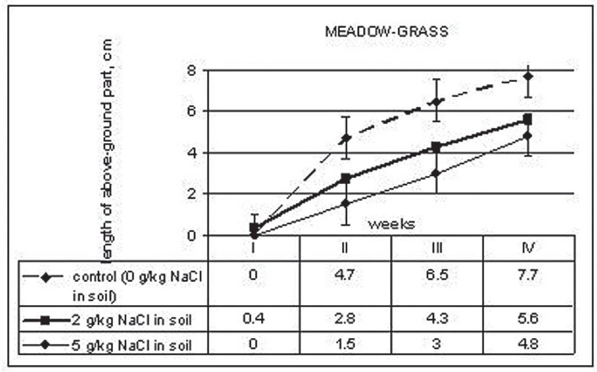


Fig. 3. The length of the meadow-grass above-ground part in soil contaminated with NaCl and containing *10% of zeolite*

Fig. 4 presenting the findings of research into plants having grown in soil with 20% zeolite content shows the situation analogous to the one when 10% of zeolite was inserted in soil, because the meadow-grass plants germinated during the 1st week only in soil contaminated with 2 g/kg of NaCl and were 0.3 cm high. The biggest difference in heights of the above-ground parts of plants that grew in soil contaminated with NaCl was on the fourth week and reached 2.1 cm. From week 2 to week 4, the meadow-grass plants in soil with 20% zeolite content were by 0.9 cm, 1.4 cm and 1.2 cm lower, respectively, than those in soil with 10% zeolite content.

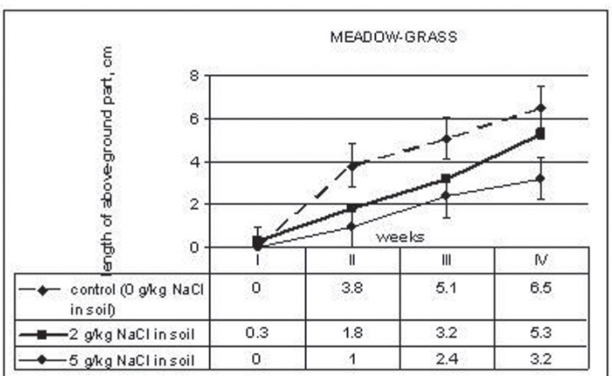


Fig. 4. The length of the meadow-grass above-ground part in soil contaminated with NaCl and containing *20% of zeolite*

The same situation was determined in experimental ryegrass plants, too (Figs. 1, 2).

Zeolite inserted into soil made the greatest influence on the change in length of fescue-grass above-ground parts (Fig. 5)

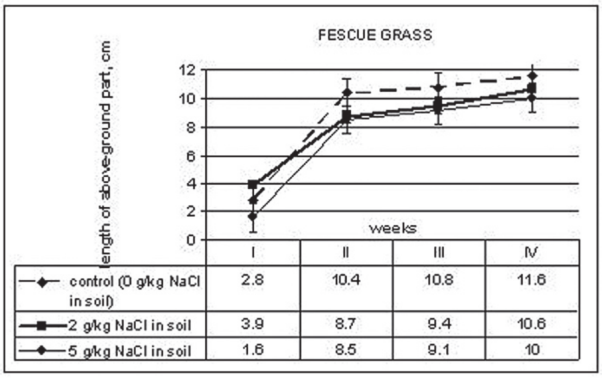


Fig. 5. The length of the fescue grass above-ground part in soil contaminated with NaCl and containing *10% of zeolite*

since the height of the above-ground part of control fescue-grass plants and those that grew in soil contaminated with NaCl differed insignificantly in the course of all the four weeks. The biggest difference in lengths was recorded in the second week of growth. Like in the case of meadow-grass plants, during the 1st week the biggest length of the above-ground part was in fescue-grass plants that grew in soil with 2 g/kg NaCl content, i. e. 3.9 cm, while that of control plants was 2.8 cm.

