MULTI-ELEMENT COMPOSITION OF *TRIGLOCHIN MARITIMA* L. FROM

CONTRASTING HABITATS INCLUDING HOT SPRINGS AND METAL

ENRICHED AREAS

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The Supervisory Committee certifies that this **disquisition** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

ABSTRACT

Sunwar, Sharmila, PhD, Environmental and Conservation Sciences Program, College of Graduate and Interdisciplinary Studies, North Dakota State University, March 2011. Multi-element Composition of *Triglochin maritima* L. from Contrasting Habitats Including Hot Springs and Metal Enriched Areas. Major Professor: Dr. Marinus L. Otte.

The aim of this PhD research was to study multi-element composition in wetland plants from contrasting habitats, including hot springs, temporary wetlands, and metal-rich areas. *Triglochin maritima* L. (seaside arrowgrass) was chosen for the study because this species is common in alkaline/saline soils and is adapted to diverse habitats. *Eleocharis rostellata, Juncus balticus, Salix exigua, S. boothii,* and *S. wolfii* were also included in the study. Field studies and greenhouse experiments were conducted to study the multi-element composition in plants. In the greenhouse experiment the effects of temperature and soil biota on multi-element uptake in *T. maritima* were studied. Root-zone soils and plant samples were analyzed for 32 - 50 elements using inductively coupled plasma OES/MS spectrometry. The expected outcomes from this research were: 1) the development of multi-element fingerprints for *T. maritima* and other plant species from contrasting habitats, and 2) a better understanding of the effects of temperature and soil biota on multi-element uptake in *T. maritima*. Habitat specific element concentration patterns in *T. maritima* were observed; concentrations of Mn, Li, and B were high in plants from hot spring influenced wetlands, whereas Ca, P, Mg, Fe, Sr, Ba, Ti, and Cu were higher in the plants of temporary wetlands. *J. balticus* and *Salix* species from mine impacted and uncontaminated sites revealed distinct differences in multi-element fingerprints. *J. balticus* showed high

concentrations of S, K, Mn, Fe, Cu, Al, As, and Cd at contaminated sites compared to un-contaminated sites. Multi-element fingerprints of *Salix* species showed that *S. boothii* had higher concentrations of Mn, Fe, Al, and Ti compared to *S. exigua* and *S. wolfii*. To our knowledge for the first time the association of mycorrhizal fungus in *T. maritima* was confirmed, and significant effects of temperature on element concentrations, contents, and their translocation in plants were observed. Generally, the distribution of the total contents of P, Na, Mn, B, Cu, Mo, Li, Sr, Ti, and Cs in both roots and leaves were lower at 40 °C compared to 20 and 30 °C, but their distribution and translocation from root to leaves were higher at 40°C. Even though the biological and physiological funtions of Li, Sr, Ba, Rb, and Ti in plants are not fully understood, these elements were substantially taken up by *T. maritima*, and significant positive correlations of these elements were found with elements that have known biological functions. Overall, concentrations of Ca, P, Mg, Mn, B, Sr, and Ba in *T. maritima* showed variation due to differences in habitats, temperature, and experimental growing conditions (greenhouse and field condition). Concentration patterns of Na, K, and Zn were species specific and affected by temperature. Li concentrations varied due to habitat differences, growth conditions, and species differences. Future research directions could include: 1) identification of the fungal species associated with *T. maritima* and studies to elucidate their possible role in survival of *T. maritima* in the elevated temperature of hot springs, 2) the effects of soil factors, such as salinity and 3) seasonal variation in uptake and translocation, particularly for the less-studied elements with yet unrecognized but potential biological functions in plants.

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CHAPTER 1. INTRODUCTION

1.1. Background, Aims and Objectives

The overall aim of this PhD research was to study multi-element composition of wetland plants from contrasting habitats, including hot springs and metal-rich areas. Factors such as temperature, salinity, and symbiosis with fungi were taken into consideration. Due to its diverse habitat adaptability, *Triglochin maritima* was selected as the main species of focus in the study, but other plant species were also investigated. The objectives of this study were (1) to assess multi-element fingerprints for *T. maritima* and other plant species, and (2) to determine the role of environmental factors, including temperature and soil biota, in element uptake in *T. maritima*. To meet these objectives the following research questions were addressed:

1. Does the element fingerprint of plant population vary between habitats?

2. Does the element fingerprint of plants in a habitat vary among plant species?

3. How do environmental factors influence the element fingerprint?

To meet these objectives two field studies in Wyoming (WY) and North Dakota (ND), and two greenhouse experiments were carried out. The findings of this research will contribute to our understanding of cycling of elements, which is essential for our understanding of trophic transfer of elements and their possible effect in ecosystems.

1.2. Elements in Plants

Transfer of elements from soil to plants is an integral part of biochemical cycling and flow of elements from non-living to living compartments of biosphere

(Kabata-Pendias, 2001). Systematic analysis of element uptake in plants can be dated back to 1938 when the Russian scientist Tkalich used plants as indicators for ore prospecting (Cannon, 1960). However, the use of plants for observation and detection of elements in the soil started as early as 1828. Plants have been used, in many parts of the world, as indicators for locating mineral ores, to identify contaminated sites, and to provide a cost-effective means for assessing short-term contamination, all through studying the plants' appearance (Rabbitt, 1954; Cannon, 1960; Cole and Smith, 1984; Baldontoni et al., 2005). During the early 1990s, analysis of elements in plants began to focus on identification of contaminated sites, biomonitoring of the environment and on the possible role of plants in remediation of contaminated sites (Cunningham and Berti, 1993; Kumar et al., 1995; Pulford and Watson, 2003). Historically, research on plant element composition has focused primarily and exhaustively on common elements, such as the macro- and micro-nutrients (e.g. N, P, K, Mg, Ca, Zn) and on pollutants (e.g. As, Cd, Zn); frequently involving only one element at a time. In agriculture, for instance, plant analysis began with examining leaves with the aim to relate nutritional status to plant response. The results of plant analysis were used as a guide in applying mineral nutrition (Smith, 1962). The lesser-known elements, including trace elements (TEs, elements which occur at low concentrations (mg kg^{-1} or less) in soils, plants, and other organisms) and Rare Earth Elements (REEs, are a chemically uniform group, which includes the lanthanides and a few other 'rare' elements) have been neglected in most studies. This is due to the perception that these 'rare' elements have no role or function in biological systems and to the

difficulty and expense of analytical techniques. Only during the past two decades have improved technologies in multi-element analysis become widely available. With the development of inductively coupled plasma mass spectrometry (ICP-MS) it is possible to measure great numbers of elements in plant samples in a short time (Fränzle and Markert, 2000; Ayrault, 2001).

Using multi-element analysis of plants as a tool for environmental studies began during the 1980s and resulted in a few scientific publications, for example, a study on *Betula alba*, *Vaccinium vitis idaea*, and *Vaccinium myrtillus* (Markert, 1988). Steinnes et al. (1992) used *Hylocomium splendens* (a moss) in multi-element analysis of atmospheric deposition in Norway. Vitorova and Markert (1995) suggested that a multi-element study in forest ecosystems in Europe could be used in identifying contaminated ecosystems. However, multi-element analysis has not yet been applied in environmental and ecological studies and very few studies have been conducted in multi-element uptake in plants.

The current status of studies on multi-element uptake in plants and the numbers of scientific publications are presented in Fig 1.1. In a search using the ISI Web of Knowledge carried out on November 20, 2009, the numbers of publications returned with the search term 'heavy metals' followed by 'plants' are higher compared to 'multi-elements' followed by 'plants'. Earlier studies were focused mainly on the so-called heavy metals, which included elements such as Cd, Zn, Pb, Ni, and Cr. The number of publications for 'heavy' metals has increased four times since the 1990s. Since then, the number of publications on multiple elements in plants has doubled, but is still less than 50. Publications on

rare earth elements in plants have tripled since 1999 (Fig. 1.1b). This may be due to research related to an increased interest in the use of fertilizers containing rare earth elements in Chinese agriculture (Xu et al., 2002).
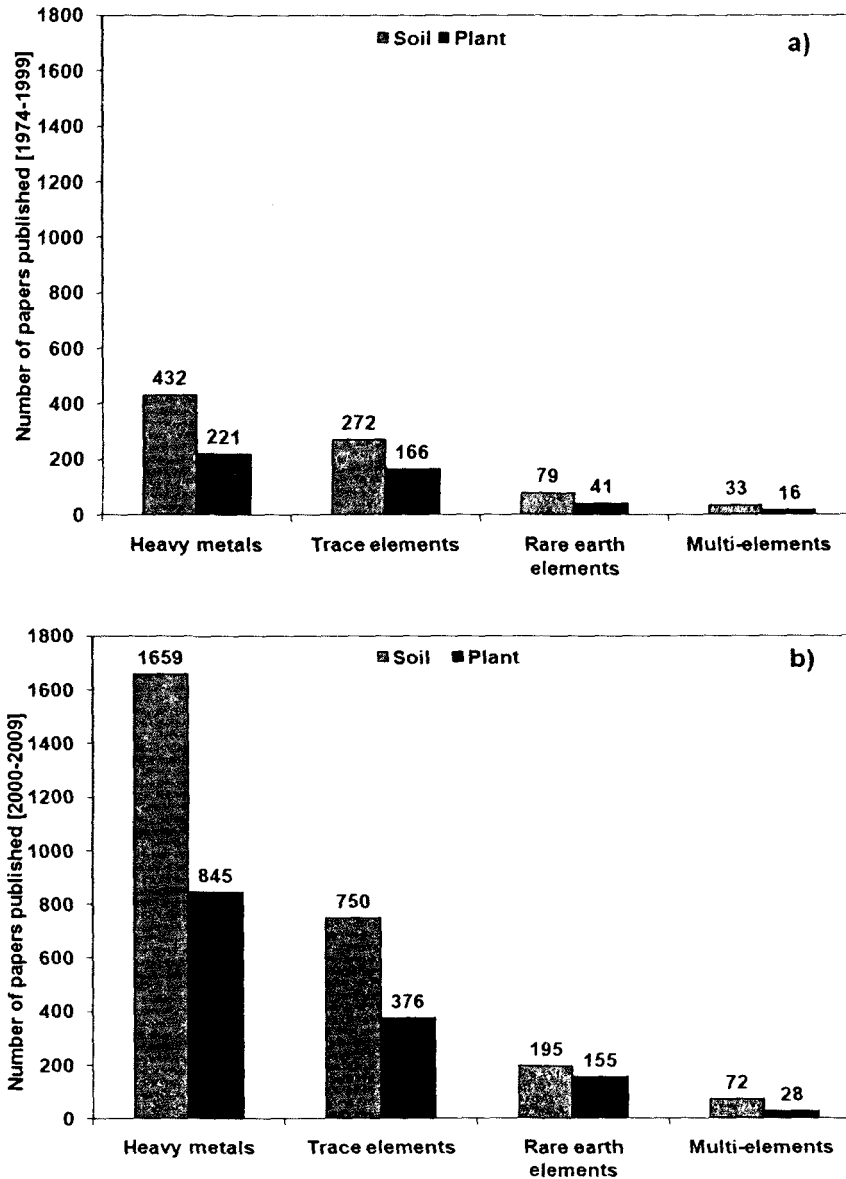
Figure 1.1. Scientific publications on element analysis for soil and plants based on a search of the ISI Web of Knowledge™, November 20, 2009. a) Number of publications during 1974 - 1999, b) Number of publications during 2000 - 2009.

Knowledge of multi-element composition of plants and soils is important, among other reasons, because of the possible antagonistic (e.g. Ca with Al, B, Cd, Fe, and Li), and synergistic effects (Ca with Cu, Mn, and Zn) among the elements (Kabata-Pendias, 2001). The occurrence of specific elements in a plant sample could indicate the presence of other elements. For example, arsenic is commonly associated with ores of Cu, Pb, and Au (Oremland and Stolz, 2003), and selenium and sulfur are associated with uranium ore (Rabbitt, 1954). Multi-element analysis can produce chemical fingerprints of plants and soils, which may be used in biogeochemical modeling studies, in understanding element concentrations in plants in different ecosystems and inter-element relations and processes, such as accumulation or exclusion of elements in organisms (Markert, 1992). Chemical fingerprinting in plants increases the power of studies when many elements in different plant species from both control and contaminated habitats are analyzed (Djingova et al., 2004).

To illustrate the importance of multi-element analysis in plants, Markert (1994) proposed a biological system of the element (BSE) (Fig. 1.2). The BSE is different from the Periodic System of the elements (PSE) in its element grouping. It is based on correlations between and among the elements in organisms. It also considers the possible functions, toxicity and physiological role of individual elements in plants and other organisms. According to the BSE, the elements are grouped into three broad categories; a) structural elements (C, H, O, N, P, S, Si and Ca), b) electrolytic elements (K, Na, Ca, Cl, Mg, Br, Cs, and Sr) involved in

maintenance of osmotic conditions in cell metabolism, and c) enzymatic elements (B, V, Cr, Mo, Mn, Fe, Ni, Cu, Zn, Sn, and Se).

Figure 1.2. Biological system of the elements from Markert (1994).

However, many other elements in the Periodic System, such as those in the lanthanide group, are not known for any roles in organisms. Markert (1994) suggests that Ge and Te, which are grouped with Se and Sn in the BSE, may be found in the near future to have essential roles in organisms. Fränzle and Markert (2000) further suggested that Br, Sr, Cs, Ge, and Te will be found to have physiological roles as well. Therefore, many elements require more study to understand their possible physiological roles and essentiality in biological systems.

1.3. Element Uptake Mechanisms in Plants

The uptake of elements in plants depends on concentrations of elements in the medium and their available forms (Markert, 1998), as well as on the activity of the plant (Jacob and Otte, 2003). In a very general way, the chemical composition of plants reflects the chemical composition of the soil and water in which they grow (Kabata-Pendias, 2001). However, in many instances there exist asymmetrical or symmetrical relationships between the available elements in substrates and their effects in organisms. Markert et al. (2000) proposed three uptake characteristics for elements in plants: i) element concentrations in the plants reflect the element concentrations in the environment, in this case plants show an indicative characteristic and such information may be useful in prospecting and biomonitoring; ii) in some cases metalliferous plants accumulate large concentrations of elements and may be suitable for applications in phytoremediation, such as phytoextraction (Schwartz et al., 2003); and iii) the reduced uptake of elements by complete or partial exclusion. Understanding such behavior is important in chemical characterization in plants. Plants take up elements mostly by absorption in their roots, either actively or passively (Kabata-Pendias, 2001). In passive uptake, the ions move by diffusion processes from the external solution into the root endodermis. The active uptake mechanism requires metabolic energy (Ernst, 1989; Kabata-Pendias, 2001). Uptake via these mechanisms varies for different elements, for example, Pb, and Ni are absorbed through passive mechanisms, whereas Cu, Mo, and Zn are absorbed by active mechanisms. Root exudates and microorganisms help to release trace elements

from the soil complexes. For example, rare earth element (REE) ions are attached to cell walls in the apoplast and combine with carboxyl groups in the cell walls, which in turn results in adsorption of selective ions into the cell wall (Liang et al., 2008). Then, the next mechanism of element uptake is the translocation of elements from the roots to the upper parts of the plants, in which the xylem tissue plays an important role.

The mobilization of elements in the upper plant parts depends on formation of complexes between elements and organic molecules such as amino acids, lipids (especially phospholipids) and carbohydrate ligands. Transpiration in plants also influences the element uptake mechanisms. Barker (1983) suggested that at high salinity levels, the transpiration rate in leaves influences the uptake of cations. At high respiration rates, the ratio of potassium to sodium decreased in the shoots. The element type also influences the uptake mechanisms. For example, Cr and Pb accumulate in roots because of high chemical affinity to the root cell wall; whereas the concentrations of mobile elements are higher in the leaves. However, in seeds the concentrations of elements remain lower since seeds possess a translocation barrier for most of the elements with unknown metabolic roles (Ernst, 1995).

Element concentration patterns are species specific, depending upon the amount of elements in the environment, and on the allocation of elements in the plant parts. Within the population, the genetic composition and its interactions with the environment will also have an effect on element concentrations in the plant tissue. The metal uptake strategies in plants are species specific and

depend on metal types. Dahmani-Muller et al. (2000) studied metal uptake strategies in three plant species. The study reported that *Armeria maritima* ssp. *halleri* populations showed differences of metal concentration between roots and leaves for Zn, Cd, Pb, and Cu. More specifically, the species showed restricted uptake of Pb and Cu in leaves suggesting an exclusion strategy for these elements. In addition, the differences in concentration of Cu, Cd, Zn and Pb between green and brown leaves in the populations suggested internal transport of these elements, and senescence of leaves as a metal detoxification mechanism. In contrast, *Cardaminopsis halleri* accumulated high concentrations of Zn and Cd in the leaf tissue and the species showed hyperaccumulation strategies with respect to Zn and Cd. In *Agrostis tenuis*, the concentration of Zn, Cd, Pb and Cu were higher in roots, suggesting immobilization of these elements in the roots.

Plant species differ in their ability to accumulate elements depending on their plant organs, seasonal pattern and spatial distribution (Marseille et. al., 2000, Quan et al., 2007). Fuhrmann et al. (2002) studied the ability of *Amaranthus retroflexus* (Redroot pigweed), *Brassica juncea* (Indian mustard), and *Phaseolus acutifolius* (tepary bean) in the uptake of cesium-137 and strontium-90 from contaminated soil. The study showed that *A. retroflexus* accumulated higher concentrations of both Cs-137 and Sr-90, and showed a linear relationship between Cs-137 concentrations in the plants and soil. The plant tissues above-ground or below-ground also differed in trace element accumulation. Quan et al. (2007) studied the seasonal patterns of above-ground biomass accumulation,

tissue element concentration, and above-ground element pools in *Spartina alterniflora* (salt cord grass), *Phragmites australis* (common reed) and *Scirpus mariqueter*. The study revealed that below-ground tissues showed higher metal concentrations than above-ground. The above-ground Zn and Pb concentrations were significantly lower in *P. australis* and *S. alterniflora* than in *S. mariqueter* in late season whereas the aboveground metal concentrations of Pb and Cu were significantly greater in *S. mariqueter* in late season.

1.4. Plant Adaptation and Environmental Factors in Element Uptake

Generally, plants are adapted to a particular type of environment, which means they perform better both physically and physiologically in certain, defined habitats. Plants may be adapted to seemingly extreme environments such as hot springs, metalliferous soils, and high salinity environments. Different environmental factors play important roles in adaptations in those habitats. Several factors also affect metal bioavailability to and mobility in soil and plants. These factors include soil variables (pH, water regime, clay content, cation exchange capacity (CEC), oxide mineral content, organic matter content), root environments, plant species, abiotic factors (salinity and temperature) and biotic factors (soil micro biota) (Kabata-Pendias, 2001). Although several factors affect element uptake in plants, in the study presented here the focus will be on temperature, salinity, and soil micro biota (mycorrhizae).

1.4.1. Temperature

Among the environmental factors, temperature plays an important role by affecting the physiological activity, growth and development of plants (Treshow,

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1970). Physiological activities within plants differ for each species, thus plants show different tolerance levels to extreme temperatures. The optimum temperature requirement for metabolic activities in plants differs from species to species and among populations. The following paragraph will discuss plants adapted to hot spring environments.

The geothermal activity in hot springs generates high temperatures, extreme pH (acidic to alkaline) and high concentrations of soluble elements (Stauffer et al., 1980). Yellowstone National Park (YNP) in Wyoming, USA, contains the highest numbers of surface-geothermal features in the world, and consists of many geothermally influenced wetlands (Fournier, 1989; Channing and Edwards, 2009). Stout and Al-Niemi (2002) studied the flowering plants of geothermal areas in Yellowstone National Park. They recorded both monocot and dicot species which are tolerant to high rhizosphere temperatures. Plant species such as *Dichanthelium lanuginosum, Agrostis rossiae, Agrostis scraba, Juncus tweedyi* were reported growing in soils with rhizosphere temperatures above 40 °C. *Triglochin maritima* and *Eleocharis rostellata* are dominant and occupy the alkali chloride-rich thermal waters within the geothermally influenced wetlands (Sunwar et al. 2008; Channing and Edwards, 2009). Different mechanisms of thermotolerance in plants have been reported. *Dichanthelium lanuginosum* is tolerant to temperatures above 40 °C and is widely distributed in hot springs of Yellowstone National Park (Stout and Al-Niemi, 2002). Stout et al. (1997) reported that heat shock proteins play roles in the adaptation of *D. lanuginosum*. Redman et al. (2002) reported that thermotolerance in *D. lanuginosum* is associated with a

plant fungal symbiosis. A fungus of the genus *Curvularia* helps to the plant tolerates soil temperatures of 50 °C to 65 °C as compared to non-symbiotic plants. Not only that, but Marquez et al. (2007) reported that the thermotolerance is generated by a mutualistic interaction of a virus with the fungus. Only *Curvularia protuberate* infected with the virus imparts heat tolerance in *D. lanuginosum,* resulting in a three-way symbiosis between fungus, virus and a plant.

Temperature is one of the important factors that control the chemical reactions within the cell in plants. Temperature also influences the availability and absorption of elements from the soil (Treshow, 1970). Roots can tolerate high soil temperatures by controlling respiratory rates and increasing respiratory efficiency, which helps to reduce ion uptake costs (Rachmilevitch et al., 2006). A few studies have reported on the effect of temperature in element uptake in plants. Fritioff et al. (2005) studied the effects of temperature and salinity on element uptake in *Elodea canadensis* (Michx.) and *Potamogeton natans* (L.). The study reported that in general Cu, Zn Cd, and Pb concentration and their accumulation were higher under increased temperatures. However, the highest temperature treatment in that experiment was only 20 °C. Similarly, Oncel et al. (2000) studied the effects of different temperature regimes, with a maximum temperature of 35 °C, on uptake of Cd and Pb in *Triticum aestivum* varieties. The study reported a significant interaction between high temperatures and high Cd concentrations demonstrating that when temperature was increased, the concentration and toxicity of Cd in roots and shoot growth were higher. Baghour et al. (2002b) studied the effect of temperature on accumulation of Ni and Co in potato. They reported that an

elevated temperature of 27 °C enhanced the uptake of Ni in roots, leaves and tubers, whereas Co uptake was higher in tubers only. Baghour et al. (2002a) also studied the effect of temperature on the uptake of Ba, Cl, Sn, Pt, and Rb in potato. This study showed that at 27 °C, the uptake of Ba, Pt, Cl and Sn were higher in the roots, leaves, and tubers compared to lower temperatures. However, plants adapted to the 'chemical soup' of hot spring environments have hardly been studied for multi-element uptake.

1.4.2. Salinity

Salinity inhibits the growth of many plants, but halophytes are well adapted to high saline environment. Halophytes may even require low concentrations of NaCl for their normal growth (Levitt, 1980). Halophytic plants use mechanisms of salt exclusion, excretion, and dilution to survive in saline environments. In some instances, the halophytes accumulate elevated ions in their tissues for their normal growth and regulate the excess ions by shedding old leaves (Albert, 1975). Plant species differ in tolerances to salinity. For example, *Suaeda maritima* and *Salicornia rubra* are two salt marsh species that grow well in high saline environment. Cooper (1982) showed that salinity did not strongly affect biomass production in *T. maritima*. Marcum and Murdoch (1992) showed negative correlation between salinity and root growth in *Sporobolus virginicus*.

Salinity also affects element uptake in plants. Many of the studies on effects of salinity can be found on salt marsh species, mainly focusing on growth response and a few on element uptakes. Levitt (1980) suggested that greater salinity suppresses nutrient uptake in plants resulting in reduced growth; for

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example NaCl competes with and reduces the uptake of elements such as Ca, Mg, and K. Cooper (1984) studied the effects of sodium on manganese uptake and its toxicity in *Triglochin maritima* and a few other salt marsh plants. Higher salinity did not affect the uptake of Mn in the shoots of *T. maritima* but significantly lowered the concentration of Ca and K. Similarly, Cooper (1982) studied the effects of salinity and water logging in salt marsh plants, including *T. maritima,* from different salt marshes. Sodium concentrations in shoots of *T. maritima* were higher. Weggler-Beaton et al. (2000) studied the effect of increased NaCl on uptake of Cd, amended with a biosolid (treated sludge) in *Beta vulgaris* cv. Fordhook Giant (Swiss chard) and *Triticum aestivum* cv. Halberd (wheat). They reported that the salinity did not affect the growth of plants. However, increased Cl concentrations elevated Cd concentrations in the tissues. Rozema et al. (1992) reported that boron content in the root and shoot of *Plantago maritima* was increased under higher salinity (200 mM). Kohl (1997) studied the effects of NaCl on *A. maritima* populations from salt marshes, from inland sandy soils, and from heavy metal-rich soils. The study showed significant differences between the inland and salt marsh populations in high salinity treatments. In the salt marsh populations, Na concentrations were higher in the leaf tissues across all salt treatments, whereas in inland populations, the Na concentrations were higher in the root tissues. This study also showed that the effect of high NaCl resulted in lower uptake of Ca in the leaf tissues. Sandy soil and salt marsh populations showed significant decreases in Mg concentrations in leaf tissue as the salinity was increased from 0 – 100 mM. However, the population from heavy-metal soils

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showed the reverse trend in Mg concentrations in leaves. Irrespective of populations, the P concentration in green leaves and roots was significantly higher in the saline treatment as compared to non-saline treatment.

1.4.3. Microorganisms

The soil biota plays a significant role in plant survival and adaptation. Mycorrhizal symbioses developed through co-evolution between plants and fungi (Smith et al., 2003). This type of symbiosis is reported in the majority of terrestrial plants. Wilkinson and Dickinson (1995) suggested that mycorrhizae play a role in the acclimation and adaptation of plants in response to metals. Mason (1928) reported the presence of endotrophic mycorrhizae in some salt marsh plants including *Plantago maritima*, *Aster tripolium*, *Glaux maritima* and *Armeria maritima*. Rodriguez et al. (2004) suggested that in high stress environments the symbiotic relationship between fungus and host plants plays an important role for the survival of both fungus and host plants. A mutualistic fungus provides several benefits to its host plants such as heat tolerance, drought tolerance, disease resistance, and metal tolerance. Rodriguez et al. (2008) demonstrated that salinity and heat tolerance in plants are habitat-specific phenomena between plant and fungal symbiosis. They isolated *Fusarium culmorum* from coastal, geothermal and agricultural habitats. The study showed coastal isolates conferred salt tolerances, but not to heat stress, and the geothermal isolates conferred heat tolerance but not salt tolerance. Similarly, fungus isolated from agricultural fields conferred disease resistant but not salt or heat tolerance. The fungus did impart drought resistance irrespective of the habitat.

Mycorrhizal fungi have an important role in nutrient cycling at the soil-root interface (Martin, 2001). The hyphae of fungi absorb inorganic nutrients through active metabolism and transport to host roots, while in turn the fungus uses carbon compounds produced by the host plant. Most of the studies on the role of mycorrhizae in nutrient uptake are limited to N and P, and a small number of studies reported on other elements, such as Ca, Cu, Zn, and Fe. Marschner and Dell (1994) observed mycorrhizae enhance the uptake of N, K, Ca Cu, Zn, and Fe in the plant. In metalliferous soils, mycorrhizae help to impart heavy metal tolerance in the plants. Bradley et al. (1982) studied the role of mycorrhizal infection and tolerance to Cu and Zn in *Calluna vulgaris, +Vaccinium macrocarpon* and *Rhododendron ponticum.* The study showed that mycorrhizal plants accumulated lower Cu and Zn in the leaves, but the concentrations of these metals were higher in the roots, compared to non-mycorrhizal plants. The authors infer that fungal hyphae prevented metal translocation in the plant. In contrast, a study by Lambert et al. (1979) reported that increased phosphorus fertilizer reduced the Zn and Cu concentrations in mycorrhizal soybean plant shoots and also reduced the mycorrhizal activity in the plants. Ambe et al. (2002) reported that rice plants inoculated with *Pyricularia oryzae* showed enhanced uptake of Mn and Zn in the shoots. Several studies also investigated the role of rhizosphere bacteria in elemental uptake in plants. De Souza et al. (1999) reported that rhizosphere bacteria enhanced the uptake of Se and Hg in *Scirpus robustus* (Pursh salt marsh bulrush) and *Polypogon monspeliensis* L. Desf. (Rabbit-foot grass). Emerson et al. (1999) also observed that iron-oxidizing bacteria present in the rhizosphere could

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play an important role in the biogeochemical cycles of P, Mn and other metals in wetland plants. Leyval et al. (1997) suggested that the role of arbuscular mycorrhizal fungi on element uptake in plants depends on the type of metals and the concentrations of metals in the growth media. Previous research on the role of mycorrhizae in nutrient uptake are limited to a small number of elements and thus needs more understanding for many other elements, which are less studied (Smith et al., 2003).

1.5. *Triglochin maritima* L.

Triglochin maritima L. (seaside arrowgrass) belongs to the family Juncaginaceae. It is a clonally propagated perennial herb, which can form irregular clumps up to 2 m across and 60 cm high (Davy and Bishop, 1991). It produces a long flowering scape and raceme inflorescence. Each fruit bears six seeds. Lambracht et al. (2007) studied the geographical distribution of genetic variation in European *T. maritima* populations. They reported two genetically different groups, which includes Atlantic Ocean group, and the North Sea and Baltic Sea group.

T. maritima L. is an ecological engineer plant (Fogel et al., 2004). The species increases plant diversity by modifying its local environment. It creates elevated rings in waterlogged areas that support plant cover (Fogel et al., 2004). The raised rings ameliorate water-logging stress, increase reductive potential, reduce salinity for neighboring plants, and promote increased species diversity. The species in many places occurs at above 60% frequency in salt marshes (Davy and Bishop, 1991). Piernik (2003) reported that *T. maritima* communities were always found above 12 mS/cm salinity, and suggested that *T. maritima* can be

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used as an indicator of soil salinity, described by electrical conductivity of the saturated extracts (EC_e).

T. maritima is an important food source for many organisms and thus is important to the food web. Several species of organisms forage on *T. maritima* (Davy and Bishop, 1991). Some animals, including geese, feed on the leaves and inflorescences, whereas others feed on below-ground tissues (Van der Wal et al., 2000; Zacheis et al., 2001). Despite being a food source for some birds, mammals and insects, the plant is poisonous, especially when tissue gets ruptured (Davy and Bishop, 1991). The plant releases the cyanogenic glucoside triglochinin in new leaves and spikes in spring (Majak et al., 1980). These authors reported lower levels of triglochinin in *T. maritima* from high saline habitats. The cyanogenic compounds in leaves became elevated in drought conditions. Thus, the aerial parts of the plants are strongly toxic to many animals, but the toxicity of the plant is influenced by habitat conditions and moisture levels.

T. maritima is a common species and widely adapted to diverse habitats such as salt marshes, brackish and fresh water marshes, hot springs, inland saline depression, and temporary wetlands (Jefferies et al., 1972; Davy and Bishop, 1991; Masuda et al., 1999; Van Der Wal et al., 2000; Khan and Ungar, 2001; Piernik, 2003; Fogel et al., 2004; Otte and Jacob, 2005). It is generally associated with neutral to alkaline pH (Davy and Bishop 1991). The chemical fingerprint of this species from different habitats could be an important tool in environmental and ecological studies. Davy and Bishop (1991) reported that *T. maritima* accumulated inorganic ions and proline for osmotic adjustment and accumulated high amounts

of sodium. Otte and Jacob (2005) reported that *T. maritima* accumulated high concentrations of lithium, beryllium, indium, thallium, germanium and scandium when grown in mineral-rich spring water. However, the mechanisms of adaptation and survival of *T. maritima* are poorly understood in high temperature and chemically enriched environments such as the hot springs of Yellowstone National Park. Similarly, it is also of interest on how the plant species differs in element uptake pattern among the populations within and between thermally enriched wetlands and other habitats. Also, the question remains unanswered whether or not there is an association between soil biota and *T. maritima*.

1.6. Multi-element Analysis of Plants

Multi-element analysis of plants is an important tool in environmental and ecological studies. Plants play a central role in chemical element cycling in ecosystems because they link the biotic and abiotic environment (Kabata-Pendias, 2001). The presence and accumulation of trace elements in soil and sediments will have potential effect on food chains. Several studies address biotransfer and trophic transfer of trace elements among different terrestrial organisms (Gnamus et al., 2000; Garrott et al., 2002; Scheifler et al., 2002; Kocar et al., 2004).

Each day greater numbers of elements are entering the environment due to increased industrial activities. For instance, the uses of chemical fertilizer enriched with REEs have increased in agriculture production, however, only a little is known about their behavior in and impacts on the environment (Hu et al., 2002; Xu et al., 2002). Rare earth element uptake and accumulation studies in agricultural crops have demonstrated significant uptake and the possible transfer of these REEs into

the food chain (Wen et al. 2001; Kucera et al., 2007). In addition, the development of nanotechnology may release more elements into the environment (Lin and Xing, 2007). Similarly, the use of platinum group elements (PGE) in automobile catalysts has increased the possible risk of accumulation of these elements in the environment and in organisms (Shäfer et al., 1998; Ek et al., 2004). Therefore, the study of element accumulation in plants and their interactions in ecosystems is important in understanding the behavior and their biological roles in the environments.

Multi-element analysis in plants produces a fingerprint, which can be an important tool for exploring the interactions and functions of elements. A multi-element fingerprint is a unique pattern of the concentrations of elements in environmental samples, for example plants and soils (Djingova et al., 2004). With the advent of new technology such as ICP-MS, the correlations of many elements with unknown functions can be established. As mentioned by Fränzle and Markert (2000), as a result of further study several trace and rare earth elements will be found to be biologically essential in the near future. Multi-element analysis in plants helps answer questions, such as: Where do elements released into environment go? How does element uptake in plants differ between species, between and among populations from different habitats and what are the implications? What are the consequences of transfer through different trophic levels? The research presented in this thesis hypothesized that:

1. Hot spring environments are characterized by elevated temperatures and are typically rich in soluble elements. Therefore, as high temperatures increase

transpiration, and because enhanced solubility of elements may enhance their uptake, the element concentrations of *T. maritima* collected from hot spring-influenced wetlands were expected to be higher compared to those collected from temporary wetlands (Chapter 2).

2. High metal concentrations are typically found in mining impacted sites. Therefore plant tissues and root-zone soils collected from such sites were expected to contain higher metal concentrations compared to those from un-contaminated sites (Chapter 3).

3. High temperatures and presence of mycorrhizae in *T. maritima* will enhance survival of the species at high temperature (40 °C) and increase element contents in tissue of the species compared to low temperature and no mycorrhizal associations (Chapter 4).

This was achieved through field work and lab experiments, as follows.

The fingerprints of *T. maritima* were determined for root-zone soils and for *T. maritima* from hot spring-influenced wetlands in YNP and from temporary wetlands in ND by analysis for multi-elements using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Multi-element concentrations along gradients away from hot springs were determined in *T. maritima*, *Juncus* balticus, *Eleocharis rostellata*, *Salix exigua*, *S. boothii*, and *S. wolfii* and their associated root zone soils. The effects of temperature and soil biota on multi-element composition of *T. maritima* were assessed under controlled environmental conditions in the greenhouse. The results of this study will help our understanding of element recycling, trophic transfer of elements and their effects in ecosystems.

The expected outcomes from this dissertation were:

1. Multi-element fingerprints of *Triglochin maritima* L. and other plant species from contrasting habitats will be obtained, and

2. Better understanding of the role of temperature and soil biota on multi-element uptake in plants.

The dissertation is structured as follows, with the three experimental chapters written as manuscripts for publication:

1. Chapter 1: Introduction

2. Chapter 2: Multi-element fingerprinting of *Triglochin maritima* L., *Eleocharis rostellata*, and *Juncus balticus* from contrasting habitats. This chapter describes fieldwork carried out in 2007 and 2008.

3. Chapter 3: Multi-element fingerprinting of *Juncus balticus, Salix exigua, Salix boothii,* and *Salix wolfii* from contaminated and un-uncontaminated sties. This fieldwork was carried out in 2008.

4. Chapter 4: Effects of temperature and soil biota (Mycorrhizae) on element content of *Triglochin maritima* L. This chapter describes the results of a controlled greenhouse experiment.

5. Chapter 5: General discussion
6. Chapter 6: Literature cited

CHAPTER 2. MULTI-ELEMENT FINGERPRINTING OF *TRIGLOCHIN MARITIMA, ELEOCHARIS ROSTELLATA AND JUNCUS BALTICUS* FROM CONTRASTING HABITATS

2.1. Abstract

This study compared multi-element content of plants of the same species from hot spring and cold water wetlands. *Triglochin maritima* L. is cosmopolitan and found in diverse habitats, including coastal and inland salt marshes and hot springs. The multi-element content in this species has been mostly investigated in plants from coastal salt marshes. *T. maritima* plants and their root-zone soils were collected from temporary wetlands in North Dakota (ND) in 2007 and from hot spring influenced wetlands in Yellowstone National Park (YNP) in 2007 and 2008. *Eleocharis rostellata* and *Juncus balticus* were also collected from YNP sites in 2008. Samples were analyzed for multi-element concentrations using inductively coupled plasma optical emission spectroscopy. Element concentrations varied between temporary and hot spring influenced wetlands which suggest habitat specific element concentrations in *T. maritima* with the exception of Na and K concentrations. Against expectations, the majority of elements were present in higher concentrations in plants from the temporary ND wetlands than hot spring sites of YNP. However Li, As, B, Mn, and S concentrations were higher in plant tissues from hot spring influenced populations. *T. maritima, E. rostellata,* and *J. balticus* revealed distinct multi-element fingerprints for elements such as Si (Structural), Na and Mg (Electrolytic), Cr and B (Enzymatic), Sr (Main/subgroup). The analyses of element concentrations in *T. maritima* and *J. balticus* along the

hot spring gradient at Elk Park revealed decreasing concentrations of Na, Mo, Li, and As away from the hot spring, whereas K and Mg concentrations increased. The variation in organic matter contents and temperature of root zone soils along the transect originating at hot spring may have contributed to the variation in element concentrations in these two species. Therefore further investigations are warranted to elucidate the effects of these factors on element concentrations in plant species from the hot springs.

2.2. Introduction

Triglochin maritima L. (Seaside arrowgrass) occurs in hot springs, saline inland wetlands, and fresh and brackish marshes (Jefferies et al, 1972; Davy and Bishop, 1991; Masuda et al., 1999; Khan and Ungar, 2001; Fogel et al., 2004; Otte and Jacob, 2005). *T. maritima* forms elevated rings that ameliorate water-logging stress and reduce salinity for neighboring plants, thus promoting increased species diversity (Fogel et al., 2004). It is an important forage species for many animals including insects, birds and small mammals (Davy and Bishop, 1991; Zacheis et al., 2001), thus trophic transfer of accumulated elements from these plants to other organisms is likely.

Multi-element fingerprinting of plant tissues is becoming an important technique in biomonitoring and ecological studies (Markert, 1991, 1992; Lasat, 2000). Multi-element fingerprinting assesses the multi-element composition in plant tissues and other matrices, based on analytic techniques such as inductively coupled plasma analysis (e.g. ICP-OES, ICP-MS). The majority of studies on element concentrations have focused on a few major elements that are important

in agricultural or toxicity contexts. Most of the trace elements (TEs) and rare earth elements (REEs) in plants are rarely studied. Recently, improved techniques and availability of instruments with low detection limits for routine element analysis have opened an arena for identification of more elements, many of which have as yet unknown functions in biological systems (Fränzle and Markert, 2000, Ayrault, 2001).

Yellowstone National Park (YNP) contains the highest number of surface-geothermal sites in the world, and thus the plants that occupy this habitat must be adapted to this unusual environment. The geothermal activity generates high temperatures and extreme pH conditions (Fournier, 1989). Hot springs produce high concentrations of soluble elements (Stauffer et al., 1980). Stout and Al-Niemi (2002) reported that both monocot and dicot plant species, including *Dicanthelium lanuginosum*, were tolerant to high rhizosphere temperatures of greater than 40 °C. Tolerance to extreme high temperatures in *D. lanuginosum* is due to a symbiosis with a fungus (Redman et al., 2002; Márquez et al., 2007). Channing (2009) reported that *T. maritima* and *Eleocharis rostellata* are dominant at alkali chloride rich thermal waters within geothermally influenced wetlands. *T. maritima* is widespread in this thermal water and was observed growing at a maximum temperature of 48.4 °C in this study. In the hot spring environment, the behavior of plants in terms of multi-element uptake is poorly understood, compared to plants growing in more common habitats. Knowledge about multiple element content of plants and soils is important for our understanding of element cycling and trophic

transfer to other organisms and of the presence and role of the many under-studied elements in the environment.

The current study investigated (i) the multi-element concentration pattern of *T. maritima* from wetlands associated with hot springs of Yellowstone National Park, Wyoming (YNP) and saline temporary wetlands of North Dakota (ND) that receive water predominantly from groundwater and from precipitation (same species, different habitat), (ii) multi-element concentrations of elements in *T. maritima* along transects of streams originating from hot springs at YNP were studied and (iii) multi-element concentrations in *T. maritima*, *E. rostellata* and *J. balticus* within wetlands associated with hot springs (different species, same habitat) were studied. Because availability of many elements in hot springs would be expected to be higher compared to non-hot spring surface waters, and because the higher temperatures near hot springs would increase evapo-transpiration, it was hypothesized that (i) element concentrations of *T. maritima* collected from the sites influenced by hot springs would be higher compared to those in plants from the temporary wetlands, and (ii) element concentrations would be higher within the hot spring sites as plants grew closer to the spring. It was further expected (iii) that patterns in element concentrations would vary between species, but how exactly was unclear, because no previous data on element concentrations of *E. rostellata* or *J. balticus* existed.

2.3. Materials and Methods
2.3.1. Sampling locations

Sampling took place during July of 2007 and 2008. In 2007, *T. maritima* was collected from populations in two contrasting habitats, saline temporary wetlands

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in North Dakota (ND) and hot springs in Yellowstone National Park (YNP), Wyoming (Fig. 2.1). A total of five study sites, two from ND; Kellys Slough, (KS) and a ditch near the Grand Forks Landfill (LF); and three from YNP; namely Boulder Spring (BS), Elk Park (EP), and Rabbit Creek (RC), were sampled. The populations of KS and LF represent temporary wetlands with relatively lower soil temperatures compared to the hot spring-influenced YNP populations. As these two regions are approximately 1,400 km apart, it is unlikely that these populations would have belonged to the same genetically recognizable population. Within the ND sites, the two populations were approximately 12 km apart. The YNP populations BS, EP, and RC represent hot spring influenced wetlands, each being fed by different hot springs. The sites within YNP were between 12 and 24 km apart. It is possible that these populations within both regions were very similar, but for the purpose of this study they were considered to be separate populations. The field sampling of 2008 was based on the findings of field season 2007. The observed variation in element concentrations in *T. maritima* species collected at different habitats raised the question of how distance from a hot spring affected element concentrations. Another question arising from the 2007 work was how much variation in element concentrations occurred between different species within the same habitat. Therefore, during the 2008 season, *T. maritima* as well as *Eleocharis rostellata* and *Juncus balticus* were sampled along streams originating from hot springs, and thus along suspected chemical and temperature gradients at YNP.

2.3.1.1. North Dakota (ND)

Kellys Slough (KS) and the landfill site (LF) at Grand Forks County were the two sampling sites in North Dakota (Fig. 2.1). These two sites are submerged temporarily during the spring and early summer. Kellys Slough is a saline depression. The site is located to the north-east of Kellys Slough National Wildlife Refuge (N 47° 59.856, W 97° 14.359). During sample collection the root-zone temperature ranged from 17 - 20 °C. The soil series, Lallie silt clay, is poorly drained, run-off is ponded, and permeability is low, with a seasonal high water table (Soil survey staff, 2009). The landfill site (LF) (N 47° 56. 467, W 097° 09.187) is located at about 6.5 Km west of Grand Forks whereas, it is about 10 Km away from Kellys Slough. The site lies within the Red River Valley physiographic region. According to Olson and Greer (1994) the area is deposited mainly with clay and silt with a pH range of 7.5 to 8.5. The root-zone temperature ranged from 17 - 21 °C during sample collection. The soil series for the site is described as Bearden silty clay loam, very deep, poorly drained, and slightly to moderately alkaline (Soil survey staff, 2009). In Grand Forks the hottest month is July and highest bare soils mean temperature during July 2007 in Grand forks was 25.8 °C (NDAWN Center, 2007).

2.3.1.2. Yellowstone National Park (YNP)

Three sites influenced by hot springs were selected within YNP (Fig. 2.1). Boulder Spring (BS) is located at N 44° 31.039, W 110° 49.575 and the root-zone temperature during sample collection ranged from 24.7 - 48.4 °C with an average temperature of 31.8 °C. Elk Park (EP) is located at N 44° 43.583, W 110° 43.255

and the root-zone temperature during sample collection ranged from 21 to 34 °C with an average temperature of 25° C. Rabbit Creek (RC) is located at N 44° 31.341, W 110° 48.783 and the root-zone temperature ranged from 28.3 to 45.6 °C with an average temperature of 32.5 °C. The soils at Boulder Spring, Elk Park, and Rabbit Creek are classified as aquic inceptisols, which indicates that the soils of these sites are wet for most of the year. The root-zone soils of these sites are influenced by geothermal activity and are derived from siliceous sinter (white, lightweight, porous, variety of silica deposited by hot spring) and altered rhyolite (igneous and volcanic rocks). The textures of these soils are transparent to large-grained crystals. The soils are silty to sandy surface textures. Inceptisols in poorly drained conditions are characterized by active leaching of minerals relative to other landscapes. Inceptisols are developed by translocation or loss of Fe, Al or organic matter, or the basic cations. The horizon contains sandy loam with finer texture. Inceptisols are formed in two situations such as soils that are developed in geologically young sediments (alluvium, colluvium, and volcanic ash) and in areas where environmental conditions (extreme temperature, parent material highly calcareous and resistant to weathering, and high water table) inhibit the soil formation process. The horizon contains sandy loam with finer texture. Inceptisols are formed in two situations such as soils that are developed in geologically young sediments (alluvium, colluvium, and volcanic ash) and in areas where environmental conditions (extreme temperature, parent material highly calcareous and resistant to weathering, and high water table) inhibit the soil formation

process. Organic matter decomposition is less efficient in such conditions and tends to accumulate more organic matter as compared to better drained soils.
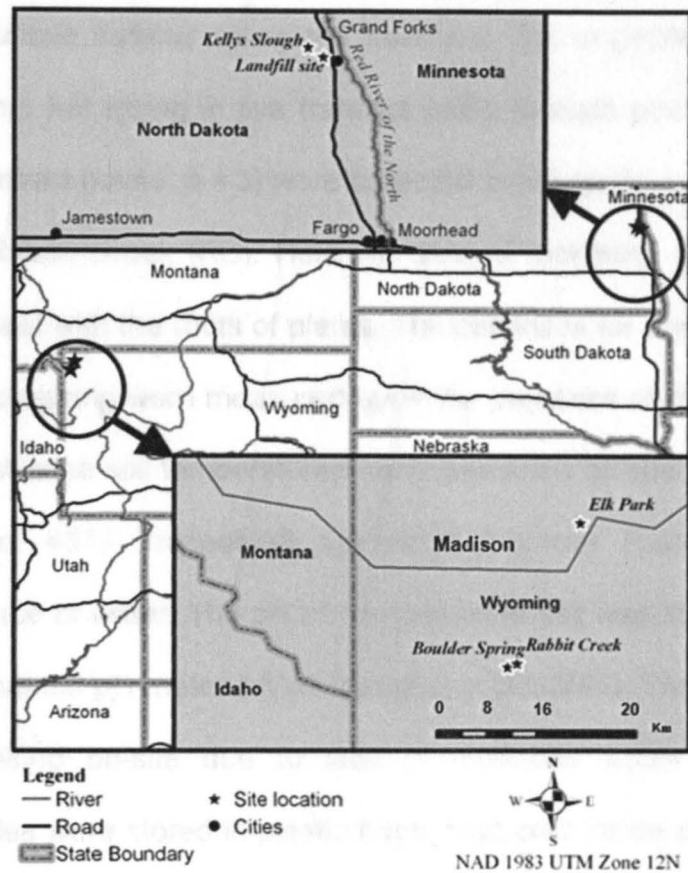
Figure 2.1. Map showing sampling sites in North Dakota (Kellys Slough and Landfill site) and Yellowstone National Park (Boulder Spring, Elk Park, and Rabbit Creek). (Map generated in ArcGIS Desktop 9.3.1 Student Evaluation Edition)

2.3.2. Sampling procedures

During the summer of 2007 sampling was carried out at both Yellowstone National Park and in North Dakota. In ND, initially three sites were sampled at $n =$ 10, but two of the sites were within close proximity, and the results were so similar, that they were deemed replications of the same population, resulting in two reported sampling sites (KS ($n = 10$), LF ($n = 20$)). In YNP, the sites BS ($n = 10$), EP ($n = 10$), and RC ($n = 10$). The samples were selected randomly within an area

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that covered 1 x 1 m^2 wooden quadrant. Root-zone soils associated with each plant sample were collected. During the summer of 2008, leaves of *T. maritima, E. rostellata* and *Juncus balticus* along the transects that originated at hot spring through away from hot spring in five transect point, in each point three replicate samples (five transect points, *n* = 3) were collected in hot spring fed streams at Elk Park (EP) and Rabbit Creek (RC). Here we defined root-zone soils as the soils that were in contact with the roots of plants. The distances for each transect point away from the hot spring were measured upon the presence of the species along the transect. Root-zone soil temperatures were measured on site using a portable thermometer (No. 4373 Traceable® Memory/Waterproof Thermometer) when there was presence of water. The pH of the root-zone soil was measured at YNP sites using a handheld pH meter (VWR Symphony SP90M5). The pH of ND sites was not determined on-site due to lack of root-zone water during sample collection. Samples were stored in plastic bags, kept cool inside a portable cooler and transported to the laboratory.

2.3.3. Plant samples and root-zone soil processing

The plant samples were separated into leaf and root compartments and washed thoroughly, first in tap water and then distilled water, to remove dust and soil particles. The samples were blotted dry and the fresh weight was recorded. The samples were oven-dried at 60 °C until constant weight. Leaves and roots were pulverized using a mortar and pestle and homogenized in liquid nitrogen. Soil samples were also pulverized. Dead roots and other large plant debris were removed from the soil prior to homogenization.

Plant and soil samples were digested using microwave digestion (CEM Mars Xpress, 230V/60HZ) in Express 55 ml PFA venting vessels. A weight of approximately 250 mg of ground plant material was pre-digested overnight in 5 ml concentrated nitric acid, 5 ml of distilled water were then added and the samples placed in a MARS Xpress Microwave Digester (16 vessels, 1600 W, 100% Power, ramped to 200 °C over 15 minutes, and held at this temperature for 5 minutes). The digested samples were transferred to culture tubes. Each vessel was rinsed with 1 ml aliquot of distilled water three times. Similarly, a known weight of approximately 500 mg of soil was pre-digested in 10 ml concentrated nitric acid. The samples were thoroughly mixed using a mini-vortexer by adding 5 ml of nitric acid at a time and was pre-digested overnight The samples were digested as above (1600 W, 100% Power, ramped to 185 °C over 15 minutes and then held for 15 minutes). The digests were filtered through Whatman® 1 filter paper into culture tubes, and the vessels rinsed as described above. Both the plant and soil samples were diluted to 1:10 except for 18 root samples from ND, which were diluted to 1:100.

2.3.4. Multi-element analyses

Analysis of 32 elements associated with the root-zone soil, leaves, and roots samples were carried out by a Spectro Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) with Crossflow nebulizer, Side-On-Plasma (SOP), with Smart Analyzer Vision v. 3.013.0752. Operating conditions for the ICP-OES instrument were: plasma power 1400 W, coolant gas flow rate 13.5 L min^{-1}, auxiliary gas flow rate was 1.2 and nebulizer flow 0.9 and 21 second sample

integration time. The ICP was calibrated using an internal calibration (ICAL solution) and a 4-point standard curve for the elements using individual or combination standards in a five percent HNO_3 matrix. A continuing control verification (CCV) standard was checked after every 10 samples for quality control. The accuracy of elemental analysis was monitored by using a certified reference material (NCS DC 73350 leaves of Poplar and NCS DC 73384 soil from the China National Analysis of Center for Iron & Steel 2004). Instrument detection limits in mg L^{-1} of the plants and root-zone soils are presented in appendix 1.

In the remainder of this paper element concentrations will be presented in molar units and presented following the biological system of the elements (BSE) of Markert (1994). In the BSE, elements are grouped in a fashion similar to the Periodic System of the Elements, but according to correlations between and among the elements, their possible physiological and biological roles, and their toxicity in organisms. According to Markert (1994), structural elements (C, H, O, N, P, S, Si, and Ca) are important in the function and structure of cell metabolism, electrolytic elements (K, Mg, and Na) are vital in maintaining osmotic conditions in cell metabolism, while enzymatic elements (B, Cu, Cr, Fe, Mn, Ni, V, and Zn) enhance the catalytic functions in cell metabolism.

2.3.5. Loss-on-ignition

Loss-on-Ignition was performed to estimate the organic matter (OM) content in the Soil Testing Laboratory, North Dakota State University. Five grams of root-zone soil were weighed into ceramic crucibles. The soils were oven-dried at 120 °C overnight. The dried soil was weighed and then samples were kept in a furnace

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for 2 hours at 360 °C. The OM content of soil samples was determined by calculating the difference in weight before and after being placed in the furnace, and were presented as OM g 100 g^{-1}.

2.3.6. Data analysis

Element concentration data were log-transformed to obtain normality and homogeneity of variance before statistical analysis. Statistical significance of differences was determined by General Linear Model (One-way ANOVA, $P <$ 0.05), and upon the significant differences in mean element concentrations between the sites for each compartment; leaf, root, and root-zone soils. Where significance was detected, multiple comparison tests were performed using post-hoc Tukey's Test ($P < 0.01$) in Minitab statistical software (Minitab [®] 15 ©2006 Minitab Inc.) to determine which of the sites were different from each other.

The purpose of the multi-element study in the transect that originated at hot spring was to examine the pattern of element concentrations in *T. maritima* and *E. rostellata* and root-zone soils associated with these plants if there are any. Thus ANOVA and mean comparison tests were not performed among the distances in the transect originated at hot spring. Principal component (PC) analysis for 2007 data was performed in R-program (R Development Core Team, 2009) using the log transformed element concentrations, which were recorded in all three compartments of *T. maritima,* and including LOI in root-zone soil, for five study sites in 2007. The PC score are centered and standardized to centroid of the axis. Only the PCs, which explained > 80% of the cumulative variation, were presented. Pearson correlation coefficients and *P*-values were calculated using Minitab. Only

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correlations that were $r > 0.55$, explaining $> 30\%$ (r^2) variation were considered important.

2.4. Results

2.4.1. Organic matter, pH, and temperature in the root-zone soil

Loss-on-ignition (LOI), an estimate of organic matter (OM) content, varied significantly among sites, with the lowest mean values at LF (3.2 ± 0.7) and RC (3.5 ± 0.7). Kellys Slough (9.2 ± 4.1) and BS (6.8 ± 2.9) had slightly higher but similar organic matter contents, while EP (33 ± 13) had the highest organic matter content at almost ten times higher than LF and RC. The root-zone temperatures of the sites varied significantly ($P < 0.01$). The temperatures (°C) of KS (19 ± 1) and LF (19 ± 1) were significantly different from BS (32 ± 8) and RC (36 ± 7) but not from EP (23 ± 2). The pH values among the YNP sites were significantly ($P < 0.01$) different, with RC (9.5 ± 0.1) the highest, and lower values at BS (8.4 ± 0.5) and EP (6.0 ± 0.2).

The LOI of root-zone soils were measured along the transect of stream that originated at hot spring during 2008 are presented in Fig. 2.2. LOI at the hot spring sites of EP and RC showed significant ($P < 0.05$) variation along the transects. The highest value of 65.3 g 100 g^{-1} in EP was observed at the distance of 29 m away from hot spring (Fig. 2.2a), whereas at RC the highest value tented to be 23.4 g 100 g^{-1} at 5 m away from hot spring (Fig. 2.2b). Organic matter content of root-zone soils did not show consistent patterns with distance from the spring. The mean, standard deviation, minimum, and maximum of temperature and pH of root-zone soils along the transect points for 2008 are presented in Table 2.1.

Table 2.1. Mean temperature and pH in the root-zone soils of *T. maritima* and *E. rostellata* along the transect originated at hot spring through distances away from hot spring at Elk Park and Rabbit Creek in 2008 (*n* = 3).

| | __Elk Park__ | | | __Rabbit Creek__ | |
Distance (m)	Temperature (°C)	pH	Distance (m)	Temperature (°C)	pH
3	33.9	5.8	5	18.9	9
10	33	6	27	20.3	9.2
14	28.6	5.1	45	23.4	8.9
29	24.7	5	67	18.5	8.8
43	22.8	5.5	125	19	9.5

2.4.2. Root-zone soil element concentrations

2.4.2.1. Hot spring and temporary wetlands

The mean element concentrations in *T. maritima* root-zone soil (μmol g^{-1}) are presented in Table 2.2. The majority of element concentrations from ND sites, except arsenic and beryllium, were significantly higher ($P < 0.01$) compared to YNP sites. Within ND sites, the electrolytic elements (K and Na) and enzymatic elements (B, Cu, Cr, and Ni) as well as Sr and Li, were higher in KS compared to LF, whereas mean element concentrations of Ca, Mg, and Ba were significantly higher in LF. In YNP, the majority of mean element concentrations were similar between BS and RC, while EP showed significantly higher ($P < 0.01$) concentrations compared to RC for S, B, Fe, Mn, Al and As. Concentrations of V, Cu, Pb, Cd, and Mo were below the ICP-OES detection limits for BS, EP, and RC soils.

Table 2.2. Mean element concentrations ($\mu mol \ g^{-1}$ dry soil) in the root-zone soils of *T. maritima* in 2007 (mean ± standard deviation, n = sample number, <dl = below the detection limit, different letters after values within one row indicate significant differences between sites at P < 0.01). Sites are North Dakota temporary wetlands, Kellys Slough (KS), and Landfill (LF); and Yellowstone National Park hot spring sites, Boulder Spring (BS), Elk Park (EP), and Rabbit Creek (RC).

Element	Element Concentration ($\mu mol \ g^{-1}$ dry weight sample)				
	KS (n = 10)	LF(n = 20)	BS (n = 10)	EP (n = 10)	RC (n = 10)
Structural					
Ca	1015 ± 570b	2182±295c	0.02±0.01a	0.04±0.02a	0.02±0.02a
P	35.1±6.8b	24.5±5.72b	2.65±1.54a	7.3±6.8a	2.85±4.89a
S	224±105c	238±149c	35.2±28ab	98.2±52.9b	19.3±29.6a
Electrolytic					
K	158±18.6c	69±23.8b	27.9±16.5a	39.4±20.9a	27.9±11a
Na	363±170c	161±49.7a	145±53.5a	285±165bc	192±42ab
Mg	538±48.8b	1174±303b	13.6±10.4a	10.3±4.42a	10.4±10.5a
Enzymatic					
B	5.85±1.7b	2.32±0.39a	2.87±2.91a	8.17±5.49b	1.91±0.85a
Co	0.087±0.02c	0.07±0.01b	0.01±0.01a	0.01±0.00a	0.007±0.01a
Cu	0.308±0.11	0.224±0.05	<dl	<dl	<dl
Cr	0.373±0.07c	0.251±0.05b	0.046±0.04a	0.03±0.02a	0.024± 0.04a
Fe	441±57.8c	344±109c	43.4±44.2ab	98.7±87.2b	20.3±37.6a
Mn	13.5±5.8c	12.9±5.04c	3.4±2.86b	6.88±6.62b	0.68±0.18a
Mo	0.005±0.001	0.007±0.00	<dl	<dl	<dl
Ni	0.258±0.04c	0.198±0.04b	0.018±0.01a	0.016±0.00a	0.01±0.02a
V	0.723±0.10	0.546±0.10	<dl	<dl	<dl
Zn	1.32±0.23b	1.39±0.89b	0.34±0.22a	0.33±0.21a	0.29±0.25a
Main group					
Al	833±102c	551±176c	89.7±63.5b	100±75.8b	33.5±12.5a
As	0.079±0.02a	0.082±0.02a	5.23±5.71bc	16.27±18c	1.86±3.82ab
Ba	1.11±0.13b	2.97±1.24c	0.14±0.14a	0.31±0.23a	0.09±0.13a
Be	0.075±0.01a	0.057±0.01a	0.104±0.09a	0.214±0.02b	0.127±0.09ab
Li	5.04±2.4b	2.96±0.61a	2.79±1.08a	4.16±2.22ab	3.81±1.09ab
Pb	0.095±0.02	0.08±0.02	<dl	<dl	<dl
Sr	5.29±2.7c	2.99±1.12b	0.05±0.03a	0.08±0.06a	0.04±0.04a
Sub group					
Ag	0.004±0.00b	0.005±0.00b	0.001±0.00a	0.002±0.00a	0.001±0.00a
Ti	3.41±0.51bc	4.09±1.37c	2.39±2.26ab	1.43±1.07a	1.26±2.36a
Zr	1.84±0.35b	1.47±0.28b	0.29±0.23a	0.26±0.2a	0.17±0.24a
Cd	0.006±0.000	0.005±0.000	<dl	<dl	<dl
Lanthanides					
Ce	0.218±0.05b	0.173±0.03b	0.031±0.03a	0.049±0.06a	0.02±0.04a
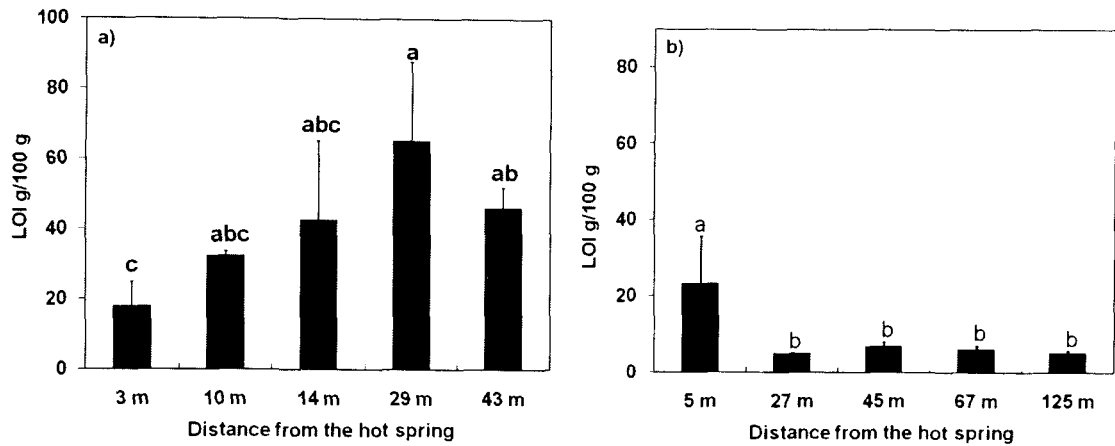
Figure 2.2. Mean Loss-on-Ignition (LOI) in root-zone soils of *T. maritima* and *E. rostellata* in (a) Elk Park (b) Rabbit Creek in Yellowstone National Park, (*n* = 15) in 2008. Error bars indicate ± standard deviations, x-axis shows the distance in meter of each transect point along the transect away from hot spring

2.4.2.2. Hot spring transects

The mean element concentrations in the root-zone soils along the hot spring transects at EP and RC are presented in Tables 2.3 and 2.4, respectively. At EP, although the patterns of element concentrations varied depending on the element, the majority of elements tended to be high in root-zone soils away from the hot spring at measured distances. Significant positive correlations of the element concentrations with LOI in root-zone soils were observed for the following elements: P (r = 0.68; P = 0.000), Fe (r = 0.56; P = 0.033), Be (r = 0.63; P = 0.012), Sr (r = 0.57; P = 0.031), Al (r = 0.73; P = 0.02), K (r = 0.71; P = 0.003), and Mn (r = 0.60; P = 0.018). In contrast, several of these elements correlated negatively with temperature: P (r = - 0.80; P = 0.000), S (r = - 0.79; P = 0.000), Mo (r = - 0.69; P = 0.005), Be (r = - 0.88; P = 0.000), Sr (r = - 0.89; P = 0.000), Al (r = - 0.76; P = 0.001), K (r = - 0.70; P = 0.004), Mn (r = - 0.60; P = 0.018), and Zn (r = - 0.82; P = 0.000).

Table 2.3. Mean element concentrations (μmol g^{-1} dry soil unless otherwise indicated) in root-zone soils of *T. maritima* and *E. rostellata* together at different sampling distances from a hot spring fed stream in Yellowstone National Park at Elk Park (EP) (mean ± standard deviation, *n* = 3).

Element	Unit	Sampling gradient distances away from hot spring at EP				
		3 m	10 m	14 m	29 m	43 m
Structural						
Ca	µmol/g	8.2±5.2	14.8±3.8	17.9±6.1	18.0±6.9	22.6±2.2
P	µmol/g	2.8±1.7	5.1±1.4	7.3±2.3	14.2±0.4	9.5±2.1
S	µmol/g	44.2±8.2	56.7±3.5	61.7±10.8	168.6±30.8	135.8±82.3
Si	µmol/g	1.3±1.2	0.3±0.1	0.4±0.4	0.5±0.0	0.2±0.1
Electrolytic						
K	µmol/g	8.4±3.8	13.3±1.8	14.6±1.3	20.8±3.5	16.8±1.6
Na	µmol/g	91.6±37.6	112.5±25.5	201.1±103.7	95. 7±28.5	45.3±5.4
Mg	µmol/g	4.5±3.1	6.4±0.6	7.5±0.9	11.5±0.8	6.3±0.1
Enzymatic						
V	nmol	25.7±14.7	29.8±9.2	47.1±16.8	76.4±18.4	32.7±15.9
Cr	nmol	16.7±10.3	21.9±4.1	29.1±2.8	42.1±8.3	21.4±1.5
Mo	nmol	68.6±21.5	30.9±10.2	76.3±30.0	198.1±80.0	229.9±186.9
Mn	µmol/g	0.57±0.4	0.8±0.1	4.8±4.3	9.6±4.9	1.8±0.5
Fe	µmol/g	16.0±11.2	19.2±5.1	47.6±31.8	87.4±25.3	18.3±6.5
Co	µmol/g	1.5±1.6	0.3±0.5	4.9±5.0	12.4±4.9	0.9±0.8
Cu	nmol	2.3±4.1	20.8±9.5	40.6±15.2	75.9±22.1	15.6±2.9
Zn	nmol	14.8±22.0	20.4±35.4	31.3±11.2	391.0±56.3	294.7±154.6
B	µmol/g	7.3±3.3	10.2±2.3	31.5±32.4	12.2±4.1	4.9±0.6
Main/sub group						
Li	µmol/g	1.8±0.9	2.3±0.5	4.2±2.4	2.3±0.6	0.9±0.1
Be	nmol	70.1±62.4	30.7±38.2	187.9±74.2	258.2±81.4	317.8±63.5
Sr	nmol	27.1±14.9	45.6±3.2	61.9±16.8	87.1±26.9	120.3±13.7
Ba	nmol	110.7±60.9	180.2±2.3	362.3±138.8	564.4±185.2	177.2±41.2
Al	µmol/g	27.6±17.6	43.3±9.1	53.1±1.2	77.4±12.2	85.8±14.2
As	µmol/g	9.0±2.3	5.8±3.1	14.8±3.7	14.8±4.6	4.2±4.1
Zr	nmol	11.7±7.2	14.7±2.0	23.6±3.8	33.6±4.9	12.3±0.0
Ti	µmol/g	0.57±0.3	0.7±0.1	1.0±0.2	1.5±0.3	0.5±0.0
Ce	nmol	11.4±8.1	11.4±3.5	20.0±4.7	39.4±9.5	11.5±2.6

At RC, the majority of elements did not show any specific concentration pattern (Table 2.4), however, the concentrations of P, S, B, and As are likely to be high nearest to the spring. Several elements showed positive correlations with LOI: Ca (*r* = 0.66; *P* = 0.000), P (*r* = 0.95; *P* = 0.000), S (*r* = 0.92; *P* = 0.000), B (*r* = 0.91, *P* = 0.000), Li (*r* = 0.80; *P* = 0.000), Sr (*r* = 0.71; *P* = 0.003), and As (*r* = 0.89; *P* = 0.000). These elements did not show any correlations with root-zone soil

temperature, whereas other elements showed positive correlations: Ba ($r = 0.80$; $P = 0.000$), Ce ($r = 0.75$; $P = 0.001$), Co ($r = 0.86$; $P = 0.000$), Cr ($r = 0.77$; $P = 0.001$), Cu ($r = 0.80$; $P = 0.000$), Fe ($r = 0.69$; $P = 0.004$), Mg ($r = 0.60$; $P = 0.018$), Ni ($r = 0.87$; $P = 0.000$), Pb ($r = 0.89$; $P = 0.000$), Ti ($r = 0.72$; $P = 0.002$), and Zr ($r = 0.71$; $P = 0.003$).

Table 2.4. Mean element concentrations (μmol g^{-1} dry soil unless otherwise indicated) in root-zone soils of *T. maritima* and *E. rostellata* together at different sampling distances from a hot spring fed stream in Yellowstone National Park at Rabbit Creek RC. (mean ± standard deviation, $n = 3$).

Element	Unit	Sampling gradient distances away from hot spring at RC				
		5 m	27 m	45 m	67 m	125 m
Structural						
Ca	μmol/g	9.5±5.6	4.4±1.4	8.6±0.41	5.3±1.4	5.5±1.5
P	μmol/g	4.5±2.1	1.3±0.15	2.2±0.29	1.9±0.49	1.2±0.3
S	μmol/g	40.5±18.1	3.9±1.4	7.6±0.84	11.1±2.7	5.9±1.8
Si	μmol/g	1.1±0.36	1.1±0.64	0.71±0.08	1.7±0.11	1.2±0.62
Electrolytic						
K	μmol/g	20.3±6	17.8±3.4	25.7±1.3	18±3.9	23±5.2
Na	μmol/g	143.2±58	117.9±33.3	65.4±2.8	93.4±27.1	103.1±26.8
Mg	μmol/g	10.5±4.6	8.1±1.2	20.7±1.8	7.6±0.23	16±4
Enzymatic						
V	nmol	34±15.4	11.4±1.8	58.7±8.2	16.2±3	32.2±11.3
Cr	nmol	22.4±10	13.4±1.4	56.1±6.3	13.9±0.76	30.2±10.5
Mo	nmol	<dl	<dl	<dl	<dl	<dl
Mn	μmol/g	0.43±0.12	0.5±0.06	1.6±0.18	0.47±0.09	0.7±0.14
Fe	μmol/g	11.5±4.3	8.5±0.39	33.6±4	9.8±0.41	19.6±5.3
Co	μmol/g	0.74±1.3	0±0	7.9±1.2	0±0	1.9±1.7
Cu	nmol	<dl	<dl	<dl	<dl	<dl
Zn	nmol	25.7±20.4	5.4±9.4	76.4±40.8	1.2±2.1	58.2±46.2
B	μmol/g	6.6±3.5	2.8±0.2	2.82±0.16	2.8±0.39	3±0.24
Main/sub group						
Li	μmol/g	7.2±3.7	3.8±0.86	3.9±0.3	3.4±0.67	4.4±0.77
Be	nmol	38.8±35.1	5.7±8.7	1.4±2.4	128.3±50.1	14.3±21.3
Sr	nmol	53.4±27.2	19.5±0.95	53.3±3.2	33.9±7.8	33±8.3
Ba	nmol	53±20	27.3±0.77	143±11	44.9±6.3	61±15.9
Al	μmol/g	45.8±17.3	24.3±1.1	89.2±7.6	42.3±6.6	54.4±16.5
As	μmol/g	5.6±4	0.03±0.05	0.36±0.11	0.96±0.18	0.09±0.08
Zr	nmol	16.2±5.8	9.8±0.72	32.8±1.7	11.8±0.82	21.2±5.3
Ti	μmol/g	0.69±0.26	0.42±0.02	2.4±0.34	0.54±0.02	1.3±0.47
Ce	nmol	12.5±6.6	3.7±0.31	39.6±4.4	9.3±2.6	18.8±7.3

2.4.3. Multi-element fingerprint of *T. maritima* roots and leaves from hot spring and temporary wetlands

The average element concentrations ($\mu mol\ g^{-1}$) and standard deviations in *T. maritima* roots and leaves for all five sites are presented in Tables 2.5 and 2.6, respectively. In most cases, similarities in multi-element concentration patterns were observed for root and leaf tissues within the ND and YNP study areas. The majority of element concentrations were much greater in roots, except for the electrolytic elements K, Mg, and Na, and the structural elements Ca and Li, which were higher in leaf tissues. Plants from YNP showed significantly ($P < 0.01$) higher concentrations of S, B, As, and Li in the roots compared to plants from ND. Elements such as B, Mn, and Li were also higher in the leaf tissues from YNP compared to ND sites.

Within ND, the element concentrations of P, K, B, Mn, and Sr were significantly ($P < 0.01$) higher in root tissues at KS compared to LF (Table 2.5). In contrast, the concentrations of Cu, Ni, V, Ti, and Zr were higher in root tissue sampled at LF. In leaf tissues, the concentrations of K, Na, Mn, Li, and Sr were higher in KS whereas Ca, Si, Mg, Fe, and Al were higher in LF samples (Table 2.6).

In YNP, the mean element concentrations of Ca, P, S, Mg, B, and Li were higher in both root and leaf tissues although these elements were low in the corresponding root-zone soil (Tables 2.5 and 2.6). Among the YNP sites, Ca, Mg, Cu, and Li concentrations were significantly higher ($P < 0.01$) in root tissue from

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RC whereas P, K, Fe, and Ba were significantly lower ($P < 0.01$) compared to BS and EP.

Table 2.5. Mean element concentrations (μmol g^{-1} dry sample weight) in the roots of *T. maritima* in 2007 for sites in North Dakota (Kellys Slough KS, Landfill LF) and Yellowstone National Park (Boulder Springs BS, Elk Park EP, Rabbit Creek RC): (mean ± standard deviation, n = 10 except for Landfill (LF) site (n = 20), <dl = below detection limit, different letters after values within one row indicate significant differences between sites at $P < 0.01$).

Element	Roots				
	KS	LF	BS	EP	RC
Structural					
Ca	116±19a	114±25a	84±21b	63±10.7c	103±10a
P	575±148a	325±89.4b	177±155c	170±84c	71.5±11.6d
S	<dl	<dl	173±70b	173±52.2b	206±42.1a
Si	55±17a	61±21a	41±37c	29±17bc	25±15c
Electrolytic					
K	403±91.7a	210±69.9c	219±169c	290±135c	116±20.8d
Mg	116±22.8a	134±36.3a	39.3±35.9b	39±17.6b	71.2±22.1a
Na	604±225b	423±205a	513±189bc	595±249c	550±169b
Enzymatic					
B	8.9±1.73b	4.2±0.97b	14.5±5.9a	15.4±4.1a	15.1±2.1a
Cu	0.08±0.02b	0.15±0.03a	0.05±0.0bc	0.02±0.02c	0.09±0.03b
Cr	0.01±0.01	0.02±0.01	<dl	<dl	<dl
Fe	127±56.3a	123±83.9a	59.8±99.7b	148±79.5a	2.54±0.6c
Mn	8.06±3.9a	4.08±2.2b	11.4±8.7a	7.4±5.2a	4.2±0.9b
Ni	0.03±0.01	0.05±0.02	<dl	<dl	<dl
V	0.11±0.04	0.20±0.11	<dl	<dl	<dl
Zn	0.21±0.05b	0.37±0.26a	0.24±0.2b	0.41±0.1b	0.16±0.1bc
Main /sub group					
Al	18.2±5.8a	24.2±10.2a	9.4±6.5b	4.4±4.1c	3.6±2.1c
As	0.19±0.08c	0.2±0.12c	3.07±2.2b	11.5±9.4a	1.32±0.9b
Ba	0.17±0.06ab	0.11±0.07bc	0.14±0.1b	0.25±0.1a	0.05±0.01c
Li	0.64±0.2a	0.34±0.11a	11.2±6.4b	7.01±3.7c	32.8±8.1a
Sr	1.66±0.4a	0.61±0.11b	0.34±0.1c	0.27±0.1c	0.35±0.1c
Ti	0.19±0.07b	0.35±0.17a	0.15±0.1b	0.06±0.05b	0.04±0.02b
Zr	0.42±0.2b	0.99±0.52a	0.32±0.2bc	0.24±0.15bc	0.1±0.05c

Table 2.6. Mean element concentrations (μmol g^{-1} dry sample weight) in the leaves of *T. maritima* in 2007 for sites in North Dakota (Kellys Slough KS, Landfill LF) and Yellowstone National Park (Boulder Springs BS, Elk Park EP, Rabbit Creek RC); (mean ± standard deviation, *n* = 10 except for Landfill (LF) site (*n* = 20), <dl = below detection limit, different letters after values within one row indicate significant differences between sites at *P* < 0.01.

Element	Leaves				
	KS	LF	BS	EP	RC
Structural					
Ca	276±47b	459±107a	95±48.3c	122±37bc	123±52bc
P	349±22.2ab	242±28b	275±140b	217±54bc	167±81bc
S	<dl	<dl	218±145	167±132	128±38
Si	6±2b	12±3a	13±3a	11±3a	15±8a
Electrolytic					
K	902±155a	390±114bc	476±291bc	591±225c	401±116c
Mg	212±25.5b	497±179a	53.4±18.5b	74.7±21.5d	118±24c
Na	2180±164a	1457±301b	1810±303ab	1770±317ab	1530±194b
Enzymatic					
B	2.41±0.4c	2.6±0.5c	4.93±0.8b	11.5±6.4a	4.4±0.4b
Cu	0.09±0.08b	0.13±0.05a	0.05±0.06b	0.05±0.04b	0.06±0.02b
Cr	0.005±0.02	0.01±0.0	<dl	<dl	<dl
Fe	2.26±0.6ab	4.15±1a	1.32±0.6b	2.19±1.2b	0.8±0.3c
Mn	5.31±2.7ab	2.45±2.5b	9.97±4.5a	8.7±5.4ab	4.5±1.4a
Ni	<dl	<dl	<dl	<dl	<dl
V	<dl	<dl	<dl	<dl	<dl
Zn	0.16±0.06a	0.14±0.06a	0.15±0.05a	0.19±0.06a	0.15±0.04a
Main /sub group					
Al	2.27±1.2b	7.0±2.6a	1.85±1.2b	1.3±0.7b	1.2±0.3b
As	<dl	<dl	0.132±2	0.26±0.21	0.14±0.06
Ba	0.03±0.008	0.09±0.03	<dl	<dl	<dl
Li	2.16±0.4d	1.3±0.3e	52±9.1b	30.2±11.2c	76.5±17.1a
Sr	2.05±0.2a	1.2±0.3a	0.07±0.0b	0.2±0.1b	0.082±0.024b
Ti	0.03±0.01	0.091±0.031	<dl	<dl	<dl
Zr	<dl	<dl	<dl	<dl	<dl

In contrast, P, K, Fe, Zn, As, and Ba were significantly higher (*P* < 0.01) in root tissues sampled from EP (Table 2.5). At Boulder Spring, P concentration was significantly higher in the roots (*P* < 0.01) compared to other sites in YNP. Similarly, Mg and Li were higher whereas P and Fe were lower in leaf tissue in RC

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(Table 2.6), but S, which was high in roots, was lower in the leaves. In Elk Park, B, Fe, and As were higher in the leaves. Similarly, P and S were higher in leaves at BS. Some elements were recorded below the detection limits. In ND sites, S was below the detection limit in both root and leaf tissues whereas V, Ni, As, and Zr were below the detection limit in leaf tissue only. Similarly at YNP sites, V, Ni and Cr were below the detection limit in both root and leaf tissues whereas Ti and Zr were below the detection limit in leaves only.

2.4.4. Principal component analysis (PCA)

Of all the elements, 12 were found in detectable concentrations in all compartments (root-zone soil, root, and leaf) from the five study sites and these were analyzed by PCA. In all compartments, two PCs explained > 80% of variation in elements concentrations (Table 2.7).

2.4.4.1. Root-zone soil

PC1 and PC2 explained 95% of the variation of multi-element concentrations in root-zone soil (Table 2.7). Three distinct patterns of multi-element concentrations in the soil can be observed from PCA analysis (Fig. 2.3). The first group tended to be clustered between ND sites with two distinct sub-groups, i.e. KS and LF, within the first group. The first group was characterized with higher PC scores of Ca, Mg, Fe, Al, P, and Mn. Calcium concentration was approximately 40-fold higher at the ND sites compared to YNP sites. The second group tended to represent the EP site of YNP. This group was characterized by a higher level of OM and lower concentrations of Ca and Mg. The third group was comprised the BS and RC sites of YNP. This group was characterized by lower concentrations of

OM, Ca and Mg. Na, Li, and Zn had no influence in discriminating the pattern of elements in root zone soil among the sites.

Table 2.7. The scores of two principal components (PC1 and PC2) calculated for three compartments (root-zone soil, root, and leaf) of *T. maritima* in five sites in North Dakota and Yellowstone National Park (*na* = not applicable)

	Root-zone soil		Root		Leaf	
	PC1	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
LOI	-0.2774	**0.4324**	*na*	*na*	*na*	*na*
Elements						
Structural						
Ca	**2.9191**	**-0.4511**	0.1072	-0.1429	**0.8074**	-0.0549
P	**0.8314**	0.2829	**0.7148**	-0.0405	0.1322	0.2677
Electrolytic						
K	0.4560	0.2314	**0.3253**	0.1594	-0.0084	0.2699
Mg	**1.7988**	-0.0147	**0.4391**	**-0.4093**	**0.9848**	-0.2250
Na	0.0217	0.1431	-0.0935	0.1274	-0.0611	0.1172
Enzymatic						
B	0.0279	**0.3390**	**-0.3983**	**0.3594**	**-0.3816**	-0.0939
Fe	**1.1003**	**0.5370**	**1.4423**	**0.7116**	**0.4181**	0.0356
Mn	0.6166	**0.3360**	0.0444	**0.3749**	**-0.4870**	0.2963
Zn	0.2337	0.0248	0.0585	0.0376	-0.0067	-0.0012
Main/sub group						
Al	**0.9859**	**0.3087**	**0.7343**	-0.2446	**0.5788**	-0.0505
Li	0.0157	0.1064	**-1.2966**	0.2819	**-1.7016**	**-0.4084**
Sr	*Na*	*na*	0.1727	-0.0253	0.4255	0.1387
Eigen values	4.56	0.34	0.85	0.22	0.84	0.13
Variability (%)	88	7	58	37	71	11

2.4.4.2. Roots

PC1 and PC2 explained 95% of the variation of multi-element concentrations in roots of *T. maritima* (Fig. 2.4). The concentrations in the roots from the ND sites follow a similar pattern to those of the root-zone soil compartment and formed a distinct group. The Rabbit Creek site showed a very
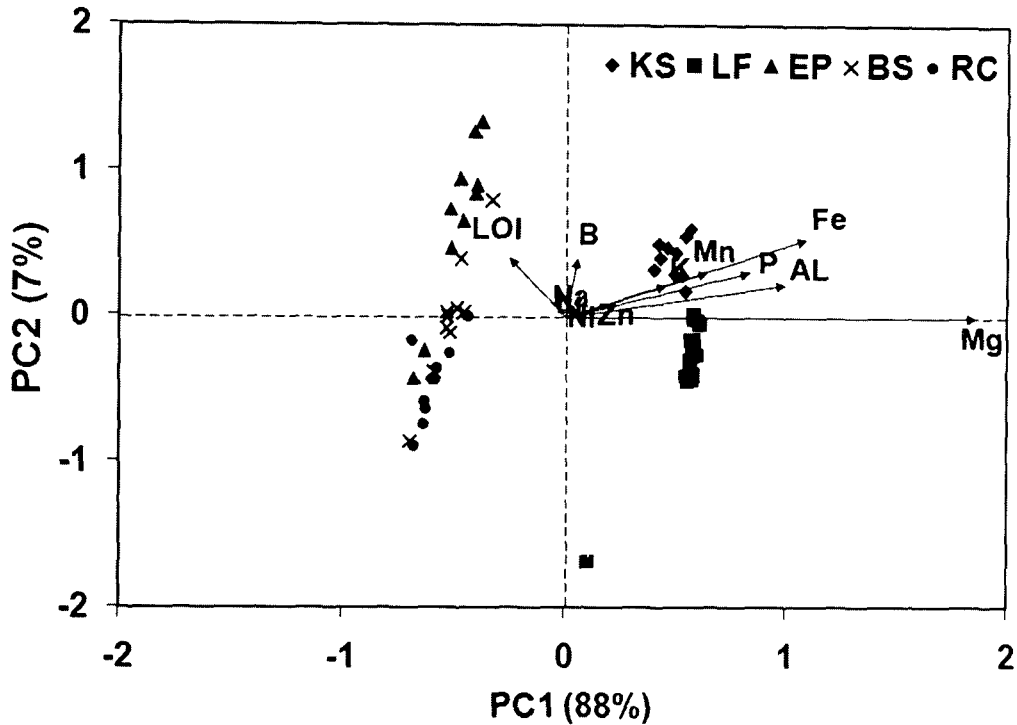
Figure 2.3. A joint plot showing score of principal components of the sites overlaid by the vectors of the elements in *T. maritima* root-zone soil in relations to the axis. Sites from hot springs of Yellowstone National Park (Elk Park [EP], Boulder Spring [BS], and Rabbit Creek [RC] and temporary wetlands in North Dakota (Kellys Slough [KS] and Landfill [LF]. Ca in PC1 2.9 is off scale.

distinct clustering of data, but the data from the other two sites from Yellowstone NP did not. Li, Fe, Al, and Ni were important elements responsible for distinguishing groups among ND and YNP study sites. Lithium had a higher negative score whereas Fe had the highest positive score. In PC2, Fe, Mg, Mn and B had the relatively highest scores.

2.4.4.3. Leaves

PC1 and PC2 explained 82% of the variation in elemental uptake in the leaf compartment whereas PC1 accounted for 71% of the variation (Fig. 2.5). The

score plot of PC1 and PC2 tended to show two distinct groups, one represented by ND (KS and LF) sites whereas the three study sites of YNP formed another group.
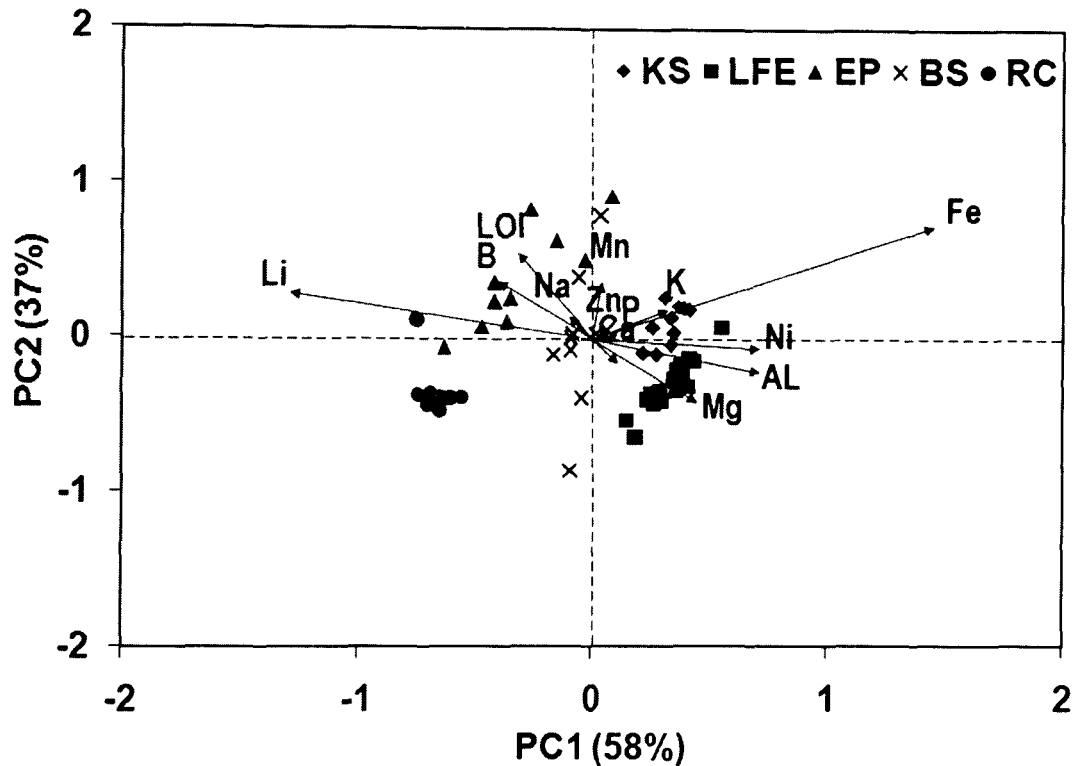
Figure 2.4. A joint plot showing score of principal components of the sites overlaid by the vectors of the elements in *T. maritima* roots in relations to the axis. Sites from hot springs of Yellowstone National Park (Elk Park [EP], Boulder Spring [BS], and Rabbit Creek [RC] and temporary wetlands in North Dakota (Kellys Slough [KS] and Landfill [LF].

Lithium, Mn, and B represented negative scores whereas Mg, Ca, Al, and Fe had positive scores in PC1. In PC2, only Li had a relatively higher score, which was negative. The values for Li, Mn and B were very specific to YNP regardless of three study sites. In contrast, the patterns of Mg, Ca, Al, Fe, Sr, and P were very specific to ND.
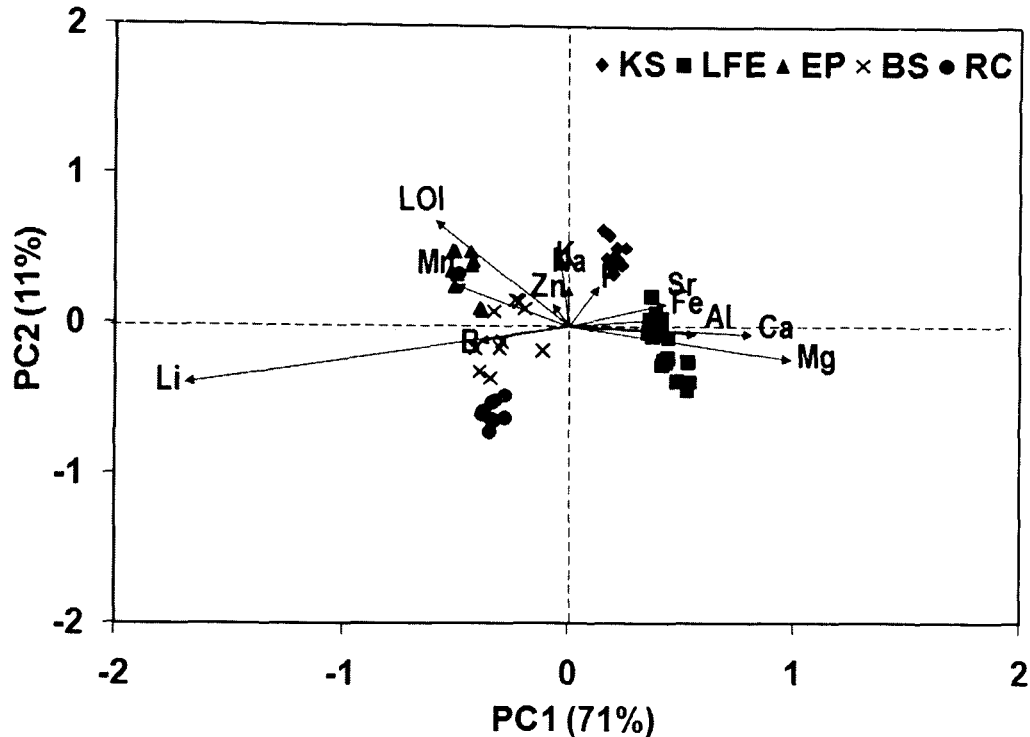
Figure 2.5. A joint plot showing score of principal components of the sites overlaid by the vectors of the elements in *T. maritima* leaves in relations to the axis. Sites from hot springs of Yellowstone National Park (Elk Park [EP], Boulder Spring [BS], and Rabbit Creek [RC] and temporary wetlands in North Dakota (Kellys Slough [KS] and Landfill [LF].

2.4.5. Multi-element fingerprint of *Triglochin maritima* leaves along hot spring stream transects

The means and standard deviations of element concentrations in *T. maritima* leaves along the hot spring stream transect are presented in Tables 2.8 and 2.9. At EP, concentrations of P, S, Si, Mg, Mo, Mn, Cu, Zn, B, Ba, and As were tend to be different in the leaves, but did not show any pattern along the hot spring transect (Table 2.8). Concentrations of As, Mo, and Si were likely to be high in the leaves of *T. maritima* at 3 m, near the spring. In contrast, concentrations of Cu and Mg were tended to be low close to the spring. The concentration of K and Fe

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showed gradually increasing patterns of with increasing distance from the spring. Element concentrations in *T. maritima* leaves and LOI in the soil did not show strong correlation except for a positive correlation for Mg ($r = 0.53$; $P = 0.043$) and negative correlations for As ($r = - 0.62$; $P = 0.013$) and Mo ($r = - 0.56$; $P = 0.029$).

Table 2.8. Mean element concentrations (μmol g^{-1} dry leaf weight) in leaves of *T. maritima* at different sampling distances from a hot spring fed stream in Yellowstone National Park, Elk Park [EP] in 2008 (mean ± standard deviation, n = 3).

Element	Unit	Sampling gradient distances away from hot spring at EP				
		3 m	10 m	14m	29m	43m
Structural						
Ca	μmol/g	125±4	79±6	94±3	139±106	89±10
P	μmol/g	43±2	50±2	44±2	63±6	44±2
S	μmol/g	98±6	82±5	95±5	121±51	250±31
Si	μmol/g	24±10	12±4	8±1	9±4	13±2
Electrolytic						
K	μmol/g	363±13	412±45	469±11	579±260	909±16
Na	μmol/g	2086±53	1811±104	1859±57	1128±979	1592±26
Mg	μmol/g	38±2	65±4.	51±3	83±17	75±3
Enzymatic						
Cr	nmol/g	12±5	6±5	6±9	4±5	8±5
Mo	nmol/g	162±5	31±5	83±16	27±24	7±4
Mn	μmol/g	7±0.3	6±0.2	11±0.5	10±7	22±0.8
Fe	μmol/g	1.46±0.1	0.99±0.1	1.5±0.2	2.8±1	3.9±0.6
Cu	nmol/g	14±4	94±73	54±9	136±65	25±13
Zn	μmol/g	0.23±0.02	0.21±0.03	0.18±0.01	0.85±1.1	0.21±0.03
Main/sub group						
B	μmol/g	12.1±0.65	14.9±1.2	7.9±0.27	4.7±3.5	3.3±0.10
Li	μmol/g	31±1	25±2	24±2	15±13	17±1
Sr	nmol/g	108±13	63±8	76±5	237±218	116±11
Ba	nmol/g	203±14	94±12	188±3	180±50	55±4
Al	μmol/g	0.56±0.07	0.59±0.05	0.56±0.01	1.7±2.1	0.7±0.15
As	nmol/g	150±20	50±10	70±20	50±50	20±10

However, element concentrations in *T. maritima* leaves showed negative correlations with root-zone soil temperature for Fe ($r = - 0.88$; $P = 0.000$), K ($r = - 0.75$; $P = 0.001$), Mg ($r = - 0.71$; $P = 003$), Mn ($r = - 0.61$; $P = 0.017$), S ($r = - 0.71$;

P = 0.003) and positive correlations for As (r = 0.63; P = 0.011), Mo (r = 0.68; P = 0.005) and B (r = 0.83; P = 0.000).

Table 2.9. Mean element concentrations (μmol g^{-1} dry leaf weight) in leaves of *T. maritima* at different sampling distances from a hot spring fed stream in Yellowstone National Park, Rabbit Creek [RC] in 2008 (mean $\pm$ standard deviation, n = 3).

| Element | Unit | Sampling gradient distances away from hot spring at RC | | | | |
		5 m	27 m	45 m	67 m	125 m
Structural						
Ca	μmol/g	85±21	74±13	71±3	72±10	76±12
P	μmol/g	43±7	39±6	45±2	42±7	30±2
S	μmol/g	93±9	183±16	160±29	135±35	67±11
Si	μmol/g	13±9	15±5	15±2	17±9	13±10
Electrolytic						
K	μmol/g	649±92	351±35	410±57	294±94	390±153
Na	μmol/g	1730±194	1826±151	1835±138	1970±184	1662±154
Mg	μmol/g	100±18	69±19	72±13	59±4	75±13
Enzymatic						
Cr	nmol/g	-	-	-	-	-
Mo	nmol/g	100±12	48±26	19±3	25±3	50±13
Mn	μmol/g	3±0.5	3±0.3	5±0.4	2±1	2±0.08
Fe	μmol/g	0.68±0.09	0.78±0.2	0.66±0.1	0.72±0.1	0.59±0.2
Cu	nmol/g	46±8	73±18	62±30	27±7	65±10
Zn	μmol/g	0.19±0.01	0.33±0.12	0.33±0.09	0.18±0.04	0.18±0.06
Main/sub groups						
B	μmol/g	3.4±0.27	3.3±0.22	3.5±0.21	3.4±0.28	2.4±0.11
Li	μmol/g	68±7	61±4	57±3	65±6	58±3
Sr	nmol/g	63±22	74±20	54±9	75±15	67±11
Ba	nmol/g	11±7	19±7	27±6	14±3	12±6
Al	μmol/g	0.49±0.33	0.77±0.51	0.61±0.43	0.83±0.28	0.95±0.74
As	nmol/g	60±20	60±30	40±4	50±10	9±8

At RC, concentrations of P, S, K, Mo, Mn, Cu, Ba, and As did not show any consistently increasing or decreasing patterns with distance from the springs (Table 2.9). However, concentrations of Mo and K in the leaves of *T. maritima* tended to be highest nearest the spring at 5 m distance. Positive correlation was observed for LOI in the soil and Mo (r = 0.66; P = 0.010) and K (r = 0.78; P =

0.001) in the leaves at RC. Root-zone soil temperatures correlated positively with Ba ($r = 0.74$; $P = 0.002$) and Mn ($r = 0.77$; $P = 0.001$) in the leaves of *T. maritima*.

2.4.6. Multi-element fingerprint of *Eleocharis rostellata* leaves along hot spring stream transect

Eleocharis rostellata leaves were collected along two streams originating from hot springs at Elk Park and Rabbit Creek in YNP. Mean and standard deviation of element concentrations of all study sites are presented in tables 2.10 and 2.11. At EP, concentrations of Ca, P, Mg, Mo, Cu, Zn, B, Li, and Sr did not follow consistent patterns (Table 2.10). Na, Mo, and As concentrations were likely to be higher at 3 m distance away from the hot spring and the Na concentration decreased gradually away from the hot spring. Ba concentration followed a similar pattern (Table 2.10). Concentration patterns of K, Mn, and Fe tended to be higher with the increase of distance away from spring.

The correlation analysis between elements in *E. rostellata* leaves and LOI at EP showed positive correlations for Fe ($r = 0.71$; $P = 0.003$), K ($r = 0.83$; $P = 0.000$), Mg ($r = 0.65$; $P = 0.008$), Mn ($r = 0.62$; $P = 0.014$), P($r = 0.62$; $P = 0.014$), and Zn ($r = 0.73$; $P = 0.002$). The correlation analysis of root-zone soil temperature with *E. rostellata* leaves showed positive correlations for B ($r = 0.66$; $P = 0.008$), Ba ($r = 0.62$; $P = 0.013$), Li ($r = 0.69$; $P = 0.004$) Mo ($r = 0.68$; $P = 0.005$) and Na ($r = 0.77$; $P = 0.001$) but negative correlations were observed for Fe ($r = - 0.58$; $P = 0.024$), K ($r = - 0.72$; $P = 0.003$), and Mn ($r = - 0.80$; $P = 0.000$).

At RC, *E. rostellata* (not present at the 125 m site) showed Na, Zn, Li, Sr, Ba, and As concentrations in the leaves did not show consistent patterns along the

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sampling transect (Table 2.11). The K concentration tended to be high at 5 m, and followed a decreasing pattern away from the hot spring, but did not correlate significantly with LOI. The correlation analysis showed negative correlations with LOI for As (r = - 0.65; P = 0.022), Ca (r = - 0.65; P = 0.023), S (r = - 0.66; P = 0.019), Sr (r = - 0.69; P = 0.013) and a positive correlation with P (r = 0.75; P = 0.005). Root-zone soil temperature showed positive correlations for As (r = 0.60; P = 0.038), Ba (r = 0.86; P = 0.000), S (r = 0.65; P = 0.024), and Zn (r = 0.84; P = 0.001).

Table 2.10. Mean element concentrations (μmol g^{-1} dry leaf weight) in leaves of *E. rostellata* at different sampling distances from a hot spring fed stream in Yellowstone National Park, Elk Park [EP] in 2008 (mean ± standard deviation, n = 3)

Element	Unit	Sampling gradient distances away from hot spring at EP
		3 m	10 m	14m	29m	43m
Structural						
Ca	µmol/g	50.4±1.9	51.1±1.3	41.7±2.4	29.0±2.1	43.8±2.1
P	µmol/g	28.0±1.2	31.4±1.40	29.3±1.8	36.6±2.9	31.7±1.8
S	µmol/g	120±4	117±7	118±6	112±4	125±5
Si	µmol/g	48.9±4.2	45.2±0.8	48.3±1.4	50.9±2.6	46.0±3.2
Electrolytic						
K	µmol/g	373±15	479±20	522±23	615±18	592±52
Na	µmol/g	460±28	319±16	290±19	183±14	127±9
Mg	µmol/g	19.3±1.4	27.9±0.8	21.9±0.99	30.6±3.0	33.9±3.0
Enzymatic						
Cr	nmol/g	14.3±18.6	13.7±9.5	10.4±9.1	15.7±10.4	20.3±14.8
Mo	nmol/g	204±42	94±26	100±34	127±11	45±6
Mn	µmol/g	8.3±0.2	10.1±1.7	11.3±0.57	12.8±1.2	14.7±0.30
Fe	µmol/g	1.2±0.44	1.5±0.02	1.9±0.14	3.3±0.65	2.6±0.34
Cu	nmol/g	30.7±8.3	58.6±4.9	66.5±6.6	47.4±11.2	25.3±10.2
Zn	µmol/g	0.34±0.03	0.36±0.04	0.53±0.06	0.41±0.02	0.42±0.01
B	µmol/g	9.91±0.72	11.4±1.5	7.5±0.62	16.8±4.7	3.0±0.24
Main/sub group						
Li	µmol/g	12.2±1.5	15.8±1.0	12.7±0.23	12.4±1.0	5.8±0.11
Sr	nmol/g	66.6±5.9	73.2±3.6	56.7±4.3	54.2±5.8	85.6±5.9
Ba	nmol/g	318.5±6.7	307.8±16.5	325.6±3.8	225.2±5.8	127.3±19.9
Al	µmol/g	0.54±0.19	0.58±0.17	0.50±0.08	0.75±0.08	0.72±0.25
As	µmol/g	0.09±0.02	0.05±0.01	0.05±0.00	0.05±0.01	0.03±0.01

Both *T. maritima* and *E. rostellata* were present at EP, which makes it possible to compare element concentrations in the two species. Similar element concentrations patterns in *T. maritima* and *E. rostellata* and their associated root-zone soils were observed for a few elements, which are presented in Fig. 2.6. Because the element concentrations values of *T. maritima*, *E. rostellata* and their associated root-zone soils had higher variations, the graphs were plotted with values normalized for the lowest concentrations of each element along the transect.

Table 2.11. Mean element concentrations (μmol g^{-1} dry leaf weight) in leaves of *E. rostellata* at different sampling distances from a hot spring fed stream in Yellowstone National Park, Rabbit Creek [RC] in 2008 (mean ± standard deviation, $n = 3$)

| Element | Unit | Sampling gradient distances away from hot spring at RC |
		5m	27m	45m	67m
Structural					
Ca	μmol/g	15.4±3.6	24.3±2.2	28.6±3.5	37.4±3.2
P	μmol/g	36.5±7.9	26.7±5.3	27.5±2.2	25.1±0.2
S	μmol/g	58±2	82±16	108±10	88±4
Si	μmol/g	48.5±7.5	41.2±5.1	45.3±2.3	41.8±3
Electrolytic					
K	μmol/g	644±14	555±33	527±22	385±7
Na	μmol/g	157±56	126±34	218±17	317±20
Mg	μmol/g	43.2±5.8	47.7±4.5	56.8±8	55.2±4.9
Enzymatic					
Cr	nmol/g	<dl	<dl	<dl	<dl
Mo	nmol/g	78±8	59±18	62±24	69±10
Mn	μmol/g	3.1±0.15	3.8±1.3	5.5±1.4	4.4±0.65
Fe	μmol/g	0.29±0.01	0.4±0.02	0.85±0.19	0.87±0.1
Cu	nmol/g	27.5±14	44.4±17.8	37±9.8	28.8±16.4
Zn	μmol/g	0.34±0.03	0 39±0.06	0.47±0.06	0.3±0.01
B	μmol/g	1.1±0.15	1±0.21	1.7±0.03	2.1±0.06
Main/sub group					
Li	μmol/g	26.9±2.9	26.6±3.4	42.7±2.5	41.9±1
Sr	nmol/g	27.6±4.7	50±3.3	47±8.3	63.5±3
Ba	nmol/g	28.8±9.1	40.8±14.1	90.8±28.4	32.6±4.3
Al	μmol/g	0.21±0.1	0.18±0.03	0.2±0.04	0.29±0.07
As	μmol/g	0.02±0.01	0.08±0.01	0.1±0.03	0.07±0.02
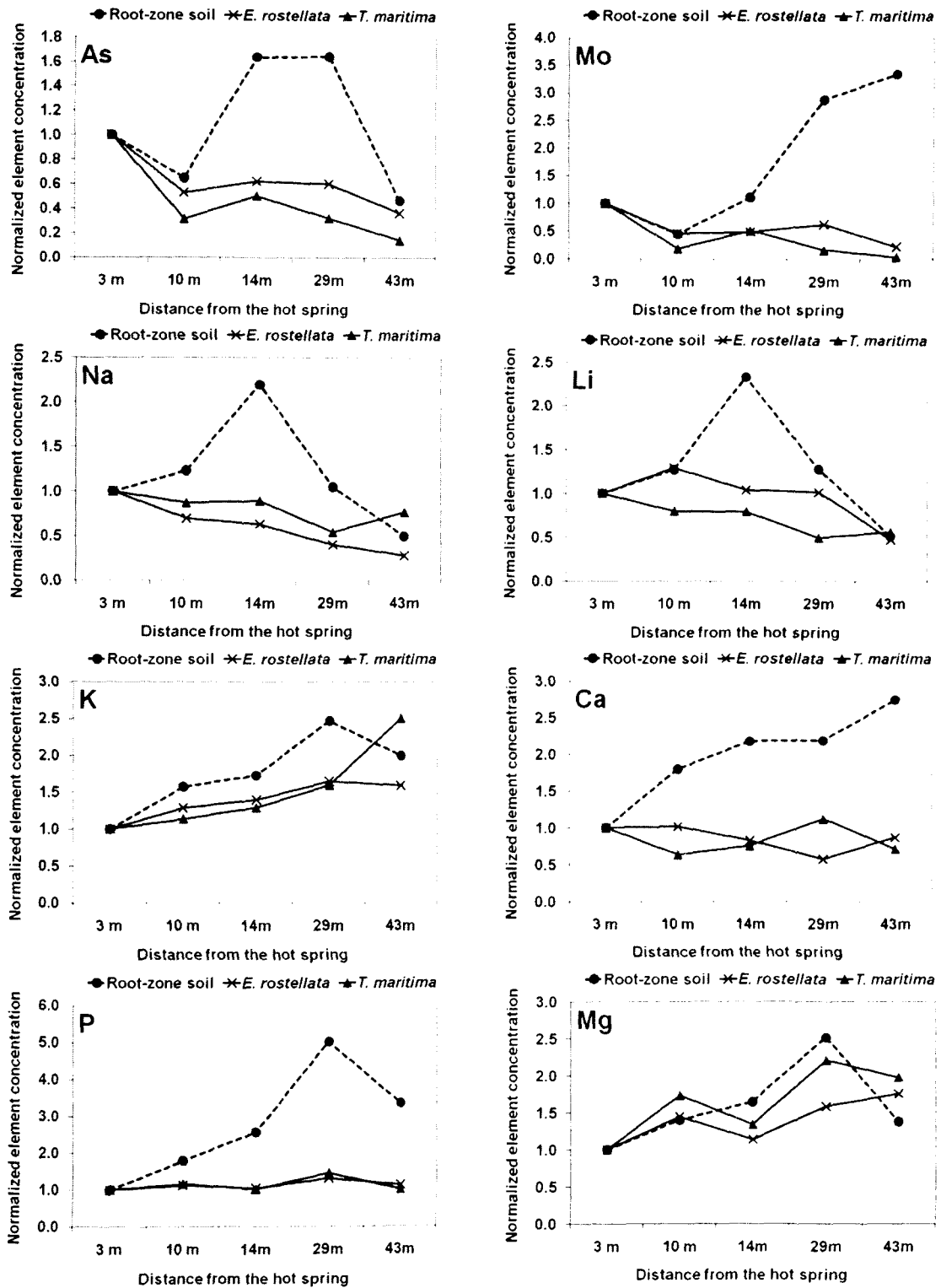
Figure 2.6. Element concentration patterns of root-zone soil and leaves of *T. maritima* and *E. rostellata* along the hot spring transect at Elk Park in Yellowstone National Park in 2008.

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As, Mo, Na, and Li concentrations in the plants showed decreasing patterns away from hot spring. In contrast, concentrations of K, and Mg increased away from the hot spring in both species (Fig. 2.6), except that Ca decreased at 29 m distance for *E. rostellata*. P and Ca in the plants were similar along the transects.

2.4.7. Multi-element fingerprint of *T. maritima, E. rostellata,* and *J. balticus* leaves from a hot spring habitat

The majority of element concentrations in the leaves between the three species *T. maritima, E. rostellata* and *J. balticus* varied significantly ($P < 0.01$). Mean element concentrations of these three species are presented in Table 2.12. The multi-element fingerprints for the species are shown in Fig. 2.7. Ca, P, and S (structural), Na, Mg (electrolytic) and Li, and Sr were significantly higher in *T. maritima* compared to the other species. Si, K, and Cr concentrations were high in *E. rostellata*. Mo and As were high in *J. balticus* compared to the other species. Mn, Ba, and Zn were similar in *E. rostellata* and *J. balticus* but higher than in *T. maritima*. B and Al were similar in *T. maritima* and *J. balticus*, but higher compared to *E. rostellata*.

The patterns in the leaves of the three species for the majority of elements did not correlate with those in the soils except for a few elements. Concentrations in *T. maritima* leaves showed positive correlations with those in root-zone soils for Ba ($r = 0.0.63$; $P = 0.012$), Mn ($r = 0.67$; $P = 0.006$), P ($r = 0.62$; $P = 0.013$). Similarly, in *E. rostellata* concentrations of Mn ($r = 0.64$; $P = 0.026$) and P ($r =$

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0.72; $P = 0.008$) positively correlated with those in the root-zone soils and negative

correlations were found for Ba ($r = -0.80$; $P = 0.002$), As ($r = -0.74$; $P = 0.006$),

and S ($r = -0.67$; $P = 0.018$). In *J. balticus* a positive correlation was observed for

S ($r = 0.78$; $P = 0.001$).

Table 2.12. Mean element concentrations (nmol or µmol g^{-1} dry leaf weight) in leaves of *T. maritima*, *E. rostellata*, and *J. balticus* at Rabbit Creek in 2008 (mean ± standard deviation, $n = 15$, different letters after values within one row indicate significant differences between sites at $P < 0.01$).

Element	Unit	*T. maritima*	*E. rostellata*	*J. balticus*
Structural				
Ca	µ mol g^{-1}	75.6±12.1a	26.4±8.5b	34.2±20.3b
P	µ mol g^{-1}	39.6±6.6a	29.0±6.1b	27.6±5.7b
S	µ mol g^{-1}	127.5±47.2a	84.0±19.7b	88.1±28.0b
Si	µ mol g^{-1}	14.3±6.6c	44.2±5.1a	18.0±3.6b
Electrolytic				
K	µ mol g^{-1}	418.5±146.6b	527.6±96.6a	393.6±61.6b
Na	µ mol g^{-1}	1804.5±173.8a	204.3±80.2b	98.3±53.8c
Mg	µ mol g^{-1}	74.9±18.3a	50.7±7.5b	31.2±13.5c
Enzymatic				
Cr	n mol g^{-1}	<dl	4.7±3.3	2.9±3.9
Mo	n mol g^{-1}	48.1±31.2b	67.0±15.1b	155.3±68.5a
Mn	µ mol g^{-1}	2.8±1.0b	4.2±1.3a	5.4±2.3a
Fe	µ mol g^{-1}	0.68±0.13	0.6±0.28	0.63±0.2
Cu	n mol g^{-1}	54.7±21.7	34.4±14.2	39.5±40.6
Zn	µ mol g^{-1}	0.24±0.09	0.38±0.08	0.39±0.20
B	µ mol g^{-1}	3.2±0.45b	1.5±0.47c	5.0±0.94a
Main/sub group				
Li	µ mol g^{-1}	61.9±6.1a	34.5±8.2b	32.3±7.3b
Ba	n mol g^{-1}	16.9±7.6b	48.3±29.0a	45.8±62.3a
Al	µ mol g^{-1}	0.7±0.44a	0.22±0.07b	0.52±0.32a
As	n mol g^{-1}	42.6±23.8b	65.2±33.6b	89.7±42.5a
Sr	n mol g^{-1}	66.6±15.5a	47.0±13.8b	25 6±20.2c

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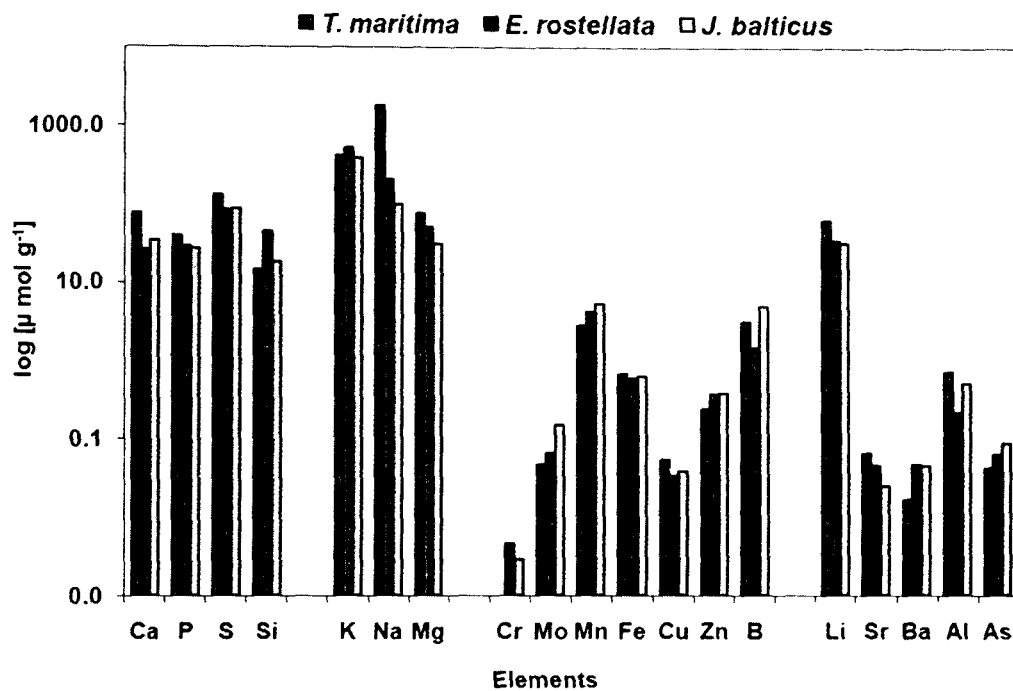
Figure 2.7. Multi-element fingerprint of leaf tissues for *T. maritima, E. rostellata,* and *J. balticus* at Rabbit Creek of Yellowstone National Park in 2008.

2.5. Discussion

2.5.1. Root-zone soils

Several studies exist on element concentrations in water in hot spring environments, but only a few exist on their soils. Studies reporting on the chemical composition of the spring waters of Yellowstone National Park include Stauffer et al. (1980) Fournier (1989), Langner et al. (2001), and Channing and Edwards (2009). Stauffer et al. (1980) reported water concentrations of Ca (0.02 μmol g^{-1}) and Mg (0.00042 μmol g^{-1}), Na (14 μmol g^{-1}), K (0.41 μmol g^{-1}), and Li (0.52 μmol g^{-1}). Channing and Edwards (2009) reported similar element concentrations in water from the Elk Park hot spring area. McCarthy et al. (2005) reported the

chemical concentrations for sediments influenced by the Champagne hot springs shallow hydrothermal vent on the island of Dominica to a depth of 10 cm. They reported element concentrations (μmol g^{-1}) for Ca (10.1), Na (303), and Mg (34.0), which were higher than the concentrations in the root-zone soils reported here. In contrast, they found concentrations of K (6.02), B (0.70), Mn (0.015), and Li (0.07), which were lower than in the current study. Here we compared the element concentrations in root-zone soil from different habitats and found distinct differences between the hot springs of YNP and the temporary wetlands of ND sites. Furthermore, the element concentrations in roots and leaves of *T. maritima* showed distinct differences between the hot springs and the temporary wetlands. Principal Component Analysis helped explain the element concentration patterns. In general, the North Dakota sites KS and LF clustered more closely together for soils, roots, and leaves than the Yellowstone National Park sites. An explanation for this is that the ND sites were closer to each other, geologically and pedologically much more similar than the YNP sites, and were probably fed by the same aquifer (the Dakota aquifer). The YNP sites were more distant from each other and were fed by different springs having diverse chemistry. On the other hand, the YNP sites were all neutral to alkaline, which is why *T. maritima* was found there in the first place. From the plant's perspective these sites were all within its natural ecological range.

In root-zone soil, elements such as As, Ca, Fe, Al, P, Mn, and B were discriminatory between sites, separating the ND sites from the NYP sites. Ca ranged from 1015 to 2182 μmol g^{-1} in ND, but was less than 1 μmol g^{-1} in YNP.

Similarly, the concentrations of P, S, K, Mg, Co, Cr, Fe, Mn, Ni, Zn, Al, Zn, and Sr in ND sites were at least twice those of the YNP sites. In contrast, all YNP sites had higher As concentrations compared to ND. Within YNP sites, EP was different from the other sites, showing relatively high LOI, Ca, B, Fe, Mn, P, K, and Al. Na, B, Ni, Li and Zn did not show any distinct patterns.

Against our expectations, the majority of element concentrations were higher in the root-zone soils of temporary wetlands compared to hot spring influenced soils. The observed differences can be explained by the soil properties of the study sites. Soil textures play a significant role in trace element retention and release (Tack, 2010). The soils of KS and LF are Lallie silt clay and Bearden silty clay loam, respectively (Soil Survey Staff, 2009). In general, fine grained soil particles retain more positively charged ions, compared to coarse-grained soil particles. The soils at the YNP sites were aquic inceptisols with relatively coarse, well-draining textures. These soils are characterized by active leaching of minerals relative to fine textured soil found in ND sites. Inceptisols are formed by leaching or loss of Fe, Al, bases, and organic matter. Furthermore, inceptisols are developed from young sediments (alluvium, colluvium, and volcanic ashes) which have poor sorption of trace elements.

2.5.2. Element concentrations in plants

Otte and Jacob (2005) reported that *T. maritima* plants from a hot spring in New Mexico contained higher concentrations of As, Fe, Li, Cs, Be, Tl, Ge, Sc, Y, U, and W compared to plants from coastal salt marshes in Europe. Despite the fact that the sites studied here were very different from those studied by Otte and

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Jacob (2005), our data confirm this pattern for concentrations of Li, and As. Furthermore, element concentrations of B, Mn, and S, were higher in root and leaf tissues in plants from hot spring-influenced wetlands from Yellowstone National Park (YNP) compared to plants from the temporary saline wetlands of North Dakota (ND). The concentrations of K (1517 μmol g^{-1}), Mg (362 μmol g^{-1}), and Fe (14.9 μmol g^{-1}) in *T. maritima* under water-logged and saline soil conditions as reported by Cooper (1982), were higher than the current study. In contrast, the Ca (79.8 μmol g^{-1}) and Mn (4.9 μmol g^{-1}) found by Cooper (1982) were lower than the current study. Otte et al. (1991) reported that the concentrations of Zn, Cu, and Cd were higher in roots compared to leaves in *T. maritima* collected from salt marshes in The Netherlands. Zn concentrations in roots (1.18 μmol g^{-1}) and leaves (0.46 μmol g^{-1}) reported by Otte et al. were almost double compared to those observed in the current study. Geological and climatic differences between sites, variation in genetic composition of plants, and soil biota present in root-zone soil play important roles in the element composition of plants (Epstein, 1972; Martin, 2001; Otte and Jacob, 2005). Furthermore the differences between operators, different sampling techniques, and time of sampling also determine different outcomes between studies. The concentration of Na (1410 μmol g^{-1}) in water-logged and saline soils reported by Cooper, (1982) is similar with the current study. Irrespective of its habitat, *T. maritima*, in this study, accumulated high concentrations of Na and K that were very similar among the study sites. *T. maritima* is halophytic species (Cooper, 1982, Davy and Bishop 1991; Piernik, 2003), and, unlike other monocotyledons, *T. maritima* accumulates high

concentrations of K and Na in the leaf tissues (Albert, 1975). These plants require salinity to complete their life cycle and adjust their osmotic conditions to maintain water potential (Unger, 1991). Naidoo (1994) reported that NaCl treatment of 200 mol m^{-3} in shoots contributed to the osmotic adjustment of 83% in *Triglochin bulbosa* and 72% in *Triglochin striata*.

The element concentration in the roots of *T. maritima* also showed distinct variation. The roots from ND sites contained higher structural (Ca, P, and Si), electrolytic (K and Mg), enzymatic (B, Cr, Ni, V, and Zn), main and sub-group (Al, Sr, and Ti) element concentrations, whereas the concentrations of the elements S, B, As, and Li were higher in roots from YNP sites. The concentrations of Na in the roots were similar across the study sites.

In leaves of *T. maritima*, the concentration of Ca, Mg, Cu, Cr, Fe, Al, Sr, and Ti varied between ND and YNP sites with high concentrations of these elements in ND. The concentrations of B, As, and Li also showed high variation between hot spring-influenced *T. maritima* and temporary wetlands. Na, P, and K concentrations in leaves were very similar across study sites. These differences in element concentrations in *T. maritima* may be due to adaptations to specific habitats. Plant genotypes and populations differ in element accumulation patterns (Clark, 1983; Ernst, 1989; Ernst, 1995; Ghasemi and Ghaderian, 2009). The accumulation of elements in plants also depends on the type of element and local variation in environmental factors. For example, Lomgi et al. (2000) found that *Thlaspi caerulescens* populations showed similar patterns for uptake of Zn but were different for Cd. Lithium concentrations in the roots and leaves were higher in

YNP sites compared to ND sites. In the root-zone soils, the concentrations of Li did not differ consistently between the study areas in ND and YNP, but its concentration was different in leaf between the study areas. The result suggests that the patterns in Li concentrations in the plants may not be explained by differences in the soil. Brown (1981), studying *Purshia tridentata* (four-winged saltbush) and *Atriplex carescens* (bitter brush), native of the Roosevelt hot springs, Utah, reported that Li uptake differs between and among species, and leaves accumulate higher Li concentration than the other parts of plants. This is true for other mobile elements in plants as well (Ernst, 1995). High Li concentrations in plants from hot spring areas could result in higher levels of trophic transfer in the ecosystem. This is an area that deserves further investigation, because Li can inhibit the growth and development in mammals. It produces teratological effects in organisms (Shkolnik, 1984; Léonard et al., 1995) and reduces the embryonic growth and development in rats (Klung et al., 1992). Bargagli (1998) proposed a role for Li in halophyte metabolism. Li is relatively non-toxic to plants and is a highly mobile element (Aral and Vecchio-Sadus, 2008). Evans and Śorger (1966) reported that Li and Na activate poly- β - hydroxybutyric acid depolymerase enzyme in halophytes, and lithium is effective in activation of chlorophylase enzyme (Shkolnix, 1984). Lithium induces membrane hyperpolarization and protects the cell from excess external stresses (Lovkova et al., 2007). The elevated concentrations of Li in *T. maritima* from geothermal influenced wetlands may play a role in reducing stress due to elevated temperatures and lower levels of macro-electrolytic elements available to the plants. Lithium increases water

retention capacity of plant tissues (Shkolnix, 1984). Under hot spring conditions, large amounts of water are required to maintain the evapo-transpiration of plants. Retention of water prevents heat injury caused by high temperature (Greulach, 1973). Lithium also inhibits physical stress in plants. Lithium showed inhibitive effects on the thigmomorphogenetic response in *Bryonia diocia* (Boyer and Chapelle, 1979) and it decreases cold-induced depolymerization of microtubules in plant cells (Bartolo and Carter, 1992).

Arsenic does not have a known biological function in plants but most plants take up this element (Kabata-Pendias, 2001). According to Langner et al. (2001), the hot spring waters of Yellowstone contained 10 to 40 µM of arsenic. Our study showed that the arsenic concentration is greater in the *T. maritima* root-zone soil, roots, and leaves from hot spring influenced sites compared to the North Dakota sites. Arsenic concentrations in soils and plants tend to correlate positively (Bech et al., 1997; Kabata-Pendias, 2001). The uptake rate of arsenic in plants depends on the concentrations in the medium (Meharg and Macnair, 1990; Carbonellu et al., 1998). In our study too, the concentrations of arsenic in root-zone soils and *T. maritima* roots were positively correlated. Concentrations were higher in the roots compared to the leaves, which is similar to results reported by Kocar et al. (2004).

Elements transferred in food chains may pose a risk to organisms (Gnamuš et al., 2000). Little is known about As transfer in food chains, but Gnamuš et al. (2000) demonstrated the transfer of mercury, an element with chemistry and environmental behavior somewhat similar to As, from soils to plant to herbivores to carnivores. Elevated concentrations of arsenic in plants increase the risk of trophic

transfer to other organisms (Kocar et al., 2004). *T. maritima* may be a possible source of As ingestion in the diet of species that feed on below-ground tissues (Van der Wal et. al., 2000).

Soil pH plays important role in arsenic uptake in plants. Generally, in lower pH arsenic sorption in soils (Arai, 2010) and uptake by plants (Marin et al., 1993) are increased. In rice (*Oryza sativa* L.), availability of arsenic III in soil solution and uptake of monomethyl arsenic acid (MMAA) increased with decreased soil pH and redox potential (Marin et al., 1993). Similarly, Meharg and Macnair (1991) found that with pH increasing from 4 to 8, arsenate uptake was decreased in *Holcus lanatus* L. This decrease was rapid in arsenate non-tolerant *H. lanatus*. Furthermore, Anke (2005) reported that high As concentrations were associated with high organic matter (OM) content in soils. In the current study within YNP sites, arsenic concentrations in EP were significantly higher than BS and RC. Low pH and high OM content of the root-zone soil at EP may have contributed to higher As concentrations in soil, roots, and leaves of *T. maritima*.

Similarly, the concentrations of the enzymatic elements B and Mn in roots and leaves of YNP sites were higher compared to ND sites. Boron is important in sugar synthesis in plants (Kabata-Pendias, 2001). Soil pH, soil texture, soil moisture, and temperature influence the concentrations of B in plants (Goldberg 1997; Kabata-Pendias, 2001). Organic matter contents in soils control the soluble B concentration as well as uptake of B in plant tissue (Yermiyahu et al., 2001). Boron retention is high in soils with high organic matter content, whereas it is easily leached in coarse textured soils with low OM content (Marschner, 1995). Hu

and Brown (1997) reported that the availability of B is high in low soil pH. In this study also, B concentrations in the root-zone soil were in agreement with previous reports. Its concentrations in leaves appeared to correlate with a low pH and high organic matter content in the soils, however, the uptake of B in roots does not correlate with soil pH. Goldberg (1997) found that uptake of B in plants is low at high pH. In this study too; at EP, which had the lowest pH and the highest organic matter content among all study sites, the highest B concentrations were observed in the root-zone soil and in leaves.

Manganese is an enzymatic element important in plants, which also influences the behavior of other micro-elements (Kabata-Pendias, 2001). In this study, no correlation between Mn concentrations in plants and soil were observed when considering all the data from the 2007 sampling season, which included sites from Yellowstone National Park, WY, and from ND. However, during the 2008 season, when plants were sampled along Rabbit Creek, YNP, *T. maritima* showed a positive correlations for Mn ($r = 0.67$; $P < 0.006$), as did *E. rostellata* ($r = 0.64$; $P < 0.026$). Considering the many factors that can determine the concentrations of elements in plant tissues, it is not surprising that element concentrations in plants do typically not correlate with those in the soil when considered over very different sites from distant geographical areas. But at Rabbit Creek it appears that the variation in factors such as pH and temperature was relatively small and so the relationship with concentrations in plants and soils became more apparent. Sulfur is another of the essential elements in growth and development of plants (Amtmann and Armengaud, 2009). Sulfur containing

compounds such as glucosinolates, glutathione, phytoalexins and other sulfur-rich proteins protect plants from extreme biotic and abiotic stress to plants (Rausch and Wachter, 2005). The production of these compounds depends on the uptake and assimilation of sulfate by plants. In our study the concentrations of S were significantly higher in root-zone soils of ND compared to YNP sites, but S concentrations in the roots and leaves of the plants showed the reverse pattern. This may indicate a higher requirement for S in plants associated with hot springs, perhaps in relation to mechanisms involved in heat and salinity tolerance. Methionine is an essential sulfur-containing amino acid, found in plant cell (Amir, 2010) which has been reported to improve salt tolerance (Gläser, 1993). Mittova et al. (2003) reported that reduced glutathione (GSH), a sulfur containing compound, decreased the salt-induced oxidative stress and increased salt tolerance in *Lycopersicon pennellii*.

Compared to 2007, more detailed studies were conducted in 2008 along gradients of hot spring influenced sites and by sampling other plant species such as *Eleocharis rostellata*. The majority of element concentrations varied significantly in root-zone soils along the hot spring gradients. Elements such as P, S, B, and As were high in the root-zone soils near hot spring gradients at RC. In contrast, the majority of element concentrations were high in root-zone soils away from the hot springs at EP. This may be due to differences in flow rates and chemistry between the two systems. Under conditions of high flow rates mobile elements will be carried further away from the spring before they precipitate out of solution or bind to substrates, also depending on pH and redox conditions. It is likely that many

elements remained in solution longer before precipitating under the conditions prevailing at EP. Arsenic concentrations at EP in the current study were higher than those reported by Kocar et al. (2004) in the Madison-Firehole watershed, Yellowstone National Park of about 2.2 μmol g^{-1}, but the values at RC were generally lower than the value reported by Kocar et al. (2004).

Organic matter (OM) plays an important role in element retention in soils (Hue et al., 1988; Tack, 2010). In this study, the majority of elements at each gradient showed a pattern that reflected to the OM content (as measured by LOI) in the root-zone soils, indicating that the variation in the element concentrations may be attributed to the organic matter content in the root-zone soils.

The majority of element concentrations in *T.maritma* and *E. rostellata* varied significantly along the hot spring gradients. Kocar et al. (2004) studied the element concentrations in different forage species around Madison Fire hole watershed area in YNP. The authors reported concentrations of As in *T. maritima* of 0.38 μmol g^{-1} and in *E. rostellata* of 0.22 μmol g^{-1}, which is higher compared to the values observed in this study. Ha et al. (2009) studied the element concentrations in *Eleocharis acicularis* at an abandoned mining site in Japan and reported that the species accumulated high concentrations (μmol g^{-1}) of Fe (1065), Zn (14.7), Mn (7.1), Cr (5.1), and Cu (3.7). In this study, the concentrations of these elements in *E. rostellata* were higher at both EP and RC compared to Ha et al. (2009), except for Mn, Cr, and Fe. In fact, Fe concentrations were more than 500 times higher compared to this study.

Many elements bind to organic matter, which explains the positive correlations between element concentrations in the soil and loss-on-ignition. However, such binding does not necessarily render elements immobile or unavailable to uptake by plants. The concentration of elements in plants is determined by many factors, including the activity of the uptake systems in the roots and of the translocation mechanisms in the entire plant, as well as temperature/season, and biomass production. Still, Padmavthiamma and Li (2010) reported that OM had a positive effect on uptake of P and Mn in *Lolium perenne* L (perennial rye grass), *Festuca rubra* L (creeping red fescue) and *Poa pratensis* L (Kentucky blue grass), and this was also found to some extent in this study for *T. maritima* and *E. rostellata*. Similarly, organic matter contents in soils control the soluble B concentration as well as uptake of B in plant tissue (Yermiyahu et al., 2001) but in this study B concentrations in leaves of *T. maritima* and *E. rostellata* did not correlate with the concentrations in the root-zone soils. Under field conditions there typically are too many factors varying and interacting to enable positive elucidation of the mechanisms that determine element concentrations in plants, and it is therefore not surprising that few correlations were observed between the element concentrations in the plants and element concentrations or LOI in the soils.

One overriding factor affecting element concentrations is temperature. Temperature influences the availability and absorption of elements from the soil in plants (Treshow, 1970), but also growth of plants and their physiological activity. Fritioff et al. (2005) reported that Cu, Zn Cd, and Pb uptake in *Elodea canadensis*

(Michx.) and *Potamogeton natans* (L) was higher at 20 °C compared to 5 and 11°C temperature. Oncel et al. (2000) also reported that at 35 °C, Cd and Pb in *Triticum aestivum* varieties increased both the concentration and toxicity compared to lower temperatures at 8 and 25 °C. Baghour et al. (2002a) also studied the effect of temperature in the uptake of Ba, Cl, Sn, Pt, and Rb in potato. These researchers reported that that at 27 °C, the uptake of Ba, Pt, Cl, and Sn were higher in the roots, leaves, and tubers compared to lower temperatures. The various correlations between temperature and element concentrations in this study may thus indicate a causal relationship, but how exactly cannot be concluded from this study and needs further investigation through experiments under controlled conditions.

To our knowledge, this is the first study ever reporting a comparison of multi-element composition of plants of the same species from hot springs and cold water environments. Against expectations, the majority of elements were present in higher concentrations in the soils of temporary wetlands compared to the hot springs. However, in plant tissues Li, As, B, Mn, and S concentrations were higher in hot spring influenced *T. maritima*. Element concentrations in plant tissues did not typically reflect the concentrations in the soil, with the exception for As, this could be, among many factors that affect element uptake in plants, the plants are selective to element uptake thus may have excluded elements in their tissue or in some instances these elements are required for physiological and metabolic activities, thus plants accumulate higher concentrations in its tissue. Soils, roots and leaves of *T. maritima* produced different multi-element fingerprints, indicating

that hot spring populations differ from temporary wetlands regarding element uptake, translocation, and potentially trophic transfer. Genetic fingerprinting may help elucidate the differences between hot spring and temporary wetland populations. To better explain the role of salinity and temperature in multi-element concentrations in plants, controlled experiments investigating the effects of temperature and other factors could provide further understanding of the processes determining patterns in multi-element concentrations in plants.

CHAPTER 3. MULTI-ELEMENT FINGERPRINTING OF *JUNCUS BALTICUS*, *SALIX EXIGUA*, *SALIX BOOTHII*, AND *SALIX WOLFII* FROM CONTAMINATED AND UN-CONTAMINATED SITES

3.1. Abstract

This study compared the multi-element fingerprints of different plant species from contaminated and un-contaminated habitats in the Greater Yellowstone area. Samples were collected from contaminated sites (mine tailings at Cooke City, CC and a mining impacted stream, Soda Butte, SB Yellowstone N.P.) and un-contaminated sites (Pebble Creek PEB, Yellowstone N.P. and Pacific Creek, PC Grand Teton N.P.). Root-zone soils and plant tissues (leaves) were analyzed for 32 elements by Inductively Coupled Plasma Optical Emission Spectrophotometry. The concentrations of Co, Cu, Zn, Sr, As, Ag and Ce followed in the order of CC>SB≥PEB≥PC from higher to lower concentrations in root-zone soils of *Juncus balticus* and *Salix exigua*. Cu, Zn, Ag, Pb, Mn, Fe, Co, Sr, P, S, Ce, and B concentration were significantly higher in SB compared to PEB. *Juncus balticus* showed marked differences in multi-element fingerprints (S, K, Mn, Fe, Cu, Al, As, and Cd) between contaminated (CC and SB) and un-contaminated sites (PEB), indicating that it can potentially be used for assessment and monitoring of soil element compositions and contaminations. *Salix exigua* showed little variation in element concentrations between contaminated (SB) and un-contaminated sites (PEB and PC). The multi-element fingerprints of *S. exigua*, *S. wolfii* and *S. boothii* from same habitat at PC showed differences in multi-element compostions among the species. *S. boothii* showed higher concentrations of Mn, Fe, Al, and Ti

compared to *S. exigua* and *S. wolfii*, and has potential to be used in phtytoremediation of these elements.

3.2. Introduction

Multi-element analysis in plants was introduced during the 1980s as a tool for environmental studies, in studying forest ecosystems and in analysis of atmospheric deposition (Markert 1988; Steinnes et al. 1992; Vitorova and Markert 1995). It can be used to develop multi-element fingerprints of plants, which is useful in biogeochemical modeling studies (Markert, 1992). This technique is also important in understanding element concentrations in plants in different ecosystems, inter-element relations and processes, such as accumulation or exclusion of elements in organisms. Multi-element fingerprinting in plants increases the power of studies when many elements in different plant species from both contaminated and un-contaminated habitats are analyzed (Djingova et al., 2004). Several plant species have been used in monitoring and evaluation of heavy metal pollution in areas contaminated with anthropogenic activities. Vitorova and Markert (1991) suggested that a multi-element study in forest ecosystems in Europe could be used in identifying contaminated ecosystems. Many plant species are also useful as biological indicators for environmental pollution and in phytoremediation (Cunningham and Berti, 1993; Kumar et al., 1995; Djingova et al., 1999; Pulford and Watson, 2003).

Past mining activities and their remaining mine tailings may result in metal pollution in the environment (Freitas et. al., 2004). Mining activities can lead to increased levels of metals in water and sediments (Dudka and Adriano, 1997;

Eisler, 2004). Rösner (1998) studied the effect of abandoned mines on surface water in Arizona, USA, where he reported pollution of groundwater by the elevated concentrations of As, Cd, and Fe. Gray et al. (2000) evaluated the effects of abandoned mercury mines in southwestern Alaska, USA. These authors reported high concentrations of mercury in stream-sediment, stream-water, and fish samples collected downstream from abandoned mines compared to regional baseline concentrations. Henriques and Fernandes (1991) studied the element contents in soils associated with pyrite mining in Portugal and metal distribution in *Juncus conglomerates*. Alvarenga et al. (2004) also studied element uptake in *Cistus ladnifer* L. in a contaminated pyrite mining area in Portugal. Freitas et al. (2004) studied the element uptake in a community of plants at São Domingos old mine located in the south of Portugal. Wong et al. (1999) studied the dispersion and toxicity of metals in plant species near an abandoned gold mine in Nova Scotia, Canada. These authors reported that *Equisetum rubiaceae* and *E. sylvaticum* contained high concentration of As and Hg in their tissues, showing the possibility of these plants for use in phytoremediation. Stoughton and Marcus (2000) studied the effect of mining activities on riparian plant populations along Soda Butte Creek, Yellowstone National Park, USA. They reported that higher level of metals such as Cu decreased the plant density and diversity in adjacent meadows. Marcus et al. (2001) studied the geomorphic process in Soda Butte Creek, which was impacted by past mining activities in Yellowstