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Effect of zeolite, clay and peat on salt stress tolerance of lettuce (*Lactuca sativa* L.)

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Abstract. The present study aimed to investigate the effects of natural zeolite, clay and peat amendments on the growth and NaCl absorptions of lettuce (*Lactuca sativa* L.) under gradually increasing salinity. Four different growing media based on quartz sands with 10% additions of zeolite, clay and peat were tested. The worst effect of NaCl on plant biomass was evident at the highest salinity levels for zeolite applications. While adverse salinity impact on leaf and root biomass was least pronounced in treatments with peat additives. As expected, the lowest Na concentrations in plant tissues were found in the growing media supplemented with zeolite. In the case of Cl, however, it was the opposite - lettuce leaves accumulated significantly higher chloride concentrations in the zeolite variants in salinity treatments above 20 mM NaCl. In the control, clay and peat treatments, as the substrate salinity increased, the Cl level in the plant increased similarly. Adverse changes in leaf chlorophyll concentration (SPAD) and photosynthetic rate (μmol m^{-2} s^{-1}) parameter appeared under salinity concentrations above 20 mM and were more pronounced in zeolite and sand substrate. According to the obtained results, peat additives can effectively mitigate the harmful effects of excessive salts by binding and immobilizing them as well as improving the water-holding capacity and nutrient availability. The study also concluded that natural zeolite successfully immobilizes cationic sodium, but the harmful effect of chlorine significantly reduced plant growth and photosynthetic performance. Clay additives to the growth medium showed the potential to reduce the adverse effects of salinity on lettuce, however, under the experimental conditions implemented, the effect was small.

Key words: sodium chloride, pot experiment, organic matter, plant biomass, photosynthesis.

INTRODUCTION

Elevated salinity of soil and irrigation water is a major and increasing problem for agricultural production in the world, especially in the arid and semi- arid regions where lives more than 75% of the world's population (Singh, 2015). It is estimated that up to 20% of arable and 50% of the irrigated land worldwide is salt-affected (Ruan et al., 2010) with estimated economic impacts as high as \$300 ha^{-1} resulting in the annual loss of 27.3 billion USD in global income (Munns & Gilliham, 2015; Elmeknassi et al., 2024). In general, the soil is considered to be saline and inhibits water uptake by plants if the electric conductivity - EC (available salts in the soil pore water) is over 400 mS m^{-1}

(equal to ~ 40 mM NaCl). While various salt compounds are present in the soil solution, sodium chloride is the most predominant one (Evelin et al., 2019). It is well known that soil salinization adversely affects the environment, agroecosystems, agricultural productivity, plant yield quality and sustainability. Many investigations have demonstrated the harmful effect of salinity on plants. Thus, cellular water deficit, inhibited membrane functions, ion toxicity, nutrient deficiencies, decreased chlorophyll concentration and the activity of several enzymes and oxidative stress can lead to disturbance of various metabolic processes and growth inhibition, molecular damage, and even plant death (Balasubramaniam et al., 2023). Additionally, osmotic stress from elevated salinity harms the germination of many plant species (Atta et al., 2023). High salt concentrations decline soil porosity and structure, soil aeration and water conductance. In addition, elevated Na^+ ion concentrations interfere with K, Ca and Mg decreasing the availability of these ions to plant uptake, as well as adversely affect the availability of Fe, Mn, Zn and Cu. The degree of salt tolerance greatly varies among plant species and varieties, also at different developmental stages (Munns & Tester, 2008).

Lettuce (*Lactuca sativa* L.), belonging to the *Asteraceae* family, is one of the main leafy vegetables produced and consumed in the world (Medina-Lozano et al., 2021). In general, lettuce is considered as a moderate salt-tolerant crop (Fernández et al., 2016) or even sensitive according to other authors (Shannon & Grieve, 1999; Xu & Mou, 2015). Previous researches show that salinity levels, in terms of EC, above 2.7 dS m^{-1} reduce lettuce growth and yield (De Pascale & Barbieri, 1995). The salinity threshold value for lettuce in different research was determined between 1.1 and 1.3 dS m^{-1} and the relative yield decrease after reaching this threshold was 9.3% to 13% (Shannon & Grieve, 1999; Ünlükara et al., 2008). However, the results of a field trial in Israel demonstrated that the yield and quality of iceberg lettuce were not significantly affected at 4.4 dS m^{-1} irrigation water salinity (Pasternak et al., 1986). Although it is difficult to determine why there are such differences in the reported results other authors point out that the experiment in Israel did not include a sufficient number of experimental treatments as well as soil in the experiment was gypsiferous and salinity effects can be partially offset by over-irrigation (Russo, 1987; Shannon & Grieve, 1999). However, there is clear evidence that indicates large differences in salt tolerance among different varieties of lettuce.

The results of studies with zeolite confirm it as a suitable material for the reduction of salinity. As reported, 15% addition of zeolite reduced salinity impact on plants by 42% (Koushafar et al., 2011). As zeolites are characterized by a high water and cation exchange ability it makes them potentially useful in the field of crop production on saline soils (Cataldo et al., 2021). On the other hand, the addition of natural zeolite can cause excessive adsorption and lead to the immobilization of essential plant nutrients with an adverse effect on plant growth. Abdelrazek (2018) reported that the addition of peat moss to saline calcareous soil improved tomato growth indices. Several other studies report that the high content of organic matter and tremendous adsorptive interfaces confirm peat as a very effective sorbent for the immobilization or removal of heavy metals and NaCl (Pelinsom Marques et al., 2020; Ondrasek et al., 2021). As an incorporation of clay into growing media positively influences the water retention characteristics of the soil (Jean-Charles, 2009) such a phenomenon could mitigate the salinity effects on plants. Additions of zeolite, peat, or clay have the potential to reduce the adverse effects of salinity on plant growth (Table 1) although their use can be limited by material cost and availability.

Table 1. The role of natural zeolite, peat and clay as soil amendments for mitigating the negative impacts of soil salinity (Jean-Charles, 2009; Pelinsom Marques et al., 2020; Cataldo et al., 2021)

	Ion exchange capacity		Absorption of toxic ions	Improving soil structure	Water retention	pH stabilization	Enhanced nutrient availability
	cations	anions					
Natural zeolite	×		×	×	×	×	×
Peat	×		×	×	×	×	×
Clay	×		×				

With the massive increase of intensive agriculture farming systems today, vegetable crop cultivation requires a high input of fertilizers and irrigation water, which can eventually lead to the elevation of soil salinity. Therefore, the importance of research on different management practices aimed at the prevention of soil and water salinization and mitigation of salinity effects on plant growth is critical.

The objective of our research was to determine the effects of salinity on lettuce growth and how management practices - addition of zeolite, clay and peat to soil media can mitigate the adverse effects of salinity on the growth and photosynthetic (SPAD, photosynthetic rate, stomatal conductance) parameters of lettuce.

MATERIALS AND METHODS

Lettuce (*Latuca sativa* L., variety 'Grand Rapids') was used as model culture. Commercial lettuce seeds were sown into 1L round polyethylene pots containing quartz sands and a mixture of quartz sands (Ltd. Saulkalne S, Saulkalne, Latvia) with 10% of studied amendments (zeolite (3–5 mm), clay and peat moss). Three pots for each treatment and three plants per pot under controlled growth conditions (photoperiod 16 h, photon flux density 250 μmol m^{-2} s^{-1} supplied by fluorescent tubes, day/night temperature 20/18 °C, relative air humidity 60–65%) were used. Individual pots were randomly located (Table 2). Substrate water content was maintained at not less than 60% of full water-holding capacity. Moisture level throughout the experiment was maintained by weighing each pot and adding deionized water. Optimal concentrations of all macro and micronutrients (mg L^{-1}): 120 N, 60 P, 150 K, 800 Ca, 50 Mg, 50 S, 30 Fe, 0.5 Cu, 1.5 Mn, 0.02 Mo, 0.2 B in the substrate were provided in each pot at the beginning of the experiment. The impact of salinity on plant growth indices was studied at four levels of salinity - 0, 10, 20, 30, 40 mM NaCl. Salt stress was imposed by gradually adding NaCl to irrigation water over 7 days until the final concentration was reached.

Table 2. Randomized complete block design used in experimental pot layout (three plants per pot)

P10	C30	S0	S10	S0	Z20	S20	Z0	P40	Z10
S40	S30	S30	Z40	Z10	P20	S40	P30	Z0	P0
Z20	C40	Z30	P20	Z30	C0	C0	S10	P10	C40
P40	C0	P0	C20	Z40	P0	C10	P30	C30	C20
S30	C10	S10	S20	S20	P10	Z20	C10	S0	S40
Z10	C30	Z30	Z40	P40	P20	C40	C20	P30	Z0

S – sand; Z – zeolite; P – peat; C – clay. 0, 10, 20, 30, 40 – salinity levels (mM NaCl).

The experiment was terminated and measurements were made 30 days after the start when visual signs of damage to plants were detected. A portable chlorophyll meter SPAD 502 (Minolta, Warrington, UK) was used for non-destructive chlorophyll determination. Photosynthetic characteristics: photosynthetic rate (A, $\mu mol\ m^{-2}\ s^{-1}$), stomatal conductance (gS, $mol\ m^{-2}\ s^{-1}$), were measured with ADC portable LCi Ultra Compact Photosynthesis system (ADC BioScientific Ltd., UK) on a fully developed young leaf. The fresh mass of plant leaves and roots was estimated immediately after harvesting, and the dry mass was determined after drying (+60 °C for 24 h).

The dry plant samples were dry mineralized with HNO_3 vapor and re-dissolved in HCl (3:100). The level of Na was estimated by microwave plasma atomic emission spectrometry (4200 MP-AES, Agilent, Santa Clara, CA, USA) according to manufacturer's instructions and Cl content was determined by $AgNO_3$ titration (Osvalde, 2011).

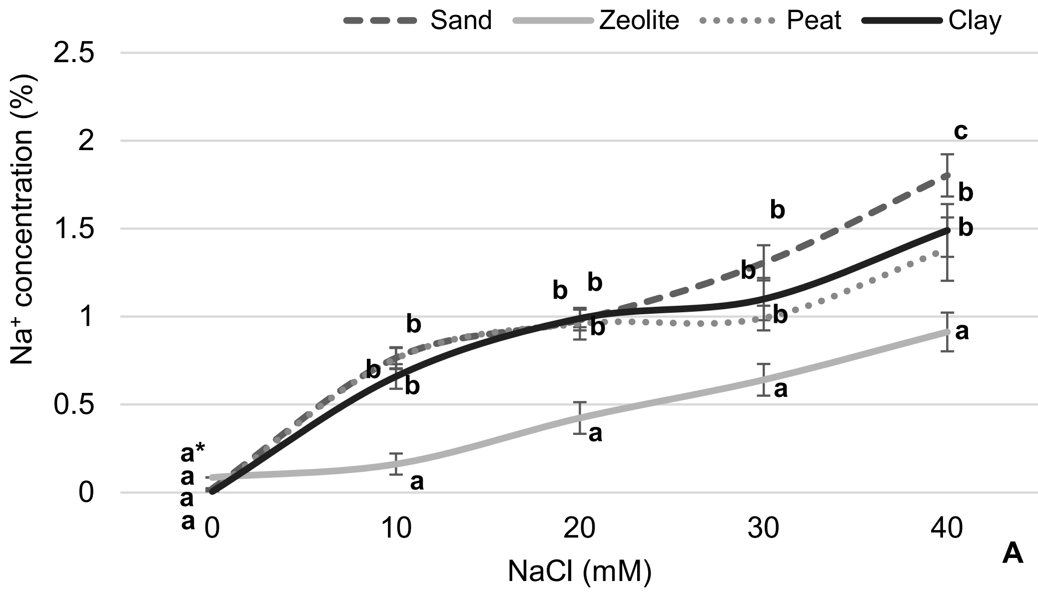
The statistical analysis of the results was carried out using MS Excel 2016 software. Standard errors (SE) were calculated to reflect the mean results of analyzed parameters. T-test 'Two-Sample Assuming Equal Variances' at $p < 0.05$ was used for testing the effect of different amendments on plant growth under salinity stress.

RESULTS AND DISCUSSION

Impact of NaCl on Na^+ and Cl^- accumulation in plant tissues

In general, Na and Cl ion concentrations drastically increased in the leaves of lettuce cultivated in the presence of increasing substrate salinity (Fig. 1). While tissue Na and Cl concentrations increased with increasing substrate salinity, the character of the response was significantly affected by the additives used.

The most pronounced increase in leaf Na concentration at a moderate to high external salinity level was detected for quartz sand substrate with the maximum concentration - 1.803% Na^+. The lowest Na concentrations in lettuce at all salinity levels were found for zeolite reaching 0.931% Na^+. Conversely, while Cl^- levels under rising salinity in treatments with sand, peat and clay increased similarly, in a case with zeolite lettuce leaves accumulated significantly higher concentrations in salinity treatments above 20 mM NaCl. Such a phenomenon can be explained by the natural zeolite's negatively charged framework with ability to effectively attract cations. Natural zeolites can also be suitable for binding anionic contaminants, such as chlorine ions, but only after surface modification by reversing their surface charge using appropriate charge-altering compounds (Senila & Cadar, 2024). Thus, our study clearly shows that natural zeolite are not an effective remedy for anion absorption due to its negative charge. However, it is undeniably a suitable material for reducing the impact of Na pollution on plants. In general, organic matter (peat) and clay have several properties with the potential to improve the structure of the soil and plant growth in saline environments, including improvement of the soil cation exchange capacity (CEC), which helps bind potentially harmful sodium ions. Our study demonstrated the significant effect of peat and clay additives on the uptake of Na in lettuce leaves when NaCl concentration in the substrate exceeds 30 mM. Although, peat amendments can serve as a sorbent for anion contaminants which is primarily attributed to its rich content of humic substances and various functional groups (carboxyl and phenolic) (Yuliani et al., 2022). As well, several studies have indicated that the presence of clays in soil enhances the sorption of anionic pollutants, but such properties are dependent on clay type and its specific physicochemical properties (Wu et al., 2022). However, our findings demonstrate insignificant peat and clay effects on chlorine ion uptake by lettuce plants (Fig. 1, B).



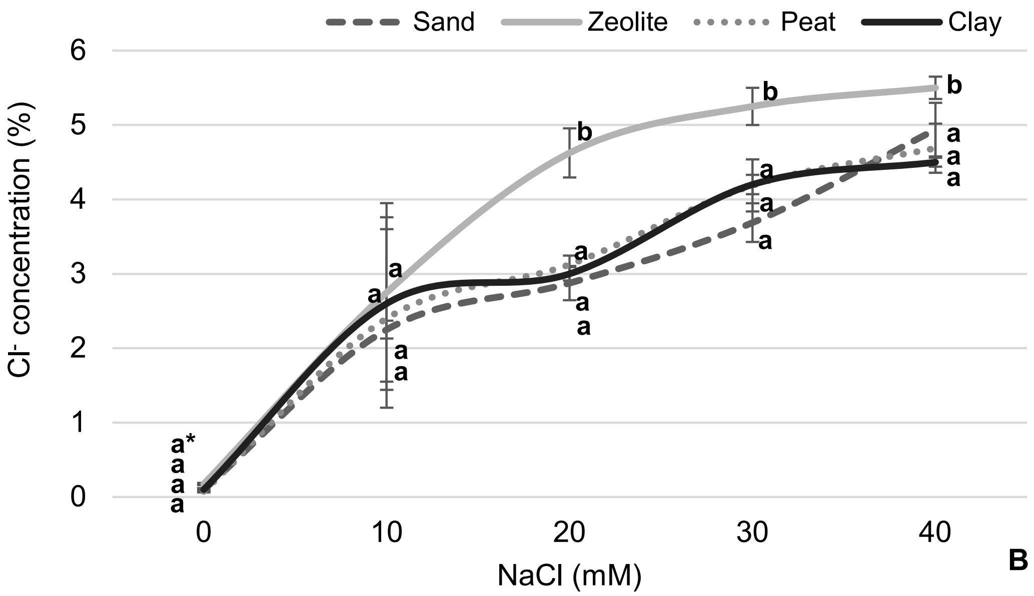


Figure 1. Accumulation of Na^+ (A) and Cl^- (B) in leaves of Latuca sativa dependent on different substrate additives (sand, zeolite, peat and clay) under increasing salinity.

*Means with different letters for each NaCl treatment level were significantly different (t-Test, $p < 0.05$).

Impact of NaCl on plant fresh and dry mass of leaves and roots

It is widely documented that elevated soil salinity affects almost all aspects of plant growth and development, including vegetative growth, photosynthesis, reproductive development and germination (Chinnusamy et al., 2006). As established in other research, lettuce is considered as relatively sensitive to elevated soil salinity, which considerably reduces its growth parameters, including root and shoots fresh and dry weight (Cheruth et al., 2016; Adhikari et al., 2019; Zhang et al., 2021). Also in our experiment reduction in fresh and dry weight of lettuce shoots and roots was observed under increasing NaCl concentrations in all substrates (Fig. 2; Fig. 3).

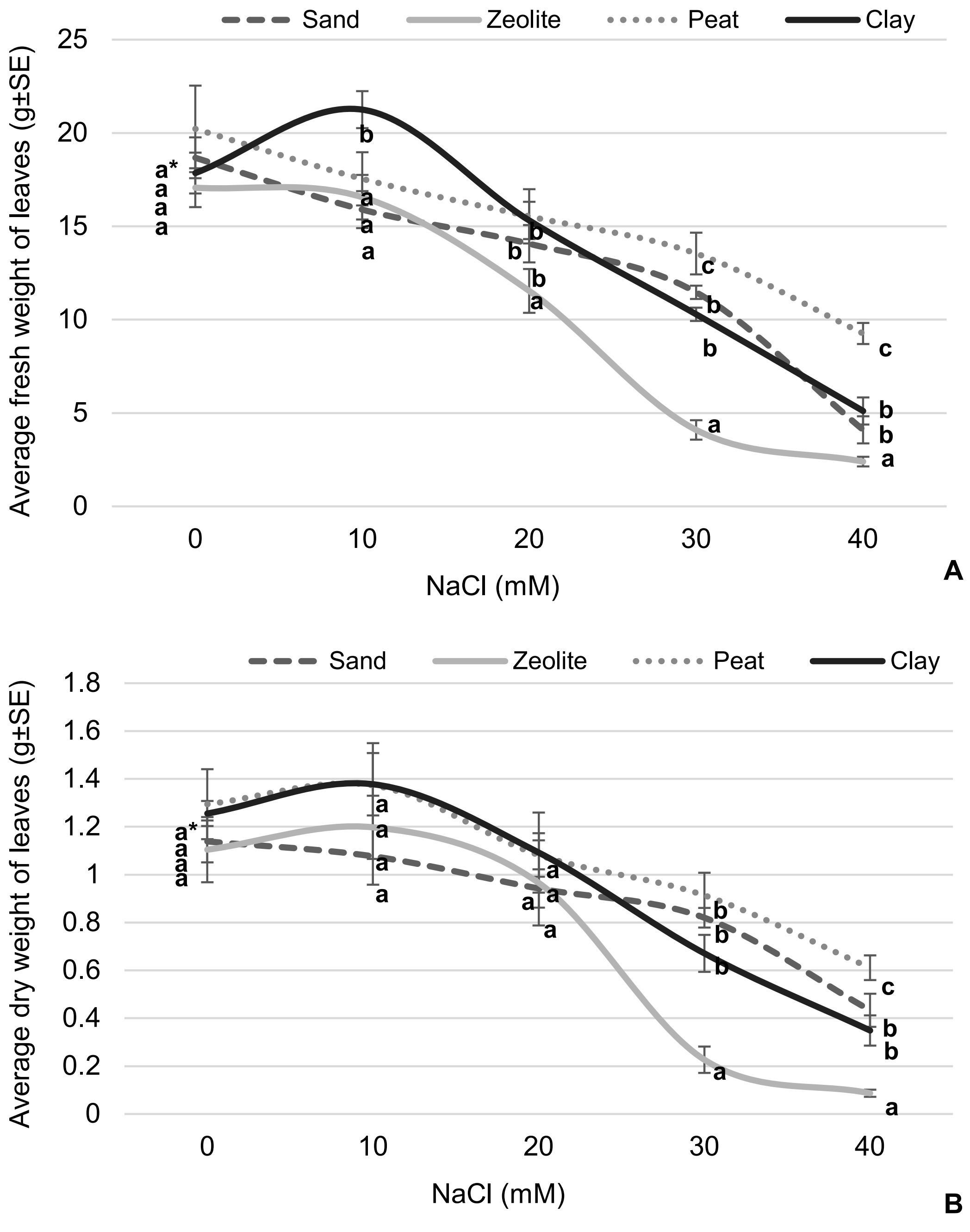
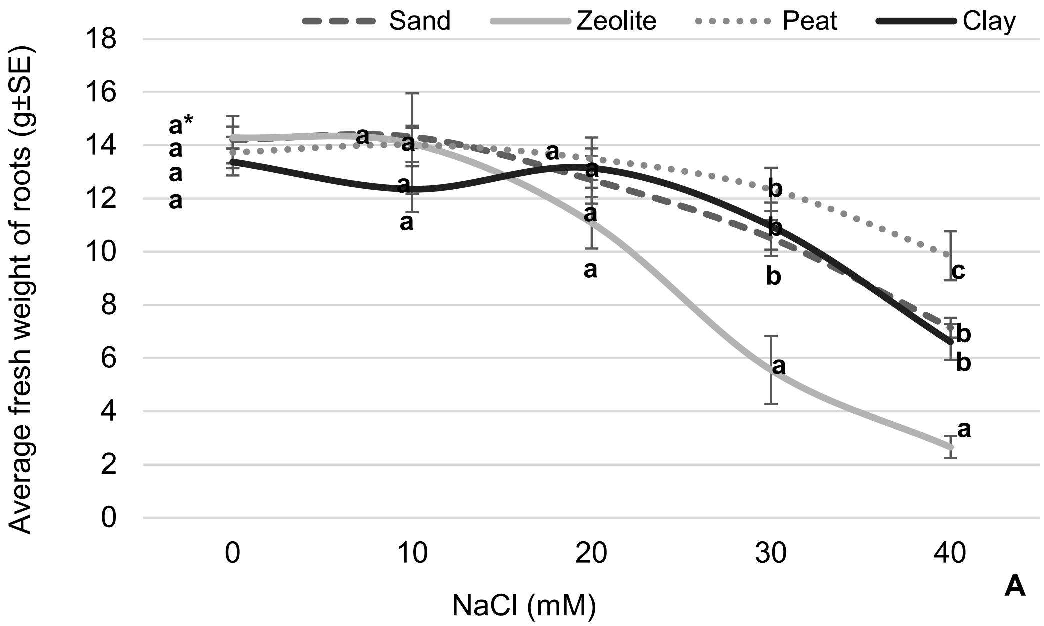


Figure 2. The effects of elevated salt (NaCl) levels on fresh (A) and dry (B) leaf mass of lettuce cultivated in substrates with different amendments.

*Means with different letters for each NaCl treatment level were significantly different (*t*-Test, $p < 0.05$).



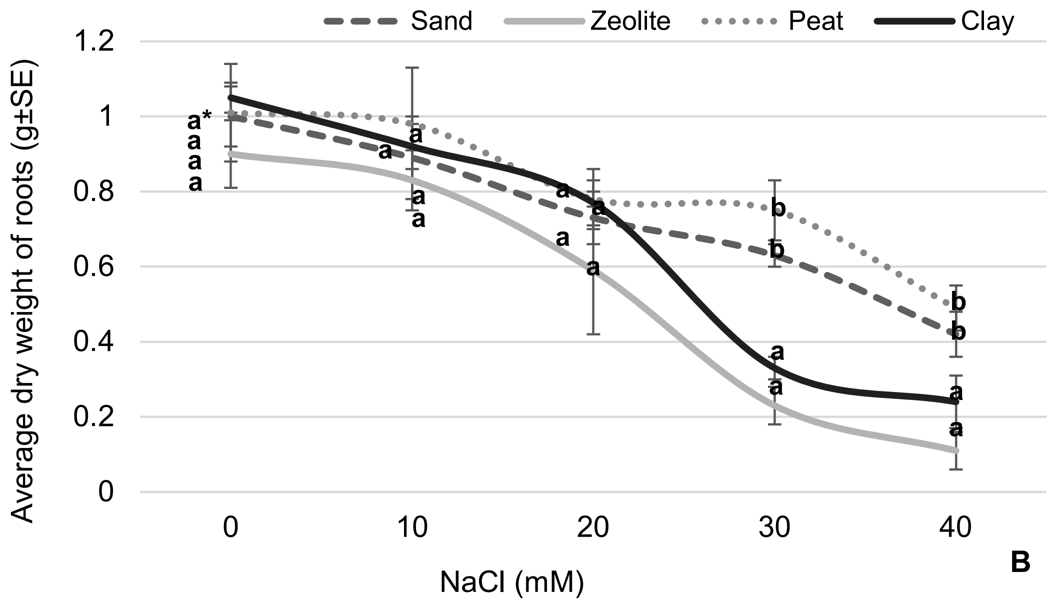


Figure 3. The effects of elevated salt (NaCl) levels on fresh (A) and dry (B) root mass of lettuce cultivated in substrates with different amendments.

*Means with different letters for each NaCl treatment level were significantly different (*t*-Test, $p < 0.05$).

While in control (0 mM NaCl) plant shoot and root fresh weight was without significant differences among different substrates, gradual reduction of studied parameters was observable above 20 mM of salinity. The highest shoot fresh and dry weight (9.26 ± 0.56 g and 0.72 ± 0.08 g) under 40 mM salinity was found in the substrate with peat additive. While the lowest fresh weight of shoots and roots was recorded in a substrate with zeolite. Moreover, measured parameters were even lower than those obtained in quartz sands. Such results are consistent with a study made by Babaousmail et al. (2022) where zeolite treatment positively influenced the fresh weight of lettuce only at low salinity concentrations. On the other hand, there are several studies where natural zeolite application mitigated the adverse effects of salinity stress on other plant species: barley (*Hordeum vulgare*) (Al-Busaidi et al., 2008), canola (Brassica napus) (Bybordi, 2016) and ryegrass (*Lolium perenne*) (Rahimi et al., 2021). It should be noted that experiments with barley and canola were conducted in open field conditions where harmful Cl ions as anions may be exposed to additional leaching. While studies with ryegrass were carried out as pot experiment in a greenhouse, chlorine concentrations were not measured in substrate or plant material. Apparently harmful effect of higher levels of chlorine in zeolite-containing substrate depressed its positive effects on sodium ion binding capabilities. As reported in other studies Cl^- ion is more toxic to plants than Na^+ (Li et al., 2017). The vast majority of the research dedicated to salinity issues has been carried out in the presence of NaCl salt, but very scarce information is available using individual salts containing Na^+ or Cl^-.

The addition of peat (organic matter) is considered as effective method in reducing the damage of salinity to plants. Organic matter improves water holding capacity, soil aeration and promotes the secretions of organic acids from roots that regulate soil pH and reduce the adverse impact of salinity. Besides, additional organic matter decomposed by soil microorganisms remarkably contributes to nutrient availability in the soil (Ondrasek et al., 2021). Our study confirms the beneficial effect of peat on plant growth parameters under increasing salinity, especially in salinity levels above 30 mM.

In general, the salinity effect on lettuce root fresh and dry weight had a similar pattern as in the case with leaves (Fig. 3). However, there was one exception. Although clay additives in the substrate under saline conditions gave similar trends in terms of leaf and root fresh weight, root dry weight was even lower than that obtained in the stand-alone quartz sand substrate.

Table 3. Effect of salinity on dry and fresh weight ratio of lettuce leaves (DW/FW)

	0 mM	10 mM	20 mM	30 mM	40 mM
Sand	0.061±0.006**a***	0.068±0.003**a**	0.067±0.004**a**	0.072±0.002**a**	0.106±0.007**b**
Zeolite	0.065±0.003**a**	0.072±0.007**a**	0.084±0.006**a**	0.089±0.004**b**	0.14±0.009**b**
Peat	0.064±0.007**a**	0.078±0.008**a**	0.07±0.004**a**	0.067±0.009**a**	0.078±0.010**a**
Clay	0.07±0.004**a**	0.065±0.009**a**	0.071±0.009**a**	0.078±0.003**a**	0.105±0.008**b**

*Means with different letters in a row were significantly different from control (0 mM NaCl) (*t*-Test, $p < 0.05$).

As reported before in experiments with lettuce, fresh weight of plant leaves was more sensitive to salinity than dry weight (Shannon et al., 1983; Pérez-López et al., 2013; Xu & Mou, 2015). Similarly, our results demonstrate a significant reduction in leaf water content under the highest salinity (40 mM) in treatments with all substrate additives except peat (Table 3). Such results underline the organic matter's positive effect on soil moisture retention and improved water availability to plants under salinity stress. Our results are consistent with the findings of Suvendran et al. (2024) and Mahmoodabadi et al. (2011) where in experiments with pea and soybean organic matter additions significantly improved plant dry weight under salinity stress.

Effect of NaCl on plant photosynthetic parameters

It is well-documented that abiotic stress caused by elevated soil salinity adversely affects the photosynthesis process in non-halophytic plants (Chaves et al., 2009). The leaf chlorophyll content serves as a key indicator of the plant's photosynthetic capacity. Chlorine ions have been traditionally considered harmful to plant growth. However, Cl^- ion levels below the toxicity threshold can be beneficial for higher plant growth under water-sufficient conditions, with physiological functions that upgrade cell water balance (Franco-Navarro et al., 2021). Our experiment also demonstrated a slight improvement in chlorophyll concentration at 10 mM salinity in all substrates where additives were used (Fig. 4). Thereby, it can be concluded, that in low salinity, clay, peat and zeolite amendments ensured sufficient water availability. Moreover, in clay and peat-containing substrates a significant decline in chlorophyll content was detected only in the highest salinity treatment. While in pure sand and zeolite-containing substrates a significant decrease in chlorophyll content was found already above 10 mM salinity.

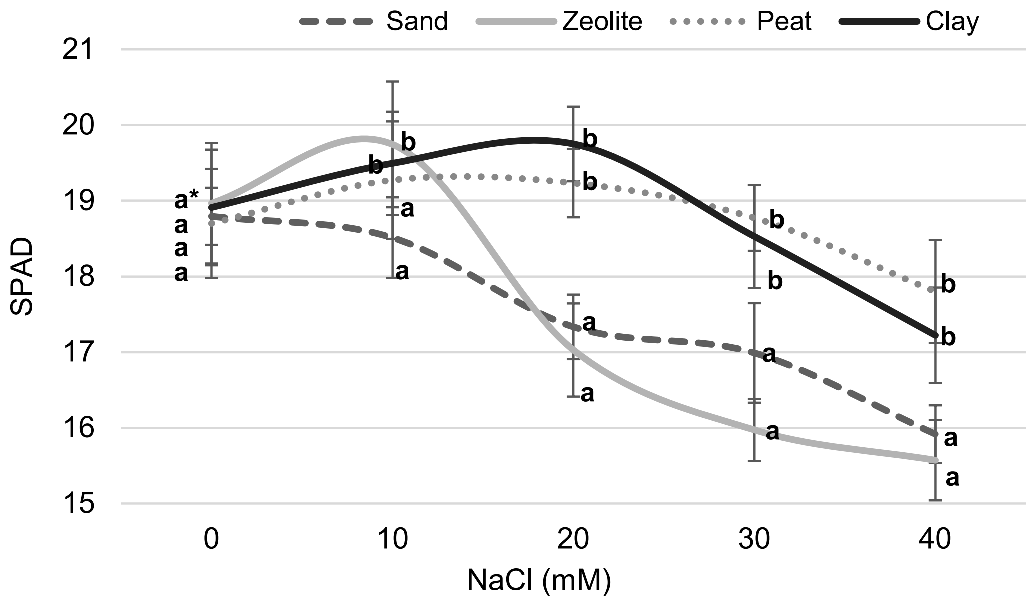


Figure 4. Effect of NaCl on leaf SPAD values of lettuce cultivated in substrates with different amendments.

*Means with different letters for each NaCl treatment level were significantly different (*t*-Test, $p < 0.05$).

Such results support previously determined salinity threshold values (1.1 to 1.3 dS m^{-1}) for lettuce (Shannon & Grieve, 1999; Ünlükara et al., 2008). It can be stressed, that peat and clay amendments significantly improve lettuce chlorophyll content and increase salt stress tolerance.

Our study approved the already-known fact that the chlorophyll content is widely proportional to the rate of photosynthesis (Fig. 5). Harmful effect of salt stress on lettuce photosynthetic rate was evident for all substrates at salinity levels above 20 mM. However, peat and clay amendments ensured significantly higher values for the highest salinity treatments.

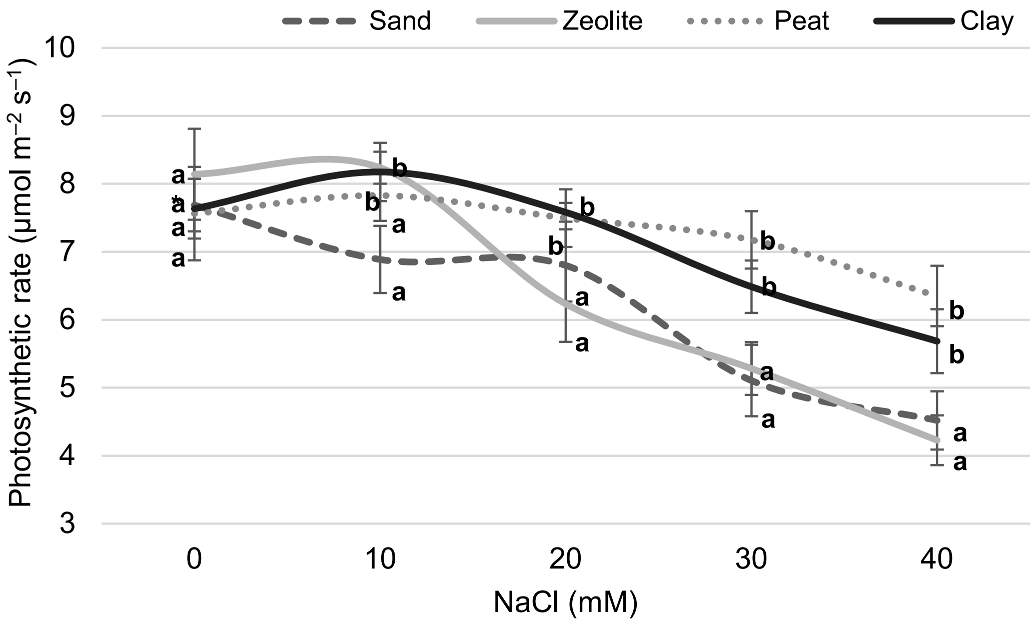


Figure 5. Effect of NaCl on leaf photosynthetic rate (µmol m^{-2} s^{-1}) of lettuce cultivated in substrates with different amendments.

*Means with different letters for each NaCl treatment level were significantly different (*t*-Test, $p < 0.05$).

A similar trend was observed in the case of stomatal conductance measurements. In general, the adverse effect of salinity on plant stomatal conductance was found (Fig. 6).

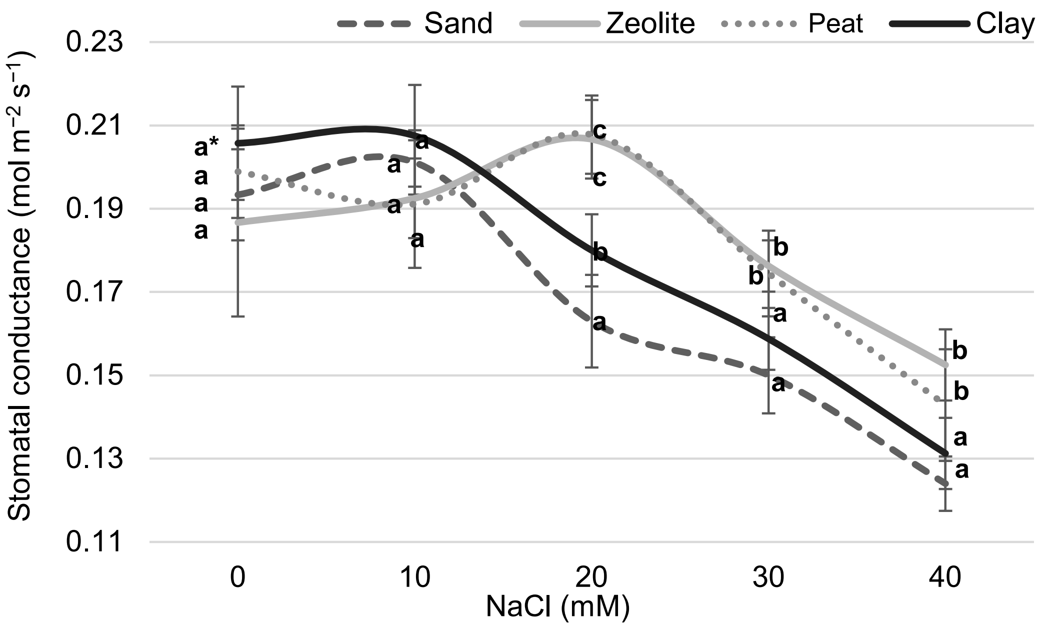


Figure 6. Effect of NaCl on stomatal conductance (mol m^{-2} s^{-1}) of lettuce cultivated in substrates with different amendments.

*Means with different letters for each NaCl treatment level were significantly different (*t*-Test, $p < 0.05$).

Such results indicate, that salt-induced decrease of photosynthetic rate was at least partly caused by stomatal closure. Despite the fact, that salinity levels above 20 mM reduced chlorophyll concentration and photosynthetic rate expressly in substrate with zeolite additions, the stomatal conductance was high and comparable with peat amendments. Such a decline in photosynthetic rate without a corresponding decline of stomatal conductance can be interpreted as a direct effect of Cl^- toxicity on plant photosynthesis, as chlorine levels in a substrate with zeolite were significantly higher (Fig. 1).

CONCLUSION

Lettuce demonstrated high sensitivity to soil salinity, as its growth was strongly reduced by salinity levels above 20 mM. Natural zeolite amendment successfully immobilized cationic sodium and improved water availability, but the harmful effect of enhanced uptake of chlorine significantly reduced plant growth and photosynthetic performance. Our study confirms the beneficial effect of organic matter (peat) on plant growth parameters under increasing salinity, especially in salinity levels above 30 mM. Although clay additives to the growth medium showed the potential to reduce the adverse effects of salinity by promoting lettuce photosynthetic rate and total concentration of chlorophyll the effect on plant biomass gain was small.

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