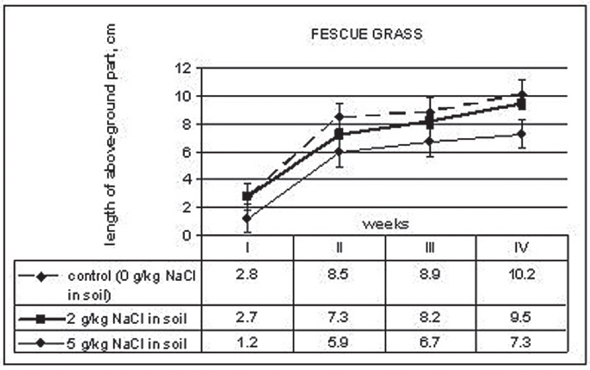


Fig. 6. The length of the fescue grass above-ground part in soil contaminated with NaCl and containing *20% of zeolite*

During this research, the length of the fescue-grass control plants and fescue-grass plants in soil contaminated with 2 g/kg NaCl differed insignificantly. Bigger differences in lengths were observed between control plants and the plants in soil contaminated with 5 g/kg –1.6 cm, 2.6 cm, 2.2 cm and 2.9 cm, respectively. Control fescue-grass plants in soil with 10% zeolite content had bigger lengths of the above-ground part.

Throughout the research period (4 weeks) the phytomass of perennial ryegrass having grown in soil with 10% zeolite content was changing consistently, the control plants had the biggest phytomass, while the plants in soil contaminated with 5 g/kg NaCl, the smallest one. The biggest difference in phytomass of control (35.37 mg) and experimental (23.82 mg and 20.67 mg) plants was recorded in the 2nd week of the experiment.

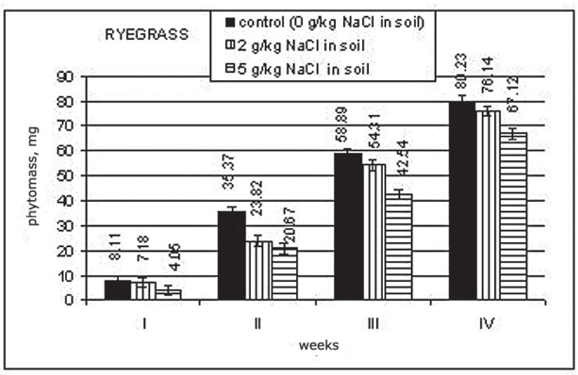


Fig. 7. The phytomass of perennial ryegrass after 4 weeks of growth in soil contaminated with NaCl and containing *10% of zeolite*

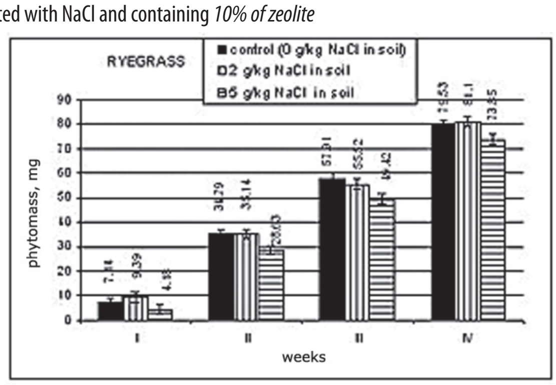


Fig. 8. The phytomass of perennial ryegrass after 4 weeks of growth in soil contaminated with NaCl and containing *20% of zeolite*

As Fig. 8 shows, the biggest phytomass of ryegrass was in the researched plants that grew in soil contaminated with 2 g/kg NaCl and containing 20% of zeolite but not in the control plants. The biggest difference (2.25 mg) between control (7.14 mg) and researched (9.39 mg) plants was determined during the 1st week of growth when the effect of zeolite was the strongest.

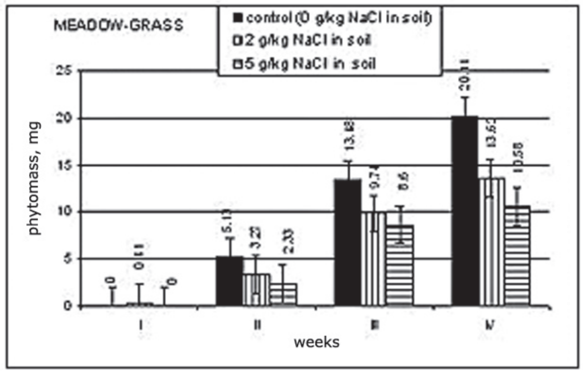


Fig. 9. The phytomass of meadow-grass after 4 weeks of growth in soil contaminated with NaCl and containing *10% of zeolite*

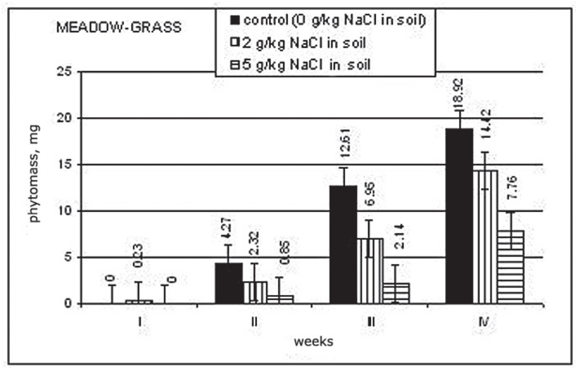


Fig. 10. The phytomass of meadow-grass after 4 weeks of growth in soil contaminated with NaCl and containing *20% of zeolite*

Due to the toxic effect of NaCl on the meadow-grass, during the 1st week of research the experimental plants germinated only in soils contaminated with 2 g/kg NaCl containing both 10% and 20% of zeolite. The control plants did not germinate. Zeolite absorbed Na^+ from NaCl and Ca^{2+}, K^+, Mg^{2+} cations from the soil in this way improving soil quality and reducing the toxic effect of NaCl. But the influence of zeolite on the growth movement of meadow-grass plants was observed only in soil contaminated with 2 g/kg NaCl.

Compared to the ryegrass and fescue grass, the phytomass of meadow-grass plants was the smallest within the entire period of growth.

The biggest increase in fescue-grass phytomass was determined after the 2nd week of growth (Figs. 11, 12). Concerning soil contaminated with 2 g/kg NaCl, in the 1st week of research the biggest increase in phytomass (2.53 mg) was determined in researched fescue-grass plants that grew in soil with 10% zeolite content. In the second week of growth the difference in phytomass of researched plants in soils contaminated with 2 g/kg NaCl

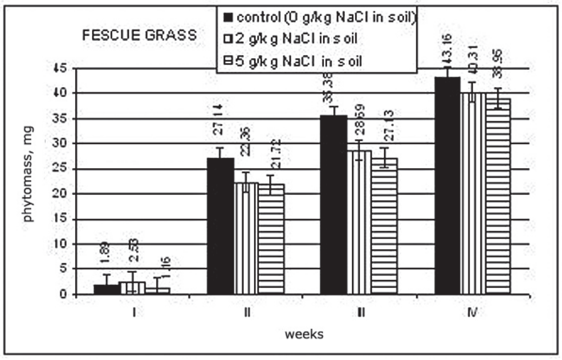


Fig. 11. The phytomass of fescue grass after 4 weeks of growth in soil contaminated with NaCl and containing *10% of zeolite*

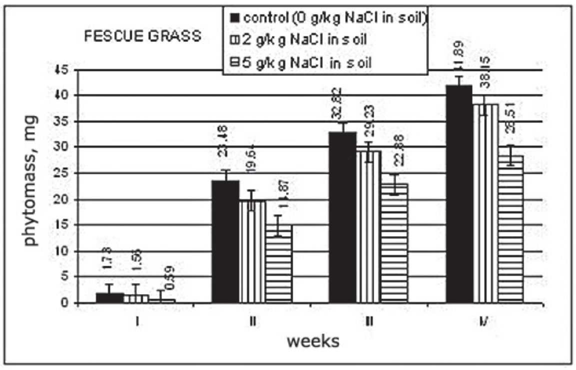


Fig. 12. The phytomass of fescue grass after 4 weeks of growth in soil contaminated with NaCl and containing *20% of zeolite*

and 5 g/kg NaCl with 10% zeolite content was only 0.64 mg. The phytomass of the researched plants in soil contaminated with NaCl and containing 20% of zeolite was by ~10% smaller than that of plants in soil contaminated with NaCl and containing 10% of zeolite.

CONCLUSIONS

1. Three researched species of grass vegetation were seeded in soils contaminated with 2 g/kg and 5 g/kg technical sodium chloride (NaCl) with inserted content of zeolite of 10% or 20% from the soil volume. During the 1st week of research, the control and experimental ryegrass and fescue grass plants as well as the researched plants of meadow-grass germinated in soils with NaCl contamination concentration of 2 g/kg NaCl with 10% and 20% zeolite contents.

2. The research findings show that the technical NaCl contained in soil has an adverse effect on the growth dynamics of grass vegetation i.e. slows down the increase of the above-ground part and reduces the phytomass.

3. The experiments have proved that it is the perennial ryegrass that shows the most rapid germination, the most rapid growth and the highest resistance to the toxic effect of salt.

4. Zeolite retains humidity in soil which was visually seen during the experiments.

Zeolite inserted into soil made the greatest influence on the change of the above-ground part and phytomass of the fescue grass. The growth process of the researched grass plants was to the greatest extent influenced by zeolite during the 1st week of growth.

5. The biggest above-ground parts as well as the biggest phytomass of all the three researched species of grass vegetation (perennial ryegrass (*Lolium perenne* L.), fescue grass (*Festuca pratensis* Huds.) and meadow-grass (*Poa pratensis* L.)) were determined in those pots where 10% of zeolite was inserted.

6. It is not rational to insert more than 10% of zeolite into soil since the researched plants that grew in soils with 20% zeolite content had by approximately 10% smaller lengths of the above-ground parts as well as phytomass than the grass plants grown in soil with 10% of zeolite.

7. It is rational to insert zeolite in soil when installing new or renewing existing road trenches for the preservation, stabilization and re-cultivation of soil in the areas of roads, and for the encouragement of growth of the plants more resistant to the adverse environmental factors (stresses).

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ŽOLINĖS AUGALIJOS AUGIMO DRUSKA UŽTERŠTAME DIRVOŽEMYJE DINAMIKA

Santrauka

Augalai aplinkoje patiria daugelį stresų: dėl druskų, sausros, sunkiųjų metalų, šalčio. Pasaulyje atliekami kelių priežiūrai naudojamų druskų poveikio įvairių rūšių augalams natūriniai ir laboratoriniai tyrimai. Lietuvoje tokio pobūdžio tyrimai kol kas tėra pavieniai. Straipsnyje pateikti Lietuvoje kelių priežiūrai daugiausia naudojamos druskos – natrio chlorido (NaCl) toksinio poveikio žolinei augalijai eksperimentinių tyrimų rezultatai. Tyrimai buvo atliekami laboratorinėmis sąlygomis. Tyrimams pasirinktos trys žolinės augalijos rūšys, kuriomis dažniausiai želdomos pakelės: daugiametė svidrė (*Lolium perenne* L.), tikrasis eraičinas (*Festuca pratensis* Huds.), pievinė miglė (*Poa pratensis* L.). Tyrimų metu nustatyta, kad NaCl, patekęs į dirvožemį, turi neigiamos įtakos žolinės augalijos augimo procesui, mažina jos antžeminės dalies ir fitomasės prieaugį. Eksperimento metu nustatyta, kad atspariausia natrio chlorido toksiniam poveikiui yra daugiametė svidrė. Į dirvožemį jo funkcinėms savybėms pagerinti ir atsparesnei neigiamiems aplinkos veiksniams (stresams) žolinei augalijai augti tikslinga įterpti ceolito ne daugiau nei 10% nuo dirvožemio tūrio.

Raktažodžiai: žolinės augalijos rūšys, kelių priežiūros druska, ceolitas