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ORIGINAL PAPER

Analysis of the Ability of Marsh Samphire (*Salicornia europaea*) to Extract Environmentally Relevant Elements from Different Culture Media: Contribution of Biochar to Plant Nutrition and Growth

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Abstract
Soil salinity is considered one of the major global challenges that agricultural production is currently facing. This condition, together with the increasing contamination with emerging pollutants, poses a serious risk for global food security. As efforts are made to develop nature-based solutions, bioremediation strategies have been implemented to harness different living organisms and mitigate environmental pollution. Halophytes grow in highly saline environment and can be the solution to valorize salt-degraded areas where other crops cannot grow. The aim of this work is to evaluate the physiological response of *Salicornia europaea* grown under different conditions and its potential to extract sodium (Na) and copper (Cu) from different culture media. Different experiments were conducted with *S. europaea* cultivated in hydroponics and in substrate with and without biochar including different Cu (0, 5 and 10 mg L^{-1} $CuCl_2$) and Na (7.5 and 15 g L^{-1} NaCl) concentrations. The growth in hydroponic media under different salinities reveals that this halophyte can extract up to 80% and 55% of the initial Na content when growing at 7.5 g L^{-1} and 15 g L^{-1} NaCl, respectively. In addition, *S. europaea* tolerates high Cu concentration, accumulating up to 1.61 mg g^{-1} DW in roots when exposed to 10 mg L^{-1} $CuCl_2$ and 7.5 g L^{-1} NaCl. Plants grown in substrate show a different behavior, being even more tolerant to higher Cu concentrations. In addition, the presence of biochar in the substrate improves plant growth and provides a greater quantity of micronutrients. These results show the potential of *S. europaea* to be used in the phytoremediation process.

Keywords Copper toxicity · Halophytes · Heavy metals · Nature-based solution · Phytoremediation · Salinity

1 Introduction

Various abiotic and biotic stress factors impair the development and productivity of plants, threatening the sustainability of the agricultural industry. Low temperature, salinity, drought, heat, and heavy metal toxicity, as well as pathogens are only a few of the stress factors that plants are constantly exposed to. Emerging pollutants, salinity and heavy metals have been proven to accumulate in soil and water bodies over time, eventually reaching dangerous levels. The term 'heavy metals' is commonly used in environmental topics to refer to metals and metalloids associated with environmental pollution, toxicity and adverse effects on living organisms, however the term has been diversely defined (Ali and Khan 2018). Many of these pollutants have increased as a consequence of various anthropogenic activities that have aggravated existing abiotic stress conditions, representing a serious threat to crops.

Soil salinity is one of the major global challenges that, together with adverse environmental conditions, has resulted in a reduction in usable agricultural land, limiting the agriculture and food sector. Primary and secondary salinization has increased salt affected land by around 800 million hectares all over the world, which is more than 6% of the world's total land area, out of which about 52% is sodic (Nikalje et al. 2017). The increased irrigation practices in agriculture, together with inadequate soil drainage, were

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the principal causes of salinization of those areas, which led to a decline in the yields of the major crops (Zaman et al. 2018). Even though salinity problems may happen with all climatic conditions, most saline soils occur in coastal areas, due to seawater intrusion, and are found in arid and semiarid regions of the world, with Australia, China, Egypt, India, Iran, Iraq, Mexico, Pakistan, Syria, Turkey, and the United States representing the most affected countries (Zaman et al. 2018). These soluble salts inhibit the growth of most major crops. An increased level of salinity in the soil, particularly of Na^+ ions, has a number of negative effects on salt-sensitive plants (glycophytes). High concentrations of Na cause osmotic imbalance, nutrient deficiency, membrane disorganization, inhibition of cell expansion and division. The low functionality of some enzymes under saline conditions, results in a strong affection of physiological and metabolic processes such as photosynthesis and reproducibility, respiration, transpiration, membrane properties, nutrient balance, enzymatic activity, cellular homeostasis and hormone regulation, ultimately leading to the production of reactive oxygen species (ROS), severe stress conditions and plant death (Mahajan and Tuteja 2005).

The presence of emerging pollutants and heavy metals is another problem we are facing today. Above certain concentrations, they are considered a critical environmental issue due to various toxic effects, as well as their persistency in the environment, which makes them easy to accumulate to dangerous levels. In addition to their negative impact on the environment, heavy metals tend to accumulate in living organisms, posing a serious health threat. The excess of heavy metals in the soil can be originated by atmospheric deposition, as well as the improper loading of solid and liquid industrial wastes on land fields and the unsustainable use of metal-rich fertilizers and pesticides. Among these causes, the discharge of industrial effluents and domestic sewage directly into the field can contribute to heavy metal contamination of the soil. The problem is even worse in developing countries where freshwater scarcity and waste management are serious problems and the irrigation of agricultural lands with wastewater effluents rich in pollutants is forced (Chopra et al. 2009). The intensive use of non-sustainable agricultural inputs represents another well-known contribution to soil contamination, which is why there is an even higher demand for sustainable agricultural practices. The degree of toxicity in plants depends on different factors like the plant species and growth stage, the concentration of the metals and the composition of the soil, but generally, in not tolerant species, long term exposure to heavy metals causes oxidative stress, which is accompanied by ROS production which may exceed the cell's natural antioxidant defenses, resulting to physiological and biochemical damaging effects such as chlorophyll degradation, enzyme inhibition though displacing of essential elements, disruption of membranes integrity and plant defense mechanisms, eventually leading to inhibition of plant growth and development and even death (Su et al. 2014).

Copper (Cu) is one of the essential plant micronutrients, however, at high concentrations can be toxic and can represent agricultural and public health issues. Long-term metal deposition in soils can result in Cur accumulation, transport, and biotoxicity due to its persistency, mobility and bioavailability. Copper levels in plant tissues normally range from 5 to 30 mg g^{-1} dry weight (DW), with concentrations above this threshold inducing various harmful effects. Excessive Cu uptake impairs physiological processes like photosynthesis, respiration, and cell wall metabolism, as well as disruption of protein structures and inactivation of enzymes, ultimately inhibiting plant growth (Printz et al. 2016; Ghori et al. 2019). Unlike other heavy metals, which are mainly released into the environment as a result of improper disposal of industrial residues and contaminated wastewaters, Cu is largely released into the environment as a consequence of improper agricultural inputs, especially Cu-based fungicides, which have been widely used in vineyards in recent decades to control fungal diseases. For this reason, the highest Cu concentrations can be found in southern countries such as Greece, Italy southern France and southern Spain (Ballabio et al. 2018). These intensive disposal of Cu in the environment, together with its well-known persistency, has led to the accumulation of this metal over time, particularly in agricultural territories, posing a big pressure on the agri-food system and representing a serious risk to consumers due to the possibility of Cu transfer from soil to food chain. In humans, the liver is the primary organ of Cu-induced toxicity. Other organs that can be affected including bones, the central nervous and the immune system, and it can also cause a deficiency of other nutrients such as iron (Stern et al. 2007).

Plants could be used to decontaminate polluted places. This process occurs naturally, and these plants can transform and break down pollutants into harmless compounds without the use of chemical catalysts. Phytoremediation refers to the use of plants to immobilize, remove, or break down a variety of pollutants from soil, water and air, including ions, hydrocarbons, pesticides and heavy metals. The use of plants in the bioremediation process has emerged as a more cost-effective, non-invasive, and socially accepted technique of addressing the removal of contaminants from the environment. Because it is cost-effective and simple to implement, phytoremediation is becoming the best option for remediation projects in less industrialized countries. Phytoremediation has several advantages over traditional techniques, including a significant cost decrease and the absence of chemical inputs, which significantly reduces the

environmental impact. The use of plants in the remediation process also improves the landscape and provides habitat for animal life.

Phytoremediation of saline environments using salt tolerant plants (halophytes) has been found to be an efficient and low-cost strategy (Turcios et al. 2021). In addition to the improved environmental and economic sustainability, using halophytes to remediate saline soils has unique advantages such as the ability to remove salt and other pollutants at the same time. The phytoremediation also considers the phytostabilization and phytovolatilization potential of metal-tolerant plants in addition to the phytoextraction process (Yadav et al. 2018). Hyperaccumulator plants are species capable of absorbing, translocating and accumulating contaminants in the tissues at concentrations much higher than those observed in the soil or in non-accumulating plant species (Reeves et al. 2021). Phytoextraction ensures that pollutants are permanently removed from polluted environments. Nonetheless, it is influenced by a number of factors such as the plant's tolerance, concentration of pollutants, and physical-chemical properties of the soil. In this context, there are materials that can positively influence the remediation process due to their physico-chemical properties, such as biochar, and in addition it can improve plant growth due to its high moisture retention and increased nutrient availability (Hansen et al. 2023).

Biochar is the carbon-rich material produced from organic feedstock under certain thermal combustion with limited oxygen. In general, biochar produced at high temperature has higher surface area and carbon content, mainly due to the increase of micro-pore volume caused by the removal of volatile organic compounds at high temperature (Nair et al. 2023). Many organic wastes can be used as feedstock to produce biochar, such as agricultural wastes and municipal solid wastes. Sludge is generated during the wastewater treatment process, which is a solid waste required to be treated and disposed. However, it is a promising feedstock for biochar production because it contains rich carbon and nutrients. Biochar has its own advantages, such as rich carbon content, carbon sequestration from the atmosphere, high cation exchange capacity, large surface area, and improves soil properties and soil fertility. Due to its own characteristics, such as large surface area, recalcitrant and catalysis, biochar has been widely used in the environment application such as soil remediation, carbon sequestration, and wastewater treatment (Wang and Wang 2019). Biochar can also stabilize heavy metals in the contaminated soils, improve the quality of the contaminated soil and has a significant reduction in crop uptake of heavy metals. Therefore, application of biochar can potentially provide a new solution for remediation of the soils contaminated by heavy metals (Zhang et al. 2013). Adsorption is the main mechanism for biochar to remove pollutants, including surface complexation, hydrogen binding, electrostatic attractions, acid-base interaction, pi-pi interactions (Qiu et al. 2022). Therefore, biochar has a high potential to be used together with halophytes in the remediation process. After the phytoremediation process and after a detailed biomass characterization, the biomass can have different possible exploitations in the industry, including food and feed, pharmaceutical and nutraceutical, cosmetic, and bioenergy sectors (Hulkko et al. 2022).

