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Tripleurospermum maritimum from a coastal shingle beach: nitrophilic status, tolerance to salinity and heavy metals

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Abstract

Tripleurospermum maritimum is a species characteristic for several protected sea coast habitats. The aim of the present study was to investigate possible nitrofilous status, tolerance to salinity and heavy metals, as well as the metal accumulation potential of *T. maritimum* plants from a sea-affected coastal habitat in controlled conditions. Plants were cultivated in an automated greenhouse in containers with a substrate made from garden soil and quartz sand. Three separate experiments were performed: (i) plants received combined treatment of three different doses of mineral fertilizer plus additional NO_3-N or NH_4-N; (ii) plants were treated with increasing concentration of NaCl; and (iii) plants were treated with increasing concentration of Cd or Pb. After seven weeks of cultivation, plant morphological characteristics, soluble ion concentration in tissue extracts and tissue Cd and Pb concentrations were measured. Increase in mineral nutrient availability stimulated shoot growth of *T. maritimum* plants. Addition of N fertilizer resulted in further increase of shoot biomass by 20 to 30%. Plants exhibited good tolerance to increased Na^+ concentration, with only about 30% reduction in shoot biomass at 5 g L^{-1}. High electrolyte accumulation potential was evident in shoots, with preferred accumulation of K^+ over Na^+. Tolerance to heavy metals was very high, as growth of plants was not affected even in 100 mg L^{-1} Cd treatment. Root biomass of plants treated with 500 mg L^{-1} Pb was reduced only by 35%, while shoot growth was unaffected. Both Cd and Pb were preferentially accumulated in roots of *T. maritimum*, with concentration in aboveground parts being less than 10% from that in roots. In conclusion, the coastal accession of *T. maritimum* from a shingle beach is moderately nitrophylic with high salinity tolerance and very high tolerance to heavy metals, with characteristic exclusion of the metals from aboveground parts.

Key words: coastal species, electrolyte accumulation, mineral fertilizer, heavy metals, nitrogen, salinity, shingle beach, *Tripleurospermum maritimum*.
Abbreviations: DM, dry mass.

Introduction

Coastal species from seawater-affected habitats are promising models in studies of plant adaptation mechanisms to soil chemical heterogeneity. These habitats are highly dynamic in respect to nutrient availability, substrate salinity as well as presence of heavy metals, creating a need for well-balanced mechanisms to provide chemical homeostasis of cells and tissues (Ievinsh 2006).

Several species of the genus *Tripleurospermum* (Asteraceae) can be found in coastal habitats, including *Tripleurospermum martimum* (L.) W.D.J.Koch (syn. *Matricaria maritima* L.) and *Tripleurospermum inodorum* (L.) Sch.Bip. [syn. *Matricaria inodora* L., *Matricaria perforata* Mérat, and *Tripleurospermum perforatum* (Mérat) M.Lainz]. Sometimes the two species are regarded as subspecies of T. *maritimum* (or *T. inodorum*) due to a large phenotypic plasticity (Kay 1994). Both species are generally considered as common weeds. *T. maritimum* is a biennial or short-lived perennial plant found on salt-affected drift-lines and sea-cliff habitats on the coasts of western and northern Europe (Kay 1972). It is a characteristic species of the European protected habitats EUH 1230 "Vegetated sea cliffs of the Atlantic and Baltic coasts" and EUH 1330 "Atlantic salt meadows (*Glauco-Puccinellietalia maritimae*)" (EC 2013).

According to recently established ecological indicator values, *T. maritimum* in Sweden is characterized as favoured by moderate salinity, but not restricted to saline habitats (indicator value 3 out of 5) and can be found on extremely N-enriched soils (indicator value 9 out of 9) (Tyler et al. 2021). Consequently, *T. maritimum* can be expected to represent coastal-specific nitrofilous species with moderate salinity tolerance. In sea-affected habitats, *T.*

maritimum can be characterized as a moderately Na^+- and K^+-accumulating species behaving as a tight regulator of electrical conductivity in tissue water (Ievinsh et al. 2021). Only one study so far aimed at experimentally revealing salinity tolerance of *T. maritimum* (syn. *M. maritima*), using seed material from littoral dunes on the Atlantic coast in France (Ben Hamed et al. 2014). As a result, it was characterized as an intermediately salinity tolerant, Na^+-including species. However, based on observations performed in the Eastern Greenland, it was suggested that *T. maritimum* represents "a long-lived halophyte well able to withstand competition from other strand species" (Corner 2012). In addition, salinity tolerance of several taxonomically related species (*Matricaria chamomila* L. and *Matricaria recutita* L.) has been assessed for practical reasons (Baghalian et al. 2008; Razmjoo et al. 2008; Heidari, Sarani 2012).

There is no information on heavy metal tolerance of *T. maritimum* available in the literature, but there are several reports concerning several taxonomically closely related species. For example, when cultivated in hydroponics, *M. chamomilla* has been shown to be tolerant to cadmium (Kováčik et al. 2006; Farzadfar et al. 2013), partially tolerant to Cu (Kováčik et al. 2008) and highly tolerant to Ni (Kováčik et al. 2009). Also, *M. recutita* has shown high tolerance to Cd (Pavlovič et al. 2006). Native presence of *M. chamomilla* in soils heavily contaminated with heavy metals Cd and Pb also indicates high metal tolerance status of the species (Voyslavov et al. 2013). Based on the mentioned results, heavy metal tolerance of *T. maritimum* can be proposed.

The aim of the present study was to investigate possible nitrofilous status, tolerance to salinity and heavy metals, as well as electrolyte and the heavy metal accumulation potential of *T. maritimum* plants from a sea-affected coastal habitat in controlled conditions of a vegetation container study.

Materials and methods

Seeds from a coastal accession of *T. maritimum* collected on shingle beach of the Baltic Sea in Ohesaare, Saaremaa Island, Estonia, growing within 2 m zone from a waterline, were used to establish the species in cultivation in conditions of an automated greenhouse. Seeds were dried in laboratory conditions for one month and then were stored at 4 °C. Procedures used for plant establishment as well as cultivation conditions in greenhouse are described in detail previously (Andersone-Ozola et al. 2021). Briefly, seeds were surface sterilized with 5% NaOCl, imbibed in water and germinated in autoclaved substrate (Garden Soil, Biolan, Finland) in plastic plant tissue culture containers in a growth cabinet. Established seedlings with the two true leaves were individually transplanted first to 250 mL plastic containers and after two weeks to 1.3 L plastic containers filled with a mixture of heat-treated substrate containing Garden Soil and quartz sand (1:1, v/v). Plants were cultivated in an experimental automated greenhouse with supplemented light (380 µmol m^{-2} s^{-1} at the plant level) with 16 h photoperiod, day/night temperature 24/16 °C, and relative air humidity 60 to 70%. Substrate water content was kept at 50 to 60% using deionized water.

Three separate experiments were performed with 3-week-old well-established plants: (i) effect of combined treatment of three different doses of mineral fertilizer plus additional treatment with nitrogen fertilizer applied as NO_3-N or NH_4-N; (ii) effect of increasing substrate concentration of NaCl; and (iii) effect of increasing concentration of Cd or Pb.

For the fertilizer and nitrogen treatment experiment, plants were randomly distributed in nine groups, five individual plants per group. Three different doses of mineral fertilizer were used as a basic treatment, consisting of 5, 10 or 15 mL or 1% mineral fertilizer Kristalon Red (Yara Tera, Norway) per individual plant per week. For each of these groups, plants either did not receive additional fertilizer or were treated biweekly with $Ca(NO_3)_2$ or $(NH_4)_2SO_4$, equalized in respect to N concentration (0.15 g N per plant). After the start of the treatment, plants were cultivated for seven weeks.

For the salinity experiment, plants were randomly distributed in five groups, five individual plants per group: control, Na 0.5, Na 1, Na 2, and Na 5 (in g of Na per 1 L substrate). Plants in each group were irrigated with 200 mL deionized water or 200 mL deionized water with 2.54 g NaCl twice a week until the respective final concentration was reached. After the start of the treatment, plants were cultivated for seven weeks.

For the heavy metal experiment, plants were randomly distributed in seven groups, five individual plants per group: control, Cd 10, Cd 50, Cd 100, Pb 100, Pb 200, and Pb 500 (in mg of metal per 1 L of substrate). For respective treatments, $CdCl_2$ and $Pb(CH_3COO)_2$ $3H_2O$ were used in necessary dilutions in deionized water. After treatment, plants were cultivated for seven weeks.

At the termination of experiments, plants were individually separated in different parts (roots, stems, leaves) and both fresh and dry mass (after drying in an oven) was measured. Water content was calculated as g H_2O per g dry mass.

Dried tissue samples (0.2 g) were used for estimation of electrical conductivity, Na^+ and K^+ concentration. Tissues were ground with mortar and pestle to a fine powder and 10 mL of deionized water was added. After filtration through nylon mesh cloth (No. 80) homogenate was used for measurement of electrical conductivity by a LAQUAtwin conductivity meter B-771 and ion concentration by LAQUAtwin compact meters B-722 (Na^+) and B-731 (K^+). Concentration of Na^+ and K^+ was calculated as mass units both on dry biomass as well as on tissue water content basis as molar units. Electrical conductivity was expressed on dry biomass was well as tissue water basis. All measurements were performed in five biological replicates, representing tissue samples from individual plants.

Concentration of Cd and Pb was measured in dried tissues of all plant parts. Each sample consisted of about 2 g plant material. Samples were fixed 2 to 3 min at 105 °C, then dried at 60 °C to constant weight and ground. The test solution was prepared by dry ashing plant tissues with HNO_3 vapour and re-dissolving in 3% HCl solution (Rinkis et al. 1987). The test solution was used for the determination of analyzed heavy metals. Microwave plasma atomic emission spectrometry (4200 MP-AES, Agilent) was used for the measurement of Pb and Cd according to manufacturer's instructions.

Results were analyzed and graphs were made with KaleidaGraph (v. 4.1, Synergy Software, USA). The Tukey HSD was used as a post-hoc test.

Results

Effect of mineral nutrition and added nitrogen on growth

In contrast to procumbent character of habitus and limited shoot growth of *T. maritimum* plants in native conditions, plants cultivated at optimum mineral nutrient availability in conditions of an automated greenhouse had pronounced upright habitus with intense branching and numerous inflorescences (Fig. 1).

Increase in mineral nutrient availability in substrate resulted in intensified shoot growth of plants, with an increase of up to 60% dry mass at triple fertilizer dose (Fig. 2A). Addition of N-fertilizer either as calcium nitrate or ammonium sulphate resulted in further increase of shoot biomass by 20 to 30%. However, root growth was not significantly affected by mineral nutrient availability or N addition in the form of ammonium sulphate (Fig. 2B). NH_4-N treatment resulted in significant root biomass increase only for plants receiving an average fertilizer dose.

Effect of salinity on plant growth and ion accumulation

Plants exhibited good tolerance to increasing substrate Na^+ concentration, growing normally even at 5 g L^{-1}, with only about 30% reduction in shoot biomass (Fig. 3A). Dry mass of roots was relatively variable between individual plants, and significant decrease in root biomass was evident at 1 g L^{-1} Na^+, with no changes at further increase of salinity (Fig. 3B). Shoot height was little affected by increasing salinity, with significant reduction by about 40% only at 5 g L^{-1} Na+ (Fig. 3C).

Increasing substrate Na^+ concentration tended to stimulate root water content, with significant increase at 2 and 5 g L^{-1} Na^+ (Fig. 3D). However, water content in aboveground plant parts – leaves and stems – significantly increased only at 0.5 and 2 g L^{-1} Na^+, but it significantly decreased in flowers at 5 g L^{-1} Na^+, evidently indicating faster senescence of generative parts.

Plant leaves and roots accumulated equal concentration of Na^+ on a dry mass (DM) basis, reaching more than 25 g kg^{-1} DM at 1 g L^{-1} substrate Na (Fig. 4A). The response was saturated at 1 and 2 g L^{-1} Na^+, for roots and leaves,

Fig. 1. Typical morphology of *Tripleurospermum maritimum* plants at reproductive stage growing in natural habitat of shingle beach (A) and in controlled conditions (B). Bar indicates 10 cm.

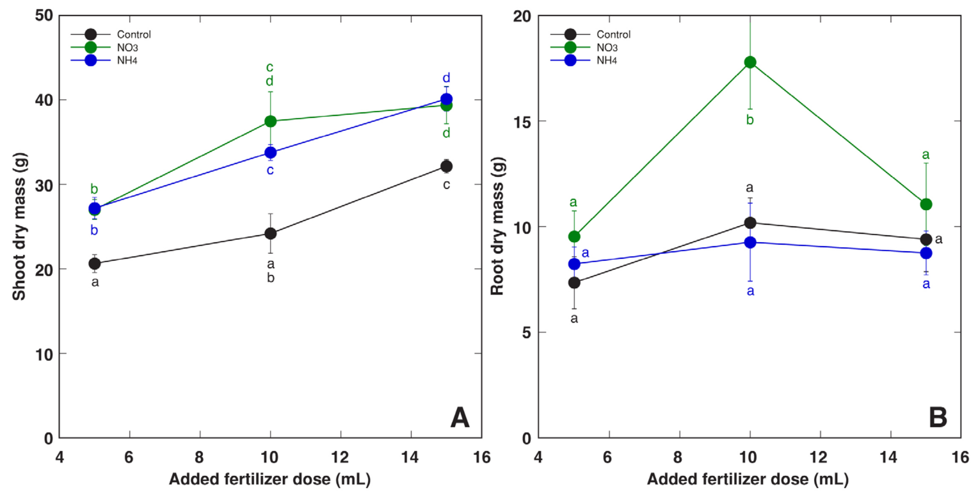

Fig. 2. Effect of increased dose of fertilizer and addition of NO_3-N and NH_4-N on dry mass of shoot (A) and root (B) of *Tripleurospermum maritimum* plants after 7 weeks of cultivation. Data are means ± SE from 5 individual plants. Different letters indicate statistically significant differences ($p < 0.05$).

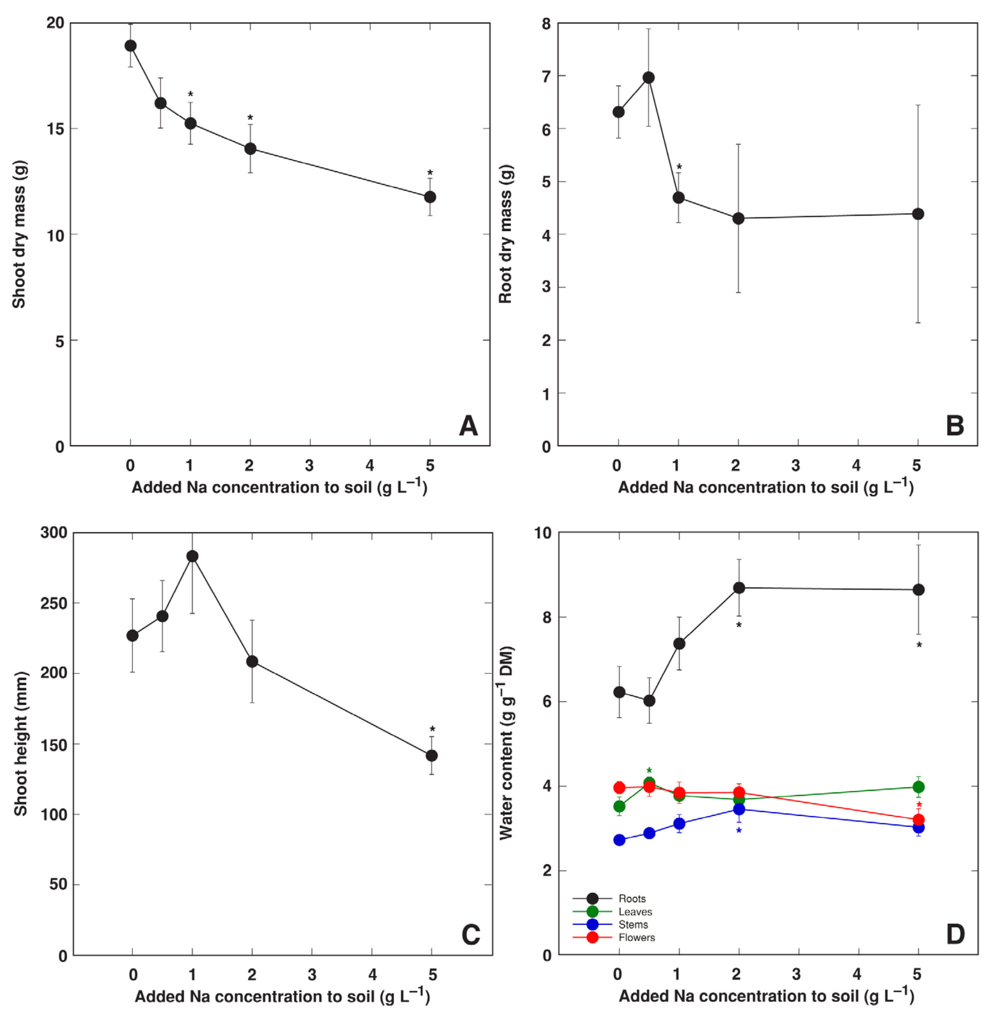

Fig. 3. Effect of increasing Na^+ concentration in substrate on shoot dry mass (A), root dry mass (B), shoot height (C) and water content in different parts (D) of *Tripleurospermum maritimum* plants after 7 weeks of cultivation. Data are means ± SE from 5 individual plants. Asterisks indicate statistically significant differences ($p < 0.05$) from control.

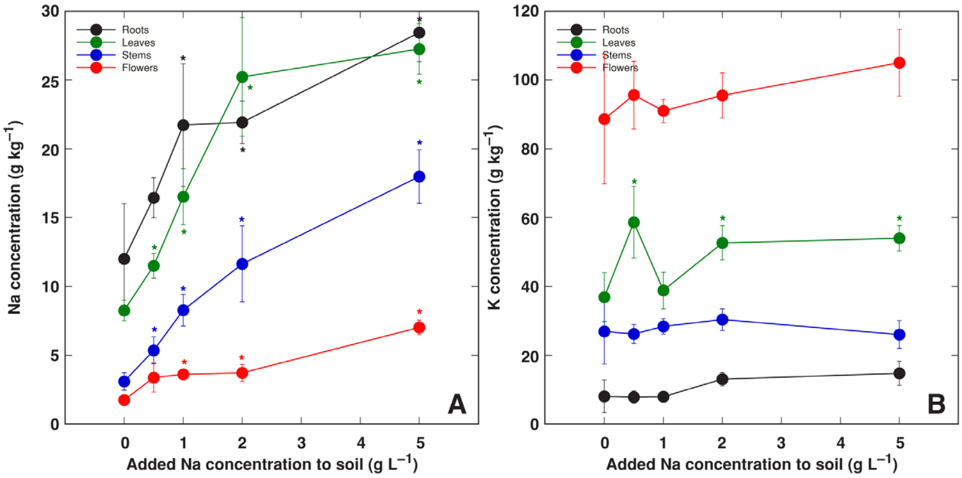

Fig. 4. Effect of increasing Na+ concentration in substrate on Na^+ (A) and K^+ (B) concentration in different parts of of *Tripleurospermum maritimum* plants on dry mass basis. Data are means ± SE from 5 individual plants. Asterisks indicate statistically significant differences ($p < 0.05$) from control.

respectively. Na^+ concentration in stem and flower tissues was significantly lower. There was a pronounced difference in K^+ concentration between plant parts, and it changed relatively little by salinity treatment, showing significant increase only in leaves at 2 and 5 g L^{-1} Na (Fig. 4B).

Due to differences in water content between plant parts, Na+ concentration on a tissue water basis was the highest in leaves, but it did not significantly increase in roots (Fig. 5A). Increase in Na^+ concentration in tissue water with rising substrate Na^+ concentration was near linear for stems, eventually reaching the same value as for leaves at 5 g L^{-1} Na^+. Changes in summed tissue water concentration of Na^+ + K^+ were relatively minimally pronounced due to preferential accumulation of K^+ over Na^+, with statistically significant increase at 5 g L^{-1} for flowers and stems, and 2 and 5 g L^{-1} for leaves (Fig. 5B).

Increase in electrolyte concentration in plant flowers, roots and leaves on a dry mass basis was relatively similar at low salinity, with significantly lower values in stems (Fig. 6A). Due to differences in tissue water content, increase of electrolyte concentration in roots was less pronounced than that in other plant parts (Fig. 6B).

Effect of heavy metals on growth and metal accumulation

Shoot growth of *T. maritimum* was not significantly affected by substrate treatment with heavy metals up to 0.1 g L^{-1} Cd and 0.5 g L^{-1} Pb (Fig. 7A). However, root growth was significantly inhibited at 0.5 g L^{-1} Pb, showing about 40% reduction of biomass (Fig. 7B). Water content in plant parts was relatively little affected by heavy metal treatment, with significant increase in flowers at 0.01 to 0.1 g L^{-1} Cd (Fig. 8A) and 0.1 to 0.2 g L^{-1} Pb (Fig. 8B), and significant decrease

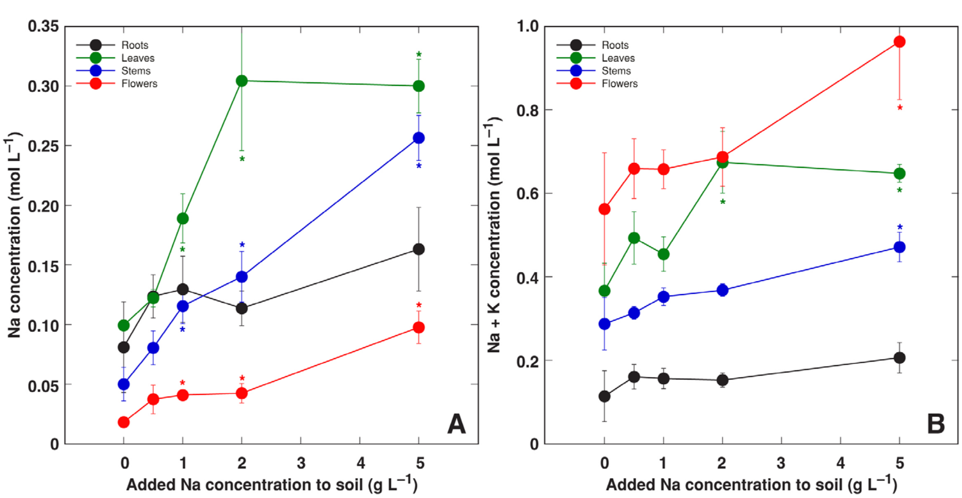

Fig. 5. Effect of increasing Na^+ concentration in substrate on Na^+ (A) and Na^+ + K^+ (B) concentration in different parts of of *Tripleurospermum maritimum* plants on tissue water basis. Data are means ± SE from 5 individual plants. Asterisks indicate statistically significant differences ($p < 0.05$) from control.

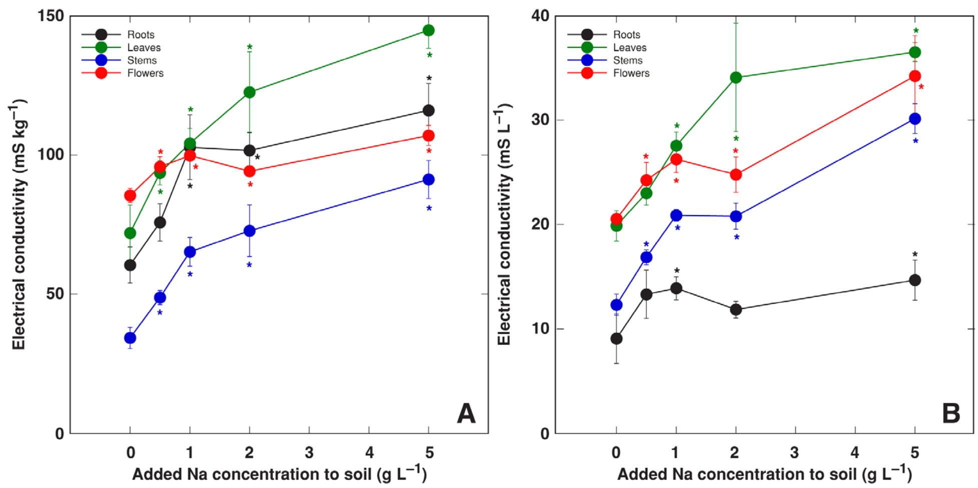

Fig. 6. Effect of increasing Na⁺ concentration in substrate on electrolyte concentration on dry mass (A) and tissue water (B) basis in different parts of of *Tripleurospermum maritimum* plants on tissue water basis. Data are means ± SE from 5 individual plants. Asterisks indicate statistically significant differences ($p < 0.05$) from control.

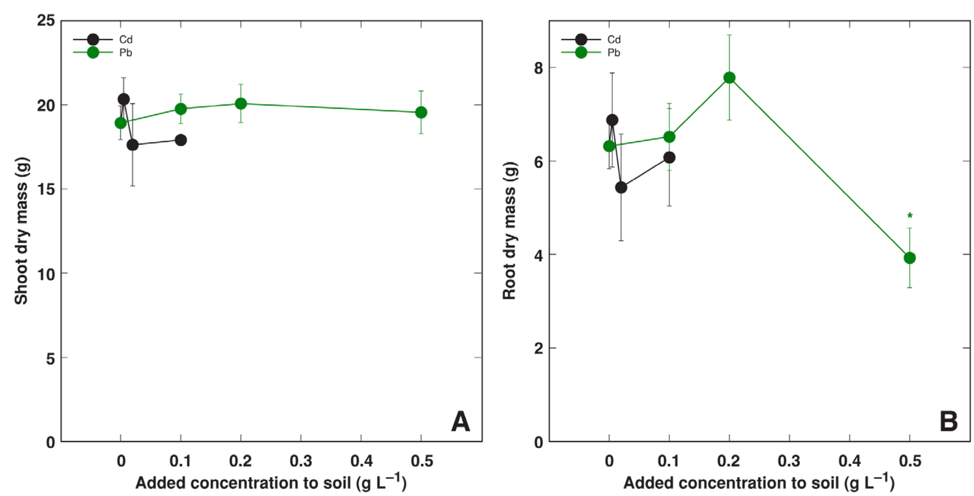

Fig. 7. Effect of increasing heavy metal concentration in substrate on dry mass of shoots (A) and roots (B) of *Tripleurospermum maritimum* plants after 7 weeks of cultivation. Data are means ± SE from 5 individual plants. Asterisks indicate statistically significant differences ($p < 0.05$) from control.

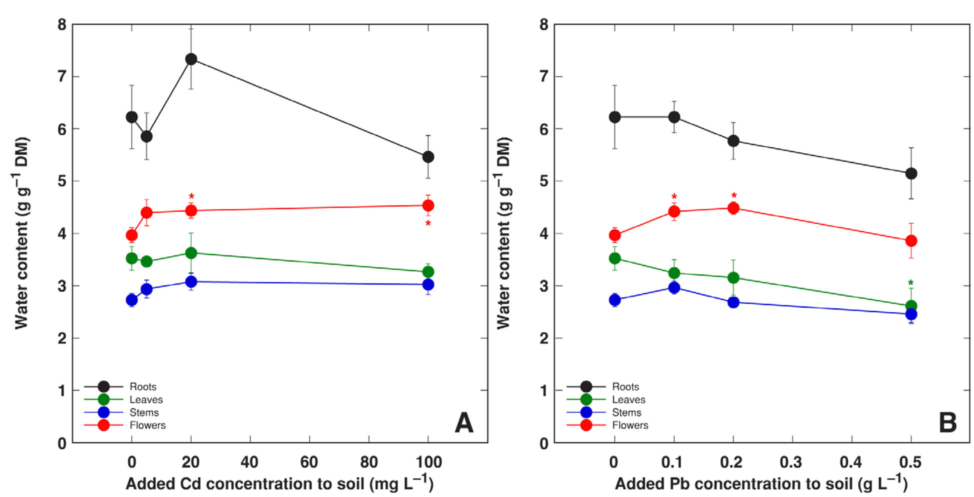

Fig. 8. Effect of increasing Cd (A) and Pb (B) concentration in substrate on water content in different parts of *Tripleurospermum maritimum* plants after 7 weeks of cultivation. Data are means ± SE from 5 individual plants. Asterisks indicate statistically significant differences ($p < 0.05$) from control.

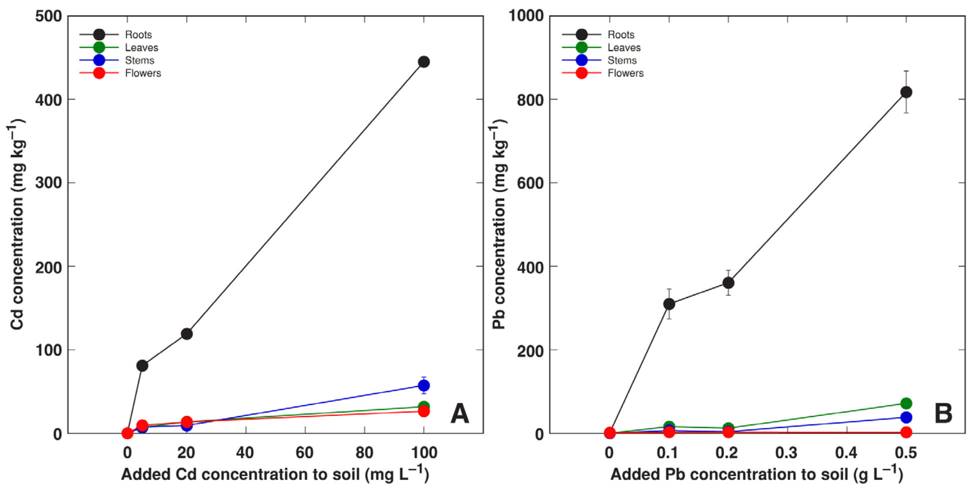

Fig. 9. Effect of increasing substrate Cd on accumulation of Cd (A) and increasing substrate Pb on accumulation of Pb (B) in different parts of *Tripleurospermum maritimum* plants after 7 weeks of cultivation. Data are means ± SE from 3 individual plants.

in leaves at 0.5 g L^{-1} Pb (Fig. 8B). No visual symptoms of toxicity on plants were evident for either of the metals.

Both metals were preferentially accumulated in roots, with concentration in aboveground parts being only less than 10% from that in roots (Fig. 9). The highest level in root tissues was 445 mg kg^{-1} for Cd and 818 mg kg^{-1} for Pb. At the maximum substrate concentration, stems (57.5 mg kg^{-1}) accumulated more Cd than leaves (32.0 mg kg^{-1}) and flowers (26.8 mg kg^{-1}). However, greater Pb accumulation occurred in leaves (71.8 mg kg^{-1}) than in stems (38.5 mg kg^{-1}) and flowers (3.0 mg kg^{-1}). The relative accumulation potential for Cd was especially pronounced, with the bioconcentration factor reaching 15 at the lowest Cd dose.

Discussion

Predominant presence of *T. maritimum* plants in a sea-affected drift-line material-dependent shingle beach habitat indicates s possible nitrophilic character and salinity tolerance of the species. In contrast to the *M. maritima* (*T. maritimum*) accession from sandy littoral dunes on the Atlantic coast of Brittany in France, being only occasionally affected by NaCl through salt sprays (Ben Hamed et al. 2014), plants from the accession of *T. maritimum* from a coastal shingle beach of the Baltic Sea used in the present study grew within 2 m from the waterline and were more or less continuously influenced by seawater.

High phenotypic plasticity of *T. maritimum* described previously (Kay 1994) was indeed supported by the results of the present study (Fig. 1). Both mineral nutrient availability and salinity significantly affected growth and morphology of the plants (Figs. 2 and 3). According to the results of the mineral nutrition experiment, *T. maritimum* plants were nitrophilous, as addition of surplus N fertilizer resulted in 20 to 30% increase in shoot biomass irrespective of fertilizer dose (Fig. 2). The species in Sweden has been characterized as having maximum possible nitrophily among vascular plants (Tyler et al. 2021). Indeed, plants growing on coastal drift-line material-affected habitats are usually considered nitrophilic (Jefferies 1977), as decomposition of wrack material consisting of vascular plant and macroalgal biomass leads to an extreme burst in local soil nitrogen concentration (Dugan et al. 2011; Rodil et al. 2019). However, no particular growth response values to added surplus nitrogen have been defined for putatively nitrophilic plant species in controlled conditions. Interestingly, another species from the same type of coastal habitat as *T. maritimum*, *Rumex maritimus*, showed a significantly more pronounced nitrophilic character, with more than four-fold leaf biomass increase by added nitrogen in controlled conditions (Ievinsh et al. 2020).

Salinity tolerance of *T. maritimum* plants in controlled conditions growing in soil can be characterized as moderate, as dry mass of shoots decreased by 38% and that of roots by 30% at 5 g L^{-1} Na^{+} (corresponding to 217 mM). For comparison, in a study with an *M. maritima* (*T. maritimum*) accession from a sandy Atlantic coast in France, shoot dry biomass decreased by 20% and that of roots by 41% at 200 mM NaCl salinity (Ben Hamed et al. 2014). For a related species *M. chamomilla*, cultivated in hydroponics at 150 mM NaCl, shoot growth was reduced by 76.3%, but root growth even increased (Heidari, Sarani 2012).

It is important to note that ion accumulation characteristics of *T. maritimum* plants in conditions of controlled experiment were similar to those observed in natural conditions of saline coastal habitats of the Baltic Sea (Ievinsh et al. 2021). In the present study, leaves of *T. maritimum* accumulated up to 27 g kg^{-1} Na^{+} on a dry mass basis (in comparison, 7 to 23 g kg^{-1} in natural conditions), up to 0.30 mol L^{-1} Na^{+} in tissue water (0.08 to 0.26 mol L^{-1}), and electrical conductivity in tissue water reached 36 mS m^{-1} L^{-1} (23 to 37 mS m^{-1} L^{-1}). Consequently, the electrolyte concentration in leaves of *T. maritimum* plants grown in controlled conditions at relatively high substrate salinity was at the top electrolyte accumulation range of that found in natural conditions, with Na^+ and K^+ concentration even exceeding the respective ranges. *M. maritima* (*T. maritimum*) plants from the Atlantic coast showed even more pronounced Na^+ accumulation potential, with shoot concentration for Na^+ reaching 4.5 mmol g^{-1} (103.5 g kg^{-1}; Ben Hamed et al. 2014). In contrast, hydroponically-cultivated *M. chamomilla* plants at 150 mM NaCl accumulated only 1.4 and 1.0 g kg^{-1} dry mass in shoots and roots, respectively (Heidari, Sarani 2012).

An important aspect of salinity tolerance was the fact that K^+ concentration in leaves of *T. maritimum* increased under salinity (Fig. 4B). In contrast, K^+ concentration in shoots and roots of *M. chamomilla* at 150 mM salinity decreased by 37.6 and 46.1%, respectively (Heidari, Sarani 2012). Increase in tissue K^+ concentration as a result of increasing substrate salinity is an important characteristic for defining salt-adapted facultatively-halophytic plant species (Munns, Tester 2008). However, obligate halophytic species are able to substitute K^+ for Na^+ as part of salinity tolerance mechanism (Belkheiri, Mulas 2013).

In the present study, root biomass of *T. maritimum* plants treated with 500 mg L^{-1} Pb was reduced only by 35% (Fig. 7B), while shoot growth was unaffected (Fig. 7A). Inhibition of root growth is a more sensitive indicator of Pb phytotoxicity in comparison to shoot growth, and even species hypertolerant to Pb show significant root growth reduction at relatively low Pb concentration in hydroponics (Mohtadi et al. 2012). Growth of *T. maritimum* plants was not affected even in 100 mg L^{-1} Cd treatment (Fig 7), indicating especially high tolerance against this heavy metal. In conditions of hydroponics, unaltered growth of plants at 120 μM (13 mg L^{-1}) Cd concentration is considered as an indication of high Cd tolerance (Kováčik et al. 2006). For Cd-sensitive plants, several-fold decrease of plant biomass at 100 mg kg^{-1} Cd in soil is a characteristic response (Anjum et al. 2014). In the case of Pb treatment in hydroponics, addition of 50 μM (10 mg L^{-1}) Pb usually results in significant decrease of shoot and root biomass for both non-metallophytes and facultative metallophytes (Fahr et al. 2015; Mohdavian et al. 2016). It is somehow more difficult to interpret response to increased Pb for soil-grown plants, due to chemical interaction of Pb in soil, considerably affecting its availability for plants (Shahid et al. 2012; Salazar et al. 2016). However, soil spiked with 400, 800 or 1200 mg kg^{-1} Pb has been frequently considered as representing low, medium, and high Pb levels, respectively (Ashraf, Tang 2017).

Preferential accumulation of Pb in roots in comparison to shoots represents a general pattern (Pourrut et al. 2011). Both Cd and Pb were preferentially accumulated in roots of *T. maritimum*, with concentration in aboveground parts being less than 10% from that in roots (Fig. 9). This feature allows the species to be considered as a typical heavy metal excluder. The taxonomically related species, *M. chamomilla*, in hydroponics also preferentially accumulated Pb in roots, with leaf Pb concentration being only 2.72 mg kg^{-1} (Grejtovský et al. 2008). A similar tendency was noted for Cd accumulation in *M. chamomilla*, but the potential for metal accumulation in leaves was higher, reaching 300 mg kg^{-1} Cd (Kovačik et al. 2009).

When characterizing plant tolerance to heavy metals, concentration of a particular metal in leaf tissues of plants is more important indicator that that in soil. The majority of plants have threshold sensitivity to tissue Cd concentration range at 5 to 10 mg kg^{-1}, and that for Pb in a range 10 to 20 mg kg^{-1} (White, Brown 2010). Consequently, given the fact that growth of *T. maritimum* plants was unaffected at 32 mg kg^{-1} leaf Cd concentration and at 12.8 mg kg^{-1} leaf Pb concentration, the species can be characterized as hypertolerant to Cd and tolerant to Pb.

In conclusion, the coastal accession of *T. maritimum* from a shingle beach is moderately nitrophylic with high salinity tolerance and high electrolyte accumulation potential in shoots, with preferred accumulation of K^+ over Na^+. The species show very high tolerance to heavy metals Cd and Pb, with characteristic exclusion of the metals from aboveground parts.

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