In many environments, especially those impacted by industrial activities, both heavy metals like copper and high salinity co-occur. Many halophytes have been already shown to accumulate toxic metals in their shoots and leaves when growing on heavy metals affected areas or wastewaters (Khalilzadeh et al. 2020). Thus, the potential of halophytes in phytoremediation is not limited to the recovery of salinity-affected areas and effluents but could also include their use in heavy metal phytoextraction. In this context, halophytes may have additional advantages over standard hyperaccumulator plants since they may be cultivated in saline environments, in addition, the presence of salt can increase metal bioavailability in soils. Based on this, halophytes can be good candidates for decontamination of saline soils with heavy metals (Manousaki and Kalogerakis 2011; Hasanuzzaman et al. 2014). Nevertheless, this area deserves further investigations on the tolerance of specific halophytic species to high concentrations of pollutants. In this sense, the ability of *Salicornia europaea* to grow in saline environments makes it a good candidate to extract considerable amounts of sodium. However, studies are needed to analyze the potential of *S. europaea* to extract salt and metals simultaneously under different conditions. Understanding how halophytes respond to and interact with both stressors is crucial for developing effective phytoremediation strategies. Moreover, investigating the interaction between Cu and salt helps elucidate how these stressors affect halophyte health, growth, and overall phytoremediation potential. For this reason, the aim of this research is to investigate the potential of *S. europaea* to extract Na and Cu under different conditions including hydroponics and substrate. Furthermore, the contribution of biochar to the remediation process and plant growth has been investigated. Different concentrations of NaCl ranging from 0 to 3% and different concentrations of $CuCl_2$ ranging from 0 to 10 mg L^{-1} in hydroponics and from 0 to 100 mg kg^{-1} in substrate culture were used. The results show a high potential of *S. europaea* to be used in the phytoremediation process of different environments contaminated with Cu and salts. Furthermore, due to the low translocation of Cu from the root to the above-ground biomass, the plant material could have other potential uses in industry. The purpose of this study is to demonstrate whether

S. europaea could be successfully used for phytoextraction of Cu as a nature-based solution in a saline environment and still be utilized for various additional purposes.

2 Materials and Methods

2.1 Cultivation of Plants

The plant experiment was conducted in a greenhouse at the Institute of Botany, Leibniz University Hannover, Germany (52°23′42″ N; 9°42′13″ E). Seeds of *S. europaea* L. var. aprica were obtained from Serra Maris bvba, Belgium. The seeds were sown and stored at 4 °C for two days to boost germination. After germination, the plants were transferred to the greenhouse and after one month of growth, they were transplanted to individual pots. *Salicornia europaea* plants were used for the implementation of two separated experiments. In a first experiment, the treatments were two different salt concentration (7.5 g L^{-1} and 15 g L^{-1} NaCl) and three different Cu concentrations (0, 5 and 10 mg L^{-1} $CuCl_2$). This salt range was selected because this is where the *Salicornia* grows best. The Cu range was selected because it covers the range of Cu concentrations found in most soils in Europe (EEA 2019). For this experiment, 18 plastic boxes containing 2 L of Hoagland solution were used, the boxes had a plastic lid with three holes, and each box contained three plants. The experiment comprised 64 plants.

The nutrient solution used was the modified Hoagland solution containing 606 mg L^{-1} KNO_3, 944 mg L^{-1} $Ca(NO_3)_2 \times 4H_2O$, 230 mg L^{-1} $NH_4H_2PO_4$, 246 mg L^{-1} $MgSO_4 \times 7H_2O$, 3.73 mg L^{-1} KCl, 1.55 mg L^{-1} H_3BO_3, 0.34 mg L^{-1} $MnSO_4 \times H_2O$, 0.58 mg L^{-1} $ZnSO_4 \times 7H_2O$, 0.12 mg L^{-1} $CuSO_4 \times 5H_2O$, 0.12 mg L^{-1} $MoNa_2O_4 \times 2H_2O$, and 9.16 mg L^{-1} Fe-EDDHA (0.56 mg Fe L^{-1}). The two salinities were reached at two different time points; first, 7.5 g L^{-1} NaCl were added to the nutrient solution of all the experimental units, reaching the first salt concentration evaluated (128.34 mM NaCl); after 2 days of adaptation, the second group of nine experimental units was added with another 7.5 g L^{-1} NaCl, reaching the second salt concentration (256.6 mM). Within the two salinities, two $CuCl_2$ concentrations (5 mg L^{-1} and 10 mg L^{-1} $CuCl_2$) and a control (0 g L^{-1} $CuCl_2$) were used. The concentrations evaluated (0.037 mM and 0.074 mM Cu) were reached by adding 5 mg L^{-1} and 10 mg L^{-1} $CuCl_2$ to the nutrient solutions. The water was aerated constantly by small compressors and one air stone in the middle of each tank (Eheim, Deizisau, Germany). The water level was adjusted constantly in each tank with tap water to compensate the evapotranspiration and therefore the salinity was kept constant. The experiment was conducted in triplicates (3 hydroponics containers per treatment). Each hydroponics container represented an experimental unit. Water samples were taken at the beginning and at the end of the experiment and stored at -30 °C before analysis.

After 25 days in hydroponics, plants were harvest. For this, plants were cut at the base of the above-ground part, separating the roots from the shoots. The fresh weights of the above-ground biomass and roots were determined per experimental unit. After harvesting, a representative sample of shoots and roots was taken from each experimental unit, frozen immediately in liquid nitrogen and stored at -80 °C before analysis. The left-over plant material was dried in a drying chamber (Memmert, Modell 600) at 70 °C until a constant weight is reached and used for the elemental analysis through ICP-OES. The water content in the plant material was determined using both values, dry weight and fresh weight of each sample.

For the second experiment, seeds of *S. europaea* were sown and then kept at 4 °C for two days to speed up germination, after that they were transferred to the greenhouse for 7 weeks (temperatures between 14 °C during night and 35 °C during the day). Then the plants were transplanted in single pots to assess different treatments, watered regularly every day with 50 mL of tap water or every two days with 100 ml of tap water. For this experiment three factors were studied: different NaCl concentrations (0, 15 and 30 g kg^{-1} substrate), different $CuCl_2$ concentrations (0, 50 and 100 mg kg^{-1} substrate) and biochar (0 and 6% w/w). Substrate (Einheitserde® Classic Profi Substrat, density 0.3076 g cm^{-3}) was sterilized and dried before use. Pots of 10 cm diameter were used, with a total volume of 260 mL. In each pot was added 100 g of dry substrate.

Plants of both experiments were exposed to 12 h of artificial light (sodium vapor lamps, SON-T Agro 400, Philips, Amsterdam, Netherlands). Light intensity ranged from 114 µmol m^{-2} s^{-1} – 640 µmol m^{-2} s^{-1} depending on the time of the day and the weather conditions. The biochar used in the corresponding treatments was produced by the company Aquagreen, Denmark (https://aquagreen.dk/). After 5 weeks of growth in pots plants were harvested. Each plant was cut at ground level, weighed for total biomass, and then divided into two parts: one was weighed and dried in the oven at 70 °C for two days then weighed again to determine the water content in the tissues; the other was immediately frozen in liquid nitrogen and stored at -80 °C.

2.2 Production of Biochar

The biochar was produced through the pyrolysis process (thermal decomposition of materials at elevated temperatures) from sewage sludge. The sewage sludge was collected from the Danish wastewater treatment plant Fårevejle

and pyrolyzed by the company Aquagreen in Denmark. The samples came from the same batch to secure consistency in its property. The sewage sludge was dried at 120 °C and then pyrolyzed under controlled conditions in an in-house slow pyrolysis continuous screw reactor at Aquagreen to ensure uniform heating and treatment. The dimensions of the in-house reactor are 288 cm × 214 cm x 154 cm (L x W x H), with a screw size of ∅ 27.2 cm. The pyrolysis temperature was 665 °C with the same residence time of 45 min. Before the biochar was used for the pot experiment, the biochar was milled on a cross-beater mill (Retsch SK1, Haan, Germany), followed by sieving on a 0.315 mm mesh. The physico-chemical characteristics of the biochar used have been reported by Hansen et al. (2023).

2.3 Extraction and Determination of Total Phenols

Total phenols can be an indicator of plant stress where, for example, under saline conditions or other abiotic factors, the amount of total phenols can increase (Boestfleisch et al. 2014). The protocol was modified for microtiter plates. Total phenols were measured with Folin-Ciocalteu reagents, using gallic acid as standard. Frozen ground leaf material (100 mg) of plant material was incubated (10 min) in ice-cold methanol (800 µL, 80%) with shaking every 2 min. After centrifugation (5 min, 15 000 x *g*), the supernatant was collected, and the pellet was re-extracted (three times) with ice-cold methanol (400 µL, 80%) to produce a total of 2 ml of extract. Based on the method of Dudonné et al. (2009), 100 µL of water was pipetted into the wells of a 96-well microplate. Triplicates of 10 µL sample, blank (80% methanol), or gallic acid standard (5–250 µg mL^{-1}) were added, and finally, 10 µL of Folin-Ciocalteu reagent. After incubation for 8 min and adding 100 µL of 7% sodium carbonate, the plate was incubated for 100 min and measured at 765 nm. Total phenols were calculated from a standard curve.

2.4 Extraction and Determination of Proline

According to method described by Carillo and Gibon (2011), standards were prepared by serial dilution of a 2 mM proline stock. The plant extract was prepared as follow: 100 mg of fine grinded fresh plant material was weighted in 2 mL reaction tube and mixed with 1 mL of 40% ethanol, then incubated at 4 °C overnight. The next day samples were centrifuged for 5 min at 14 000 x *g*, the supernatant, which contains > 93% of the proline was transferred to a clean labelled reaction tube. 50 µL of extract or standard or blank (methanol 40%) were mixed with 100 µL reaction mix (ninhydrin 1%, acetic acid 60% and ethanol 20%). Samples were incubated in a water bath at 95 °C for 20 min, cooled at room temperature and centrifuged at 2500 rpm for 1 min. 100 µL of sample were transferred into a microwell-plate and measured at 520 nm.

2.5 Elemental Analysis

The elemental analysis was performed in triplicates on homogeneous dried samples of each experimental unit as well as on the samples taken from nutrient solution or substrate at the beginning of the experiment and after the harvesting. The dry plant samples were ground to a fine powder in a grinding machine (MM 400, Retsch GmbH, Haan, Germany) and stored at room temperature. The extraction of elements was carried out using about 0.038 g of dried plant material. After weighting, the samples were incinerated for a minimum of 10 h at 480 °C in a muffle furnace (M104, Thermo Fisher Scientific Corporation, Waltham, Massachusetts, USA).

The ashes were digested by adding 1.5 mL of 1:3 HNO_3. After 10 min of incubation, 13.5 ml of ultra-pure water were added to the samples. The solutions were then filtered by gravity directly into 15 ml falcon tubes through 0.45 µm pore size filters (Carl Roth, Karlsruhe, Germany). The extraction of elements from the hydroponic samples was carried out from 1 mL of the sample following the same digestion protocol used for the plant material (addition of 1.5 mL 1:3 HNO_3 and ultra-pure water till 15 mL volume) and the same filtration method as mentioned above. For the determination of bioavailable elements in the substrate of the second experiment in pots, dried soil samples were extracted with the following procedure: 150 mg of dry soil were weighted in 2 ml reaction tube and 1.5 mL of HNO_3 1 M were added. Samples were shaken at room temperature for 2 h at 1500 rpm. Then centrifuged at 15 000 x *g* for 5 min, the supernatant was collected in clean reaction tubes to which 13.5 mL of ultrapure water were added. The solution was then filtered into 15 mL labelled tubes and stored at 4 °C until the final analysis. Samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6000 ICP Spectrometer, Thermo Fisher Scientific Corporation).

2.6 Bioaccumulation and Translocation Factor

The bioaccumulation factor (BF), an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the growth media, was calculated as follows: BF of shoot = C_{shoot}/C_w, and BF of roots = C_{root}/C_w, where C_{shoot} and C_{root} are the element concentrations (mg g^{-1} DW) in the plant shoots and root, respectively, and C_w is the element concentration in culture solution (mg mL^{-1}). The translocation factor (TF) or mobilization ratio, assessed

to determine the translocation of elements from the roots to the above-ground tissues, was calculated as: $TF = C_{shoot}/C_{root}$, where C_{shoot} and C_{root} represent the element concentrations in the plant shoot and root, respectively (Galal and Shehata 2015).

2.7 Statistical Analysis

All statistical analyses were conducted using R, version 3.1.1 (R Core Team, Vienna, Austria) and InfoStat software, version 2020e (InfoStat Team, Cordoba, Argentina). The experiments were performed in a randomized way under controlled conditions in the greenhouse. For each analysis three biological replicates were considered. The effects of the different main factors: NaCl, biochar and $CuCl_2$ were analyzed through the analysis of variance (ANOVA). For the factor salt, in the hydroponic experiment a control (0 g/L) was not considered because *S. europaea* is an obligate halophyte and can not grow and develop under non-saline conditions. The Tukey multiple comparison test with a significance level of 0.05 was done to determine which means differ from the rest.

3 Results

3.1 Biomass Production in Hydroponics

In the first experiment, the response of plants to different concentrations of salt and Cu under hydroponic conditions was investigated. According to the results, high concentration of $CuCl_2$ in the media leads to drastic changes in the uptake of essential micronutrients, which results in morphological, physiological, and biochemical changes. The effects are visible on both shoot and root organs, with yellowing of the shoots in the plants exposed to Cu, especially between nodes, resulting in an unusual alternation of light green and yellow areas. The effect of Cu in the cultivation medium on the general development of *S. europaea* is validated by the analysis of variance (ANOVA), which highlights a significant effect on biomass yield with respect to the $CuCl_2$ treatment (p value < 0.0001). The results reveal an expected decrease in the general development of the plant proportional to the increase of Cu in the medium, with the highest biomass yield obtained in the control (0 mg L^{-1} $CuCl_2$) cultivated at the two salinities tested (7.5 g L^{-1} and 15 g L^{-1} NaCl). The different salinities have no significant effect on biomass production (Table 1).

3.2 Biomass Production of Substrate-Grown Plants

The production of biomass is significantly affected by the treatments. The analysis of variance shows that all three factors (NaCl, $CuCl_2$ and biochar) have significant effects on biomass yield, with the highest production using the combination of 15 g kg^{-1} NaCl, 50 mg kg^{-1} $CuCl_2$ and biochar. However, there is an interaction effect between the factors NaCl and $CuCl_2$. Using biochar and under non-saline conditions, the higher the concentration of Cu the higher the biomass production, nevertheless under saline conditions the highest biomass is obtained with a concentration of 50 mg kg^{-1} $CuCl_2$ and at higher Cu concentrations the production is negatively affected. This may be caused by an antagonistic effect between the two elements. By not applying $CuCl_2$ the higher the salt concentration the higher the biomass production. By using biochar, higher yields are generally obtained. This may be due to several reasons including a higher availability of nutrients for the plants, a higher moisture holding capacity of the substrate and at the same time high concentrations of elements can be adsorbed in the cation exchange complex of biochar and thus avoid possible toxic effects. The lowest biomass production is obtained under non-saline conditions and without the use of biochar (Fig. 1). It is clear that *S. europaea* plants produce higher yields under saline conditions, being a true halophyte, it requires salt in the medium for optimal growth. As for Cu, being a micronutrient plants requires it at low concentration, while after exceeding a threshold (which is different among plant species) it becomes toxic, decreasing the biomass yield.

The Na concentration in the substrate has a positive effect on biomass production, however, it does not have a linear behavior. The lowest biomass production is obtained with the lowest Na concentrations, increasing rapidly with increasing Na concentration to approximately 2 mg g^{-1} and stabilizing biomass yield from this value onwards (Fig. 2). A relatively high dispersion can also be observed due to the biological characteristics of the species itself.

Table 1 Biomass gain of *S. Europaea* cultivated under hydroponic conditions. Values represent the mean of three replicates (g/container). Plants cultivated for 25 days under two salt treatments (7.5 g L^{-1} and 15 g L^{-1} NaCl) and three copper treatments (0 mg L^{-1}; 5 mg L^{-1} and 10 mg L^{-1} $CuCl_2$). Different letters indicate significant differences between treatments ($p < 0.05$)

Salt treatment (g L^{-1} NaCl)	Cu treatment (mg L^{-1} $CuCl_2$)		
	0	5	10
7.5	185.14 ± 21.33 **a**	77.44 ± 6.69 **b**	42.94 ± 5.47 **b**
15	171.12 ± 0.50 **a**	67.07 ± 11.5 **b**	61.91.14 ± 8.59 **b**

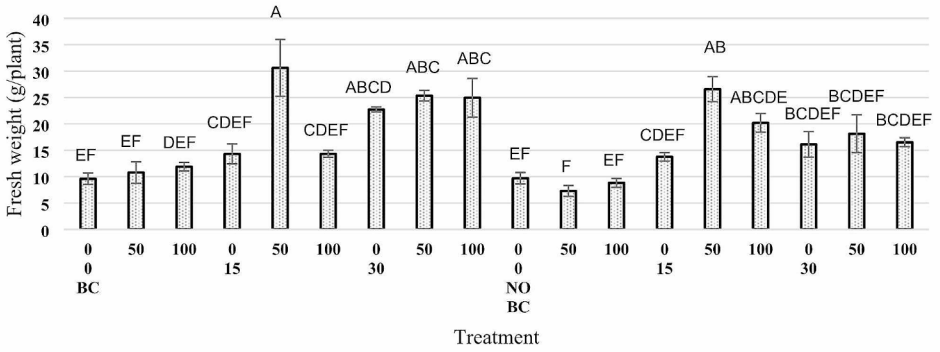


Fig. 1 Biomass production of *S. europaea* cultivated under different conditions. BC: Biochar. The values 0, 15 and 30 indicates the applied NaCl concentration in g kg^{-1} substrate. The values 0, 50 and 100 indicates the $CuCl_2$ concentration in mg kg^{-1} substrate. Bars represent the mean value ± S.E. Different letters indicate significant differences between treatments ($p < 0.05$)

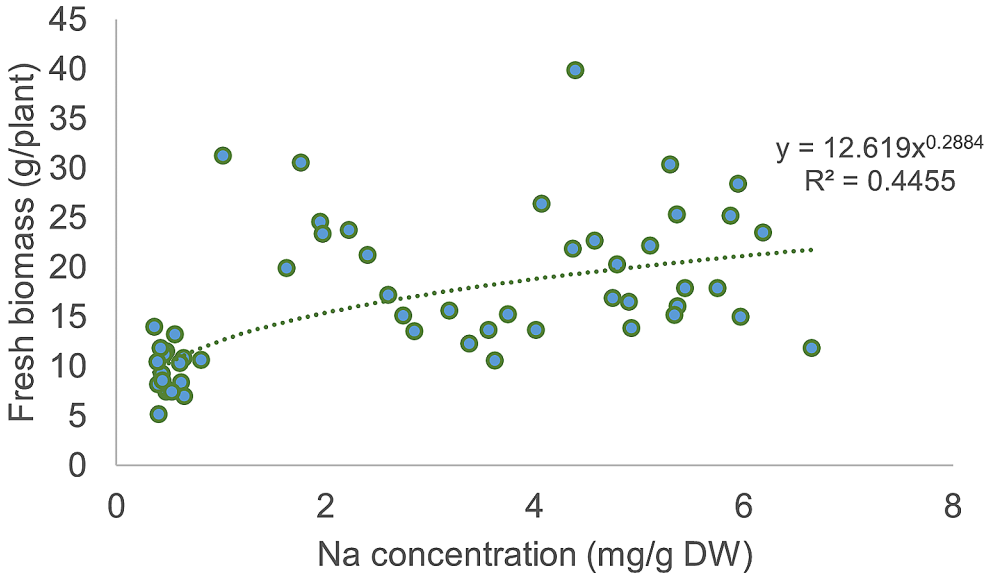


Fig. 2 Correlation between the variables Na concentration in the plant tissue and biomass production

3.3 Effect of Salinity and Copper on Mineral Composition of the Plants Cultivated in Hydroponics

The results obtained indicate that, although essential for plants, metals like Cu start to be toxic when found at high concentrations. The plants exposed to $CuCl_2$ exhibited drastic changes in the mineral composition compared with the control plants, confirming an imbalance in the uptake and accumulation of the other essential elements and, consequently, affecting the plant development. The results show a general decrease in the concentration of most of the elements along with the increase of Cu concentration in the medium. Interestingly, the variation in the concentration of the elements in the shoots is not always the same in the roots. The concentration of elements like Cu, K, Mg, and Na in the roots, seems to be more subjected to variation than in the shoots. On the other hand, the concentration of Ca is shown to be highly affected in the shoots, while not altered in the roots, and micronutrients like Al and B do not vary among the treatments neither in the shoots nor in the roots. The analysis of variance indicates that the treatment with $CuCl_2$ has high significance differences in the accumulation of most elements analyzed (Ca, Cu, Fe, K, Mg, Na, P, and S), with highest effects on Ca, Fe, Mg and Na in the shoots (p values < 0.001) and Cu, K, Fe, Mg and S in the roots (p values < 0.005) (Tables 2 and 3). The variation in the accumulation of important nutrients is reflected in the growth of the plants and implies a reduction in biomass production along with the Cu treatment, confirming the strong effect of a high concentration of Cu in the media on the general physiology of *S. europaea*.

The $CuCl_2$ treatment shows to have a big impact on the mineral concentration in both shoots and roots, however, the effect of the salt treatment is found only in the shoots, with the highest impact on Ca and Na (p values < 0.0001), together with Mg and S (p values of 0.0037 and 0.0441 respectively), while in the roots the only element that shows a significant difference according to the salt treatment is Na, indicating that the Na uptake and accumulation in the roots doesn't affect the mineral composition. The combination of NaCl and $CuCl_2$ treatment doesn't have a significant impact on the accumulation of nutrients, except for Mg and S, whose concentrations are significantly affected in both shoots and roots. Interestingly, while the accumulation of

Table 2 Mineral composition of *S. europaea* shoots cultivated under hydroponic conditions. Values represent the mean of three replicates. Plants cultivated for 25 days under two salt treatments (7.5 g L^{-1} and 15 g L^{-1} NaCl) and three copper treatments (0 mg L^{-1}; 5 mg L^{-1} and 10 mg L^{-1} $CuCl_2$). Different letters indicate significant differences between treatments ($p < 0.05$). DW = dry weight

Salinity	Elements (mg g^{-1} DW ± SE)	Copper treatment (mg L^{-1} $CuCl_2$)		
		0	5	10
7.5 g L^{-1}NaCl	**Al**	0.55 ± 0.13 **a**	0.71 ± 0.12 **a**	0.4 ± 0.03 **a**
	B	0.47 ± 0.12 **a**	0.66 ± 0.07 **a**	0.4 ± 0.05 **a**
	Ca	8.62 ± 0.18 **bc**	11.23 ± 0.68 **a**	10 ± 0.32 **ab**
	Cu	0.02 ± 0.01 **a**	0.03 ± 0.01 **a**	0.02 ± 0.01 **a**
	Fe	0.07 ± 0.01 **a**	0.01 ± 0.0025 **b**	0.02 ± 0.0013 **b**
	K	16.34 ± 2.72 **a**	18.58 ± 2.52 **a**	11.48 ± 2.39 **a**
	Mg	1.34 ± 0.07 **a**	1.31 ± 0.05 **a**	0.66 ± 0.11 **b**
	Na	150.19 ± 1.15 **a**	129.17 ± 2.92 **b**	120.48 ± 2.11 **b**
	P	2.78 ± 0.13 **ab**	3.48 ± 0.3 **ab**	2.78 ± 0.16 **ab**
	S	3.26 ± 0.48 **a**	2.31 ± 0.1 **ab**	1.68 ± 0.16 **b**
15 g L^{-1}NaCl	**Al**	0.7 ± 0.05 **a**	0.7 ± 0.05 **a**	0.56 ± 0.06 **a**
	B	0.5 ± 0.14 **a**	0.6 ± 0.06 **a**	0.48 ± 0.06 **a**
	Ca	5.13 ± 0.36 **d**	7.32 ± 0.43 **c**	7.81 ± 0.41 **c**
	Cu	0.03 ± 0.01 **a**	0.04 ± 0.0038 **a**	0.04 ± 0.01 **a**
	Fe	0.06 ± 0.01 **a**	0.02 ± 0.0025 **b**	0.01 ± 0.0019 **b**
	K	9.94 ± 1.1 **a**	16.64 ± 1.98 **a**	15.8 ± 1.69 **a**
	Mg	0.86 ± 0.01 **b**	0.96 ± 0.02 **b**	0.94 ± 0.08 **b**
	Na	168.46 ± 7.74 **a**	154.54 ± 4.36 **a**	160.31 ± 1.25 **a**
	P	2.37 ± 0.07 **b**	3.97 ± 0.38 **a**	3.47 ± 0.42 **ab**
	S	2.01 ± 0.1 **b**	1.76 ± 0.1 **b**	2.18 ± 0.22 **ab**

Table 3 Mineral composition of *S. Europaea* roots cultivated under hydroponic conditions. Values represent the mean of three replicates (g/container). Plants cultivated for 25 days under two salt treatments (7.5 g L^{-1} and 15 g L^{-1} NaCl) and three copper treatments (0 mg L^{-1}; 5 mg L^{-1} and 10 mg L^{-1} $CuCl_2$). Different letters indicate significant differences between treatments ($p < 0.05$). DW = dry weight

Salinity	Elements (mg g^{-1} DW ± SE)	Copper treatment (mg L^{-1} $CuCl_2$)		
		0	5	10
7.5 g L^{-1}NaCl	**Al**	0.59 ± 0.11 **a**	0.43 ± 0.04 **a**	0.43 ± 0.04 **a**
	B	0.45 ± 0.1 **a**	0.47 ± 0.05 **a**	0.37 ± 0.04 **a**
	Ca	6.74 ± 0.5 **a**	7.77 ± 2.04 **a**	6.99 ± 1.62 **a**
	Cu	0.04 ± 0.0035 **c**	0.91 ± 0.21 **ab**	1.6 ± 0.35 **a**
	Fe	0.35 ± 0.05 **b**	0.98 ± 0.05 **ab**	0.85 ± 0.29 **ab**
	K	41.1 ± 6.31 **a**	19.48 ± 0.87 **b**	16.42 ± 1.85 **b**
	Mg	0.54 ± 0.03 **b**	0.76 ± 0.01 **a**	0.49 ± 0.07 **b**
	Na	51.3 ± 5.05 **bc**	47.27 ± 3.41 **bc**	36.25 ± 3.06 **c**
	P	4.28 ± 0.48 **a**	6.02 ± 0.92 **a**	5.39 ± 1.08 **a**
	S	2.39 ± 0.09 **a**	2.15 ± 0.16 **ab**	1.46 ± 0.21 **c**
15 g L^{-1}NaCl	**Al**	0.47 ± 0.07 **a**	0.7 a ± 0.11 **a**	0.52 ± 0.05 **a**
	B	0.41 ± 0.06 **a**	0.57 ± 0.1 **a**	0.43 ± 0.05 **a**
	Ca	6.86 ± 1 **a**	4.99 ± 0.2 **a**	4.65 ± 0.09 **a**
	Cu	0.06 ± 0.02 **bc**	0.86 ± 0.03 **abc**	1.55 ± 0.17 **a**
	Fe	0.55 ± 0.01 **ab**	1.14 ± 0.02 **a**	0.95 ± 0.21 **ab**
	K	31.69 ± 4.11 **ab**	20.63 ± 2.39 **b**	21.15 ± 1.01 **b**
	Mg	0.49 ± 0.03 **b**	0.6 a ± 0.02 **b**	0.62 ± 0.02 **ab**
	Na	72.91 ± 6.37 **a**	58.87 ± 2.37 **ab**	57.52 ± 3.27 **ab**
	P	5.27 ± 0.82 **a**	5.96 ± 0.22 **a**	5.17 ± 0.24 **a**
	S	2.11 ± 0.02 **ab**	1.72 ± 0.12 **bc**	1.87 ± 0.01 **abc**

Na seems to be highly affected by the treatments in both shoots and roots, with a decrease in the concentration of $CuCl_2$ and an increase in salinity, the accumulation of Cu is affected only in the roots, with an proportional increase with the Cu treatment, while in the shoots, the statistical analysis doesn't show a significant difference in the accumulation of this element among the treatments, suggesting that Cu is poorly translocated to the above-ground biomass (Tables 2 and 3).

3.4 Sodium and Copper Uptake by Plants in Hydroponics

The results shown in Tables 2 and 3 indicate a higher concentration of Na in the shoots and roots under saline conditions and a general decrease when the concentration of Cu in the medium increases. The analysis of variance shows a significant difference of Na concentration in the shoots with respect to the $CuCl_2$ treatment (p-value 0.0007). This shows that there is a mutual suppression in the uptake of both elements. The same trend is observed in the roots, with the Na concentration decreasing with increasing $CuCl_2$ concentration, nevertheless the Cu concentration in the roots is not significantly influenced by the salt concentration in the culture medium. In the roots, the results of the mineral composition show an increased accumulation of Cu proportional with the raising of the $CuCl_2$ concentration in the medium. These results confirm the competition between Na and Cu in the uptake and accumulation in shoots and roots of *S. europaea*, where high concentrations of Cu somehow limit the Na uptake. Unlike Na, which is mainly translocated and accumulated in the shoots, Cu is absorbed and stored in the roots, with the highest concentration of 1.6 mg g^{-1} DW found in the roots of the plants grown at 10 mg L^{-1} $CuCl_2$, and 7.5 g L^{-1} of NaCl (Table 3), while the values found in the shoots are in the range of the concentration found in the controls (0.01–0.04 mg g^{-1} DW). According to these results, roots play a fundamental role in the accumulation of this element.

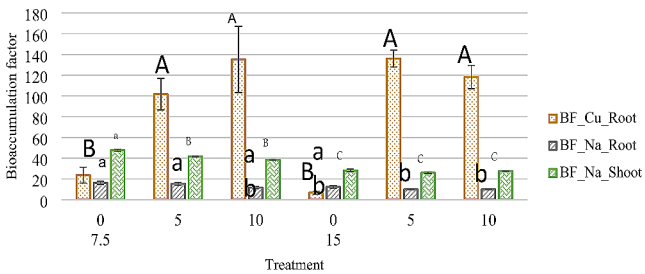


Fig. 3 Bioaccumulation factor (BF) for cupper and sodium in roots and shoots of *S. europaea* cultivated in hydroponics under two salt treatments (7.5 g L^{-1} and 15 g L^{-1} NaCl) and three copper treatments (0 mg L^{-1}; 5 mg L^{-1} and 10 mg L^{-1} $CuCl_2$). Bars represent the mean value ± S.E. Different letters indicate significant differences between treatments ($p < 0.05$)

As can be seen in Table 3, the concentration of Cu in the roots under $CuCl_2$ treatment is significantly higher than in the control, which is not the case in the above-ground biomass, indicating that *S. europaea* accumulates Cu in the roots as mentioned above but it is poorly translocated to the above-ground tissues. This is confirmed by the bioaccumulation factor (BF) and the translocation factor (TF). A plant's ability to accumulate metals from soils can be estimated using the BF, while its ability to translocate metals from the roots to the shoots is measured using the TF. Both BF and TF can be used to estimate a plant's potential for phytoremediation purposes, where TF > 1 indicates a very efficient ability to transport nutrients from roots to shoots, most likely due to efficient metal transport systems. Enrichment coefficient (EF) is an important factor when considering the phytoremediation potential of a plant species. In addition, EF > 1 shows a special ability of the plant to absorb metal ions from soils and transport it to aerial parts (Galal and Shehata 2015). According to the results, in the plants cultivated at 7.5 g L^{-1} NaCl, the Cu BF significantly increases from 23.6 in the control to 101.7 at 5 mg L^{-1} $CuCl_2$ and then to 135.1 at 10 mg L^{-1} $CuCl_2$. The TF without the addition of $CuCl_2$ is 0.33, however, in the presence of $CuCl_2$ is below 0.04, this confirms the poor Cu translocation to the shoots. For the case of Na, it is stored in both organs, roots and above-ground tissues with BF varying from 9.9 to 16.3, however, it is translocated and stored in greater quantity in the shoots with a TF varying from 2.3 to 3.4, therefore even higher EFs have been obtained than those of BFs, varying from 38.5 to 47.6 with a salinity of 7.5 g L^{-1} NaCl and from 25.9 to 28.2 at 15 g L^{-1} NaCl (Fig. 3). It should be noted that although the higher the salt concentration in the medium, the higher the Na uptake in the different organs, however, the EF is higher at a salinity of 7.5 g L^{-1} NaCl because *S. europaea* is more efficient at absorbing Na when the concentrations in the medium are relatively low.

3.5 Mineral Composition of Substrate-Grown Plants

The elemental analysis shows the variation in the mineral composition of the shoots in relation to the different treatments. With respect to macronutrients such as P, K, Ca, Mg, and S, salinity has a significant effect on their concentrations in the shoots. Plants grown under non-saline conditions accumulate the highest concentrations of those macronutrients, decreasing significantly under saline conditions (Table 4). The other factors ($CuCl_2$ and biochar) have no significant effect on their concentrations, except for S, where the use of biochar significantly increases (p-value 0.0163) its concentration in the plant material with a mean

Table 4 Elemental analysis in the shoots of *S. europaea* cultivated in substrate. BC: Biochar. The values 0, 50 and 100 indicates the $CuCl_2$ concentration in mg kg^{-1} substrate. The values 0, 15 and 30 indicates the applied NaCl concentration in g kg^{-1} substrate. Values represent the mean of three biological replicates ± S.E. different letters indicate significant differences between treatments ($p < 0.05$)

$CuCl_2$ (mg kg^{-1})	NaCl (g kg^{-1})	Biochar	P	K	Ca	Mg	S	Na	Cu	Mn	Zn	Fe	B	Al
0	0	BC	5.98 ± 0.88 abcd	25.84 ± 4.35 a	48.23 ± 2.58 a	2.54 ± 0.15 a	15.19 ± 1.73 a	40.65 ± 3.35 d	0.03 ± 0.0043 abcd	0.37 ± 0.04 abcd	0.06 ± 0.0027 bcdef	0.12 ± 0.03 ab	0.12 ± 0.04 b	0.17 ± 0.06 b
0	0	NO BC	6.9 ± 0.35 a	27.54 ± 3.6 a	41.94 ± 5.48 a	2.74 ± 0.18 a	12 ± 1.97 a	36.82 ± 0.42 d	0.03 ± 0.01 abcd	0.42 ± 0.04 ab	0.06 ± 0.0045 abcdef	0.08 ± 0.0046 ab	0.35 ± 0.1 ab	0.34 ± 0.16 ab
0	15	BC	4.54 ± 0.43 abcde	19.73 ± 1.26 ab	26.2 ± 1.58 b	1.48 ± 0.07 b	5.8 ± 1.21 bcde	108.99 ± 2.76 c	0.01 ± 0.0041 d	0.23 ± 0.03 e	0.07 ± 0.01 abcd	0.13 ± 0.04 ab	0.22 ± 0.09 ab	0.35 ± 0.04 ab
0	15	NO BC	3.98 ± 0.52 abcde	11.51 ± 0.62 b	25.53 ± 0.36 b	1.38 ± 0.07 b	3.21 ± 0.18 e	113.61 ± 3.64 c	0.02 ± 0.0044 abcd	0.23 ± 0.01 e	0.05 ± 0.0027 cdef	0.07 ± 0.01 ab	0.46 ± 0.07 a	0.51 ± 0.08 ab
0	30	BC	2.74 ± 0.09 e	10.55 ± 0.69 b	29.13 ± 1.88 b	1.24 ± 0.03 b	4.26 ± 0.3 e	159.76 ± 4.87 a	0.03 ± 0.01 abcd	0.26 ± 0.01 cde	0.05 ± 0.01 cdef	0.04 ± 0.01 b	0.1 ± 0.06 b	0.1 ± 0.08 b
0	30	NO BC	3.44 ± 0.36 cde	10.1 ± 0.65 b	24.92 ± 2.22 b	1.21 ± 0.02 b	3.2 ± 0.54 e	149.68 ± 6.91 ab	0.03 ± 0.01 abcd	0.26 ± 0.02 cde	0.04 ± 0.0036 ef	0.04 ± 0.0034 b	0.18 ± 0.08 ab	0.27 ± 0.11 ab
50	0	BC	5.97 ± 0.34 abcd	24.75 ± 2.97 a	47.29 ± 2.31 a	2.85 ± 0.27 a	12.01 ± 1.4 ab	40.14 ± 2.72 d	0.04 ± 0.01 abc	0.38 ± 0.0031 abc	0.08 ± 0.0025 abc	0.1 ± 0.02 ab	0.25 ± 0.04 ab	0.25 ± 0.11 ab
50	0	NO BC	6.54 ± 1.7 ab	27.62 ± 0.42 a	46.04 ± 1.36 a	2.58 ± 0.12 a	10.94 ± 0.7 abcd	42.68 ± 4.32 d	0.02 ± 0.0039 bcd	0.43 ± 0.02 a	0.06 ± 0.01 abcde	0.12 ± 0.05 ab	0.2 ± 0.05 ab	0.22 ± 0.13 ab
50	15	BC	3.17 ± 0.14 de	18.98 ± 2.6 ab	28.77 ± 3.39 b	1.65 ± 0.18 b	4.79 ± 0.98 de	120.14 ± 11.19 c	0.05 ± 0.01 a	0.24 ± 0.02 e	0.05 ± 0.00066 def	0.07 ± 0.01 ab	0.09 ± 0.03 b	0.12 ± 0.04 b
50	15	NO BC	3.02 ± 0.1 de	12.4 ± 1.57 b	29.83 ± 2.71 b	1.54 ± 0.06 b	4.38 ± 0.58 e	114.21 ± 9.2 c	0.04 ± 0.01 abc	0.3 ± 0.02 bcde	0.04 ± 0.0025 f	0.05 ± 0.0032 b	0.16 ± 0.05 ab	0.15 ± 0.06 b
50	30	BC	2.98 ± 0.14 e	10.28 ± 1.41 b	25.34 ± 0.78 b	1.11 ± 0.13 b	4.15 ± 0.02 e	123.73 ± 9.46 bc	0.04 ± 0.0043 abcd	0.22 ± 0.02 e	0.06 ± 0.01 abcdef	0.11 ± 0.01 ab	0.28 ± 0.05 ab	0.36 ± 0.07 ab
50	30	NO BC	3.33 ± 0.14 de	11.68 ± 0.8 b	24.05 ± 1.04 b	1.28 ± 0.09 b	3.77 ± 0.41 e	126.01 ± 2.71 bc	0.01 ± 0.0031 d	0.26 ± 0.01 de	0.03 ± 0.0011 f	0.07 ± 0.02 ab	0.15 ± 0.05 ab	0.2 ± 0.07 ab
100	0	BC	6.33 ± 0.7 abc	26.6 ± 3.47 a	46.03 ± 2.37 a	2.67 ± 0.16 a	13.25 ± 3.26 a	38.5 ± 1.39 d	0.03 ± 0.0025 abcd	0.39 ± 0.03 ab	0.09 ± 0.01 a	0.08 ± 0.01 ab	0.21 ± 0.06 ab	0.18 ± 0.06 b
100	0	NO BC	5.42 ± 0.55 abcde	23.89 ± 0.75 a	47.6 ± 0.85 a	2.57 ± 0.04 a	11.28 ± 0.8 abc	35.18 ± 1.7 d	0.05 ± 0.00021 abc	0.4 ± 0.02 ab	0.06 ± 0.01 abcde	0.07 ± 0.01 ab	0.19 ± 0.01 ab	0.11 ± 0.01b
100	15	BC	3.6 ± 0.34 bcde	12.36 ± 0.78 b	27.65 ± 0.18 b	1.49 ± 0.06 b	5.57 ± 0.35 cde	112.53 ± 3.31 c	0.05 ± 0.01 ab	0.21 ± 0.04 e	0.08 ± 0.0045 ab	0.17 ± 0.02 a	0.4 ± 0.01 ab	0.65 ± 0.04 a
100	15	NO BC	3.77 ± 0.16 bcde	13 ± 0.71 b	28.44 ± 1.28 b	1.46 ± 0.05 b	3.95 ± 0.7 e	115.81 ± 6.98 c	0.02 ± 0.0035 cd	0.3 ± 0.01 bcde	0.05 ± 0.0027 cdef	0.07 ± 0.02 ab	0.37 ± 0.11 ab	0.42 ± 0.12 ab
100	30	BC	3.04 ± 0.35 de	12.72 ± 1.76 b	27.43 ± 0.99 b	1.3 ± 0.04 b	4.28 ± 0.34 e	136.58 ± 4.43 abc	0.02 ± 0.01 abcd	0.24 ± 0.02 e	0.04 ± 0.01 ef	0.08 ± 0.03 ab	0.17 ± 0.06 ab	0.21 ± 0.14 ab
100	30	NO BC	3.05 ± 0.17 de	13.22 ± 0.75 b	24.1 ± 0.76 b	1.26 ± 0.15 b	4.03 ± 0.08 e	128.95 ± 5.55 bc	0.03 ± 0.0047 abcd	0.26 ± 0.01 de	0.04 ± 0.004 ef	0.05 ± 0.0047 b	0.22 ± 0.02 ab	0.25 ± 0.01 ab

value of 7.70 mg g^{-1} DW, whereas without the use of biochar its mean concentration is 6.61 mg g^{-1} DW.

With regard to micronutrients, salinity and biochar factors have significantly influenced their concentrations in the plant material. For example, the concentrations of Zn, Mn and Fe decrease under saline conditions (Table 4). With the use of biochar the concentrations of Fe and Zn increase in the shoots. The Na concentration in the shoots increases proportionally with the concentration of salt in the medium, while biochar has no significant influence on the Na uptake by plants. Regarding the Na concentration in the plant material, there is an interaction between the two factors (NaCl and $CuCl_2$). The highest Na concentration is found in plants grown at a salinity of 30 g kg^{-1} and without the addition of $CuCl_2$ with an average value of 154.72 mg g^{-1} DW while the lowest Na concentration is found in plants grown under non-saline conditions and with a concentration of 100 mg kg^{-1} $CuCl_2$.

3.6 Bioavailable Elements in the Substrate

To evaluate the contribution of biochar to plant available elements, elements were extracted from substrate samples and then analyzed with ICP-OES. According to the results, salinity but mainly biochar contributes significantly to the availability of elements in the substrate. Most macro- and micronutrients are found at higher concentration under non-saline conditions. This is mainly due to the fact that the plants produced less biomass, extracting less nutrients than those grown under saline conditions. Interestingly, biochar contributes significantly to the higher availability of most nutrients. For example, the mean value of P using biochar is 3.02 mg g^{-1} DW while in the absence of biochar the concentration decreases significantly to 0.35 mg g^{-1} DW. The mean concentration of Mg is also higher using biochar (Fig. 4). This trend is observed for most elements including micronutrients. For example, Fe has an average value of 2.19 mg g^{-1} DW in the substrate where biochar was applied, while in the absence of biochar, the average value is only 0.89 mg g^{-1} DW.

With the exception of Al, biochar does not contribute to increasing the availability of heavy elements. The Al concentration determined in the substrate using biochar is 2.83 mg g^{-1} DW while in the substrate without biochar it is significantly lower with an average value of 0.84 mg g^{-1} DW, however, there is no difference in its concentration in the plant shoots, being poorly translocated to the above-ground tissues. It must be taken into account that the concentration of the different elements in the biochar depends on the source of the raw material, and it is necessary to characterize the biochar before it is used in the field, taking into account local regulations.

3.7 Principal Components Analysis (PCA) for the Plants Cultivated in Substrate

According to the PCA, the highest variability is explained by PC1 (58.7%) and to a lesser extent by PC2 (30.2%). Macronutrient concentrations (S, K, Ca, Mg, P) in the plants are highly correlated with each other, being directly proportional to plants grown under non-saline conditions. This is due to the fact that the plants cultivated under non-saline conditions produce less biomass. Therefore, the concentration of elements is higher, however, the plants are under stress because *S. europaea* is an obligate halophyte requiring salt for its optimal growth. This is also confirmed by the concentration of phenols and proline, being higher under non-saline conditions. Plants grown under saline conditions produce a higher amount of biomass, with a higher Na uptake efficiency. These variables are in direction with the 30 g L^{-1} NaCl treatment and with the use of biochar so that under these conditions a higher plant growth is obtained (Fig. 5).

As can be seen in Table A1. biomass production is highly correlated with the sodium concentration in the plant material (0.91) and in soil (0.78), so its production is highly dependent on the salinity of the substrate. There is also a high correlation between the variables Na concentration in the soil and in the plant material (0.94), being a plant species that uptakes sodium proportionally to the amount present in the substrate. Biomass has a negative correlation with the concentration of elements in the plant material, but this is due to the dilution effect, because the plants produced more biomass, diluting the concentration of nutrients. Phenols and proline are also negatively correlated with sodium in plants and soil, an indicator that plants are under stress under non-saline conditions.

In Fig. 6 it can be seen that there is a positive correlation between the concentration of Na in the soil and the concentration of Na in the plant material, however, its behavior is not linear, the concentration of Na in the tissues stabilizes at concentrations higher than 2 mg g^{-1} Na in the substrate. The same happens with phenols, but with a negative correlation with respect to soil salinity, where their concentrations decrease rapidly as the concentration of Na in the substrate increases, stabilizing at concentrations higher than 2 mg g^{-1} Na in the substrate.

3.8 Accumulation of Polyphenols in the Plants Cultivated in Hydroponics

The results reveal a general increase of the accumulation of metabolites proportional to the amount of Cu in the culture medium, with the highest concentration found at 10 mg L^{-1} $CuCl_2$ and 7.5 g L^{-1} NaCl for both phenolic compounds and flavonoids (5.55 mg GAE g^{-1} DW and 4.02 mg CE g^{-1} DW, respectively), which may be a result of plant stress caused by high Cu concentration (Table 5). According to the analysis of variance, there is a significant difference between the different $CuCl_2$ treatments (p value 0.0313), while the salinity doesn't indicate a significant effect on the metabolite concentration found in the plant material. However, except for the control (no $CuCl_2$ added), the concentration of total phenols quantified at 15 g L^{-1} NaCl is always lower compared with the one measured at the same Cu treatment but lower salinity, suggesting a role of Na in the reduction of the plant stress created by the presence of Cu. These findings could confirm once again the importance of salt for the obligate halophyte *S. europaea* which requires salt for its optimal growth and shows a reduced accumulation of these bioactive compounds in presence of moderate salt concentrations, even when exposed to a high concentration of contaminants.

3.9 Accumulation of Phenolic Compounds and Proline in the Plants Cultivated with Substrate and Biochar

Higher levels of proline and phenols have been found in the plants cultivated under non-saline conditions. In particular the presence of biochar is shown to decrease significantly the concentration of these organic compounds, meaning that the plants are under less oxidative stress. For example, the proline concentration under non-saline conditions using biochar is 2.3 mol g^{-1} DW while without the use of biochar it increases significantly to 7.8 mol g^{-1} DW (Fig. 7).

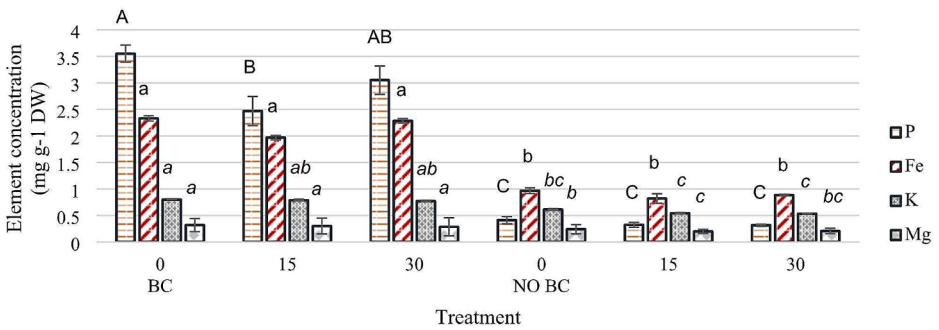


Fig. 4 Concentration of elements in the substrate after harvesting *S. europaea* plants. The values on the x-axis (0, 15 and 30) represent the different salt concentrations in g kg^{-1} substrate. BC: Biochar; NO BC: No biochar applied. Bars represent the mean value ± S.E. Different letters indicate significant differences between treatments ($p < 0.05$)

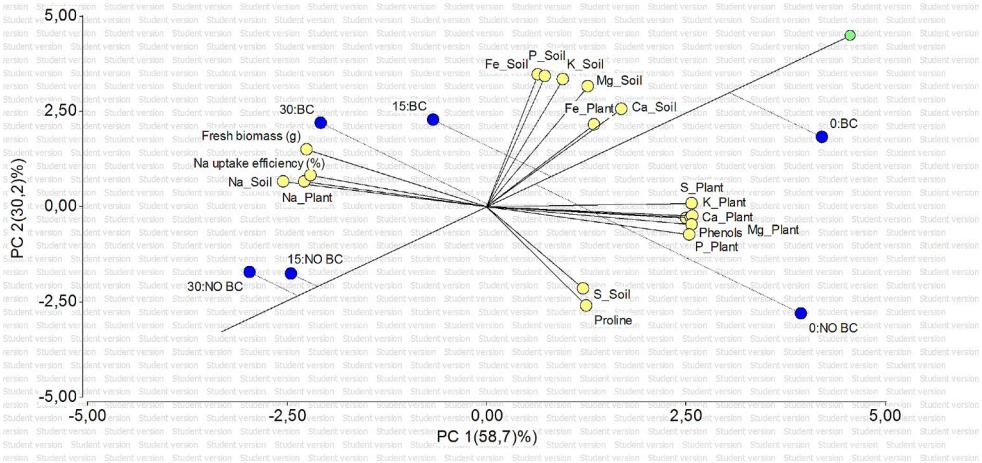


Fig. 5 Principal components Analysis (PCA) of the different parameters of plants cultivated in substrate. The blue dots represent the different combination of treatments: 0, 15 and 30 g L^{-1} NaCl, and the use of biochar (BC) or without biochar (NO BC)

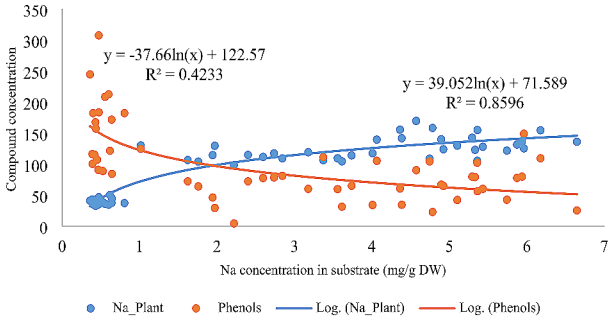


Fig. 6 Correlation between Na concentration in the substrate and its concentration in the plant material and total phenols. Na_Plant concentration of Na in the shoots (mg g^{-1} DW). Phenols in µg GA g^{-1} FW

3.10 Phytoextraction Capacity of *S. Europaea* Cultivated in Hydroponics

The elemental analysis reveals that *S. europaea* is a salt accumulator, confirming what has previously been reported in the literature about the potential of this species to extract salt from a salt-rich medium and highlighting the plant's adaptation to higher salinities through improved Na^+ uptake when the salt concentration is higher. The results with the different Cu treatments indicates that *S. europaea* can tolerate large concentrations of this metal, supporting the already reported tolerance of salt-accumulating halophytes to some heavy metals. In this case, the highest concentration of Cu (1.6 mg g^{-1} of Cu) is found in the roots of plants grown at 7.5 g L^{-1} NaCl and 10 mg L^{-1} $CuCl_2$, whereas the highest concentration of Na (162 mg g^{-1} DW) is found in the shoots of the controls (plant grown without $CuCl_2$) at 15 g L^{-1} NaCl, with very similar results in plants cultivated in the absence of $CuCl_2$. It is important that plants with high remediation capacity also produce a high amount of biomass, so that the plants have a greater potential to extract and accumulate pollutants. This means that for real technical phytoremediation purposes, the objective doesn't refer to achieve the highest Na^+ concentration in shoots and roots, but to ensure the highest absorption rate associated with the best plant development possible. In this context, Table 6 refers to the total amount of Na and Cu removed from the medium.

4 Discussion

The $CuCl_2$ treatments significantly decreased the biomass yield in hydroponics, producing plants with pronounced chlorosis. The differences in the biomass production confirm the impact of Cu in the plant performance, with lower growth at 10 mg L^{-1} $CuCl_2$. This effect is also visible at both salinities, however, the different concentrations of

Table 5 Concentration of total phenols and flavonoids determined in the shoots of *S. Europaea* cultivated under hydroponic conditions. Values represent the mean of three replicates. Plants cultivated for 25 days under two salt treatments (7.5 g L^{-1} and 15 g L^{-1} NaCl) and three copper treatments (0 mg L^{-1}; 5 mg L^{-1} and 10 mg L^{-1} $CuCl_2$). Different letters indicate significant differences between treatments ($p < 0.05$). DW = dry weight

Phenolic compounds (mg GAE g^{-1} DW)	Salt treatment (g L^{-1} NaCl)	Cu treatment (mg L^{-1} $CuCl_2$)		
		0	5	10
	7.5	1.57 ± 0.11 **b**	3.73 ± 0.2 **ab**	5.55 ± 1.81 **a**
	15	2.84 ± 0.61 **ab**	1.74 ± 0.12 **ab**	3.58 ± 0.47 **ab**
Flavonoids (mg CE g^{-1}DW)				
	7.5	0.84 ± 0.04 **a**	2.24 ± 0.17 **a**	4.02 ± 1.76 **a**
	15	1.73 ± 0.65 **a**	0.76 ± 0.09 **a**	2.08 ± 0.14 **a**

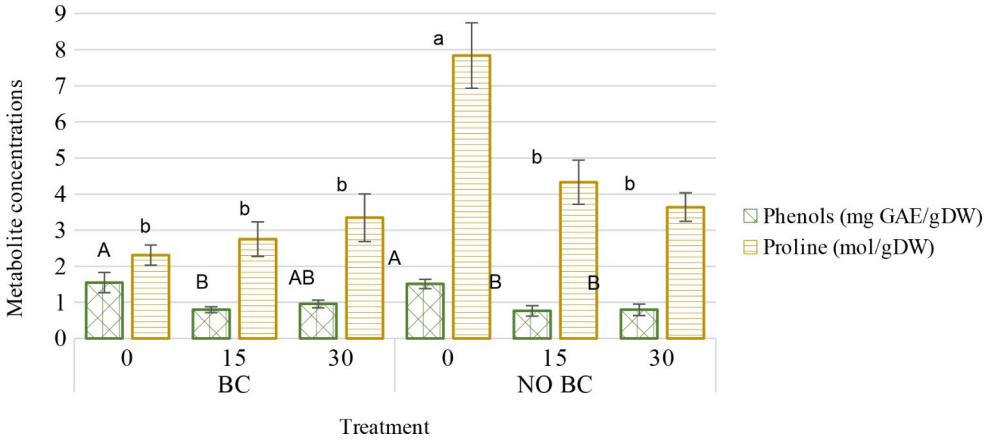


Fig. 7 Metabolite concentration in the plant tissues of *S. europaea* cultivated in substrate at different salinities (0, 15 and 30 g kg-1 substrate), and with biochar (BC) and without biochar (NO BC). Bars represent mean value ± S.E. Different letters indicate significant differences between treatments ($p < 0.05$)

Table 6 Summary of the total amount of na (g) and Cu (mg) found in *S. Europaea* (shoots + roots) per experimental unit. Different letters indicate significant differences between treatments ($p < 0.05$)

Initial concentration		Element uptake	
Na (g)	Cu (mg)	Na (g)	Cu (mg)
6.43 ± 0.07	5.97 ± 0.00017	1.5 ± 0.22 **a**	0.15 ± 0.04 **c**
6.22 ± 0.08	18.72 ± 0.00048	0.72 ± 0.08 **b**	1.05 ± 0.2 **abc**
6.25 ± 0.01	23.98 ± 0.0013	0.49 ± 0.04 **b**	1.61 ± 0.41 **a**
11.97 ± 0.03	7.08 ± 0.00049	1.77 ± 0.09 **a**	0.37 ± 0.09 **bc**
11.92 ± 0.03	15.81 ± 0.00031	0.76 ± 0.15 **b**	0.77 ± 0.09 **abc**
11.68 ± 0.07	25.01 ± 0.00039	0.72 ± 0.12 **b**	1.32 ± 0.26 **ab**

NaCl do not affect significantly the biomass yield within the same Cu treatment. This chlorosis could be caused by the dramatic changes in the mineral composition found in the shoots and roots of the plants exposed to Cu and, therefore, by a possible deficiency in one or more micronutrient like Fe, Mg or Mn, where chlorophyll biosynthesis can be impaired. The stronger chlorosis (Fig. A1) of the shoots of the plants exposed to $CuCl_2$ at the highest salinity (15 g L^{-1} NaCl) could be due to the combined effect of Na and Cu to impede the uptake of other essential nutrients, and the chlorophyll synthesis and photosynthesis can be impaired. According to Printz et al. (2016) Fe in biological molecules can be substituted by Cu which is able to perform similar functions. For this reason, many Cu-proteins have a functional counterpart that uses Fe as cofactor and plant growth on a substrate with a high Cu concentration is commonly linked to a decreased Fe-content in plant tissues. Therefore, plants with Cu toxicity share similarities with those related to Fe-deficiency, such as the presence of leaf chlorosis, decreased leaf chlorophyll content and enhanced oxidative stress (Printz et al. 2016). The decrease in chlorophyll due to high Cu concentrations may also be due to inhibition of aminolevulinic acid dehydratase involved in early steps of chlorophyll biosynthesis (Hirve and Jain 2022).

The chlorosis linked to a decrease of the chlorophyll content and chloroplast damage have been reported in *S. europaea* plants irrigated with heavy metal-polluted wastewater containing 0.4 mg L^{-1} to 0.6 mg L^{-1} Cu (Khalilzadeh et al. 2020). However, it must also be considered that different Cu valences can have different results with respect to toxicity (Young and Santra 2014). Hajihashemi et al. (2020) also confirmed the impact of metal concentrations and exposure time on the light-dependent reactions, chlorophyll-a fluorescence and electron transport. In this sense, chlorosis may be caused by the uptake inhibition and translocation of other essential elements like Fe and Zn under exposure to high concentrations of heavy metals, which has an antagonistic effect on the synthesis of different pigments (Bazihizina et al. 2015). Pätsikkä et al. (2002) also suggested that the reduction of leaf chlorophyll concentration, caused by the Cu-induced iron deficiency, can cause high photosensitivity of photosystem II in Cu^{2+}-treated plants.

The highest concentration of Cu has been determined in the roots, suggesting that the roots are affected more severely by the exposure to Cu than other parts of the plant. The inhibitory effect of Cu on the root development agrees to what was found in other studies and may be linked to reduced water uptake and reduction in mitotic cells in the

meristematic area of the roots (Kabir et al. 2010). These outcomes elucidate the negative effects of high Cu concentrations on the physiology and development of hydroponically grown plants, with a strong inhibition of the root system leading to substantial changes in the shoot development and chlorophyll production.

Plants grown on solid substrate behave differently from hydroponic culture, showing no visible toxic effects at high concentrations of Cu, moreover, optimal growth is obtained with a concentration of 50 mg kg^{-1} $CuCl_2$, a salinity of 15 g kg^{-1} and with the use of biochar. This may be due to the fact that both substrate and biochar have the ability to adsorb high amounts of the different elements within their cation exchange complex, so their bioavailability to plants may be different compared to hydroponics. Castaldi and Melis (2004) also suggested that most metals interact strongly with organic matter limiting their uptake by the different crops.

Salt has an impact on the uptake of other elements such as Ca, K, and Mg, where increasing the concentration of NaCl in the different culture media decreases the uptake of those elements in the plant tissues. This could be an antagonist effect of salt on other ions, as the plants uptake more Na^+ excluding other cations. Biochar doesn't show an effect on Ca, K and Mg, however, it increases the uptake of S and microelements such as Fe and Zn. The significant decrease of macronutrients under higher salinities may also be due to the dilution effect. Plants grown in substrate under non-saline conditions produce a lower amount of biomass compared to plants grown under saline conditions, so elements may accumulate more than in plants with better growth. Based on these results obtained from the plants cultivated in substrate, biochar positively benefits the concentration of micronutrients in the plant tissues, because it contains different micronutrients which are available to the plants. However, biochar does not contribute to increase the concentration of certain heavy elements in the plant material such as Pb and Al. In future phytoremediation projects on saline soil the parallel application of biochar to the soil might increase the efficiency of the remediation process due to increased nutrient availability, plant health and water holding capacity (Hansen et al. 2023).

The concentrations of Na and Cu in plant tissues vary significantly depending on the treatment. In the case of Na, its concentration in both roots and shoots increases with increasing concentration of NaCl in the culture medium, having translocation factors greater than 2.3, which indicates a very efficient ability of *S. europaea* to transport Na from roots to shoots. Instead, Cu accumulates in the roots but is poorly translocated to the above-ground tissues. For example, under non-saline conditions, the highest Cu concentration determined in the shoots is 0.04 mg g^{-1} DW in the plants grown at 100 mg kg^{-1} $CuCl_2$ followed by the 50 mg kg^{-1} and 0 mg kg^{-1} $CuCl_2$ treatments with approximately the same concentration of 0.03 mg g^{-1} DW. This indicates that under high Cu concentrations, Cu is poorly translocated from the roots to the above-ground tissues as confirmed by the hydroponic experiment, where the different $CuCl_2$ concentrations in the growing medium have no significant effect on the Cu concentration in the shoots, with concentrations very similar to the control (cultivated in the absence of $CuCl_2$). Therefore, *S. europaea* is suitable for soil phytostabilization due to its ability to retain large amounts of Cu in the root system but it is not translocated to the shoots. This pattern is confirmed in other plant species able to accumulate moderate Cu contents (Cao et al. 2018; Valderrama et al. 2013), suggesting that, even at high concentrations in the medium, in many plants, Cu is in general not translocated to the shoots.

The potential of salt-tolerant plants in the immobilization or removal of heavy metals from saline environments has been already suggested by Manousaki and Kalogerakis (2011) but, to date, this is the first study presenting the ability of the halophyte *S. europaea* to accumulate Cu cultivated under different salinities and different growth media. Regarding the hydroponic experiment, *S. europaea* plants treated with 5 mg L^{-1} and 10 mg L^{-1} of $CuCl_2$ (0.037 mM and 0.074 mM Cu respectively), accumulated in the roots a concentration ranging from 0.8 to 1.60 mg g^{-1} DW of Cu. These values are below the range found by Cao at al. (2018), who highlighted that the highest concentrations were found in the roots of seven willow species with values ranging from 1.9 to 26 mg g^{-1} DW, however, the species were exposed to higher Cu concentration ranging from 0.015 mM to 0.12 mM, and for a longer time period (40 d). The Cu concentrations used in this study are more similar to those reported by Kamal et al. (2004) who found in 12 different plant species including *Marsilea drummondii*, *Hippuris vulgaris*, *Cyperus pseudovegetus*, and *Baumia rubiginosa*, Cu concentrations varying from 0.015 mg g^{-1} and 0.095 mg g^{-1} DW in the shoots and from 0.2 mg g^{-1} to 1.15 mg g^{-1} DW in the roots. The results are also in the range found by Valderrama et al. (2013), who reported 1.9 mg g^{-1} DW and 1.2 mg g^{-1} DW of Cu *in Azolla filiculoides* grown at 5 mg L^{-1} and 10 mg L^{-1} $CuCl_2$ respectively, and then the Cu concentration increased to 6.01 mg g^{-1} DW in plants cultivated at 25 mg L^{-1} $CuCl_2$, indicating that the accumulation in this plant species is proportional to the Cu exposure. However,

it must be considered that Cu concentration varies as a function of species, plant organs, stage of growth, and environmental factors. Although the Cu content found in the roots of *S. europaea* increases proportionally to the concentration of $CuCl_2$ in the hydroponic medium, it is important to take into account that plants exposed to high concentrations may show toxic effects and their phytoextraction capacity is compromised. It should also be noted that most Cu accumulates in the roots and is poorly translocated to the aboveground plant tissues regardless of the Cu concentration in the growing medium as discussed above.

Differences in the amount of Na and Cu uptake and translocation are also due to the different nutrient requirements and transport mechanisms used by plants, which differ between species. The uptake of Na^+ by halophytes like *S. europaea* involves several specialized mechanisms that enable these plants to thrive in high-salinity environments. Na^+ influx across the plasma membrane in the roots occur mainly via nonselective cation channels. At low levels, Na^+ is harmless and uptake in the high affinity range may be a purely 'passive' process. Long-distance transport to the shoot relies on loading into the xylem. The exact transport mechanisms for xylem loading of Na^+ have yet to be determined but may comprise both passive loading (mediated by Na^+-permeable ion channels located at the xylem–parenchyma interface (Wegner and De Boer 1997), and active loading mediated by Na^+/H^+ exchangers (Maathuis 2014). The first mechanism by *S. europaea* to overcome high Na^+ concentrations is the water storage in the parenchyma. This dilutes the accumulated salts, thus helping to maintain cellular turgor, allowing the plant to cope efficiently with high salinity. Regarding Cu there are no specific studies regarding its uptake and translocation in *S. europaea*, although the high-affinity Cu transporter protein (COPT) family is a selective Cu(I) transporter in roots (Ryan et al. 2013). Then the Cu translocation in some species like in tomato can involve complexing with amino acids in the xylem sap (Liao 2000), however, each plant species may have different transport mechanisms. For future applications, more halophytic plant species and even more *Salicornia* genotypes need to be tested for their soil phytostabilization and phytoremediation potential to select the best suited halophytes for a particular soil type, salinity and heavy metal abundance as they follow different physiological strategies to avoid toxicity. Results from constructed wetlands of saline waters in phytoremedition could be taken into account (Turcios et al. 2021). Using the knowledge already obtained from constructed wetlands up-scaling from greenhouse experiment might be facilitated.

The different $CuCl_2$ treatments in the hydroponic medium increase the concentration of polyphenols in the plant tissues. This increase may be due to an increase in plant stress. The stress generated by the exposure to Cu is primarily associated with damages on the plasma membranes, which are frequently attributed to lipid peroxidation and lead to leakage of K^+ and other substances, in addition to the production of a large number of active oxygen free radicals, which attack all types of biomolecules like nucleic acids, proteins, lipids and amino acids (Chen et al. 2015; Khalilzadeh et al. 2020). Halophytes may tolerate high concentrations of heavy metals through their high production of different antioxidants including polyphenols, carotenoids and vitamins when growing under stress conditions. To transfer these finding into praxis, glycophytes could be treated with extracts of halophytes to mitigate stress effects caused by both high salinity and heavy metals. Promising results have been already obtained by foliar application of extracts of the halophyte *Arthrocnemum macrostachyum* on soybean plants (Osman et al. 2021). The protein pattern in soybean was modified indicating a fundamental effect of the halophytic extract on molecular level. The oxidative stress generated by Cu and other heavy metals may be neutralized by the same protection mechanisms involved in the tolerance to salinity, including the accumulation of non-enzymatic and enzymatic antioxidant compounds and osmoprotectants like proline (Manousaki and Kalogerakis 2011). This is also confirmed by our results, where the concentrations of total phenols and flavonoids in the plants cultivated in hydroponics at 5 mg L^{-1} and 10 mg L^{-1} $CuCl_2$, are significantly different compared to the control, with the highest values always found at 10 mg L^{-1} $CuCl_2$, confirming the stress condition of the plants at these high Cu concentrations and the involvement of phenols and flavonoids in the antioxidant activity. This approach was implemented to determine the potential for Cu nanoparticles as foliar application to increase the salt tolerance of *Trigonella foenum-graecum* L. and to better cope with the detrimental impacts of salt stress by inducing protection mechanism and therefore mitigating the effects (Fouda et al. 2024). It is important to keep in mind that Cu-based nanoparticles offer a variety of possible agricultural applications, but their persistent and prolonged use may have negative impacts on the soil and on the ecosystems. However, it must also be considered that under stress conditions, enzymatic reactions may be involved. For example, Khalilzadeh et al. (2020) reported the enhancement of the antioxidant-enzymatic activity under heavy metal-induced oxidative damage, with catalase and peroxidase as the most important ROS-scavenging enzymes in

S. europaea. Additionally, the synthesis of other bioactive molecules and osmolytes like soluble sugars and proline, is a well-known biochemical response in plants, with the latter known to increase the tolerance to heavy metals through metal chelation in the cytoplasm, inhibition of lipid peroxidation, maintenance of the water balance and protection of the protein synthesis (Li et al. 2013).

Regarding the plants cultivated in substrate, interestingly Cu treatments haven't shown any significant effect on the concentration of total phenols (data not shown), where the different Cu concentrations used in the substrate do not significantly affect the physiology of the plants. Comparing this experiment using substrate with hydroponics, the substrate buffered the possible toxic effect of the relatively high Cu concentrations, where the substrate and biochar particles can adsorb sufficient amounts of the element on their surface, whereas in hydroponic media the different elements are in solution, being more available to the plants, reaching a toxic level at lower concentrations. Interestingly, the presence of biochar in the substrate significantly decreases the proline concentration in the plant tissues. This characteristic of biochar to decrease the plant stress can be related to its ability of retaining high moisture in the substrate and providing the plants with necessary microelements like Fe and Zn. The positive and supporting role for plant health of biochar should be taken into account for large scale phytoremediation projects in different types of soil, saline and non-saline (Hansen et al. 2023). The impact of salinity is clear as well, being *S. europaea* a true halophyte it needs salt in the medium for optimal growth, in fact the highest concentration of proline and phenolic compounds is found in plants without salt treatment.

5 Conclusions

The halophyte *S. europaea* has a high potential to be used in the phytoremediation process in saline environments contaminated with metals such as copper (Cu). Its highest biomass production is obtained in saline environments, with salinities varying from 1 to 3%, its optimum production depends on other factors including the type of substrate and environmental conditions. Interestingly, salinity does not significantly affect Cu uptake, however, the presence of Cu affects growth and physiology more severely in hydroponic conditions compared to substrate culture, with reduced plant growth and pronounced chlorosis. The use of substrate with biochar helps to mitigate the toxic effects of copper and at the same time improves plant growth through better moisture retention and increased availability of nutrients to the plants. *Salicornia europaea* has the ability to uptake relatively high amounts of Na and translocate it to the above-ground tissues with translocation factors higher than 2.3, but Cu is mostly accumulated in the roots and poorly translocated to the above-ground biomass, with translocation factors lower than 0.33. This may have the advantage of being able to use this plant species for the phytoextraction of Cu through the roots while the aerial part can still be used for different purposes in the industry, including food and feed, pharmaceutical and nutraceutical, cosmetic, and bioenergy sectors. As part of bioeconomy approaches this fact paves the way for larger scale phytoremediation projects on saline soil using *S. europaea* and other halophyte plants species as attractive industrial markets are currently being developed.

Appendix

Table A1 Correlation factors

Correlation matrix/Coefficients																		
	Fresh mass	Phenols	Proline	Ca_Plant	Fe_Plant	K_Plant	Mg_Plant	Na_Plant	P_Plant	S_Plant	Ca_Soil	Fe_Soil	K_Soil	Mg_Soil	Na_Soil	P_Soil	S_Soil	Na uptake
Fresh mass	1																	
Phenols	-0,85	1																
Proline	-0,6	0,52	1															
Ca_Plant	-0,86	0,98	0,5	1														
Fe_Plant	-0,18	0,29	-0,09	0,37	1													
K_Plant	-0,9	0,92	0,51	0,96	0,54	1												
Mg_Plant	-0,91	0,96	0,51	0,99	0,41	0,98	1											
Na_Plant	0,91	-0,94	-0,54	-0,98	-0,4	-0,98	-1	1										
P_Plant	-0,94	0,94	0,57	0,97	0,39	0,98	1	-1	1									
S_Plant	-0,87	0,97	0,4	0,99	0,44	0,97	0,98	-0,97	0,97	1								
Ca_Soil	-0,22	0,54	-0,05	0,55	0,84	0,6	0,51	-0,47	0,46	0,61	1							
Fe_Soil	0,2	0,21	-0,52	0,19	0,6	0,16	0,12	-0,06	0,04	0,27	0,84	1						
K_Soil	0,09	0,27	-0,41	0,28	0,77	0,3	0,23	-0,18	0,16	0,36	0,93	0,97	1					
Mg_Soil	-0,07	0,39	-0,35	0,39	0,79	0,43	0,36	-0,31	0,29	0,49	0,95	0,94	0,99	1				
Na_Soil	0,78	-0,81	-0,47	-0,89	-0,43	-0,88	-0,92	0,94	-0,91	-0,86	-0,4	-0,01	-0,14	-0,24	1			
P_Soil	0,16	0,24	-0,55	0,23	0,59	0,19	0,16	-0,1	0,08	0,32	0,82	0,99	0,96	0,94	-0,06	1		
S_Soil	-0,54	0,42	0,95	0,42	0,16	0,52	0,47	-0,5	0,53	0,35	0,07	-0,45	-0,28	-0,23	-0,47	-0,49	1	
Na uptake	0,9	-0,92	-0,51	-0,86	-0,08	-0,82	-0,85	0,83	-0,86	-0,87	-0,35	-0,05	-0,08	-0,23	0,6	-0,08	-0,38	1

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Declarations

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Consent to Participate Not applicable.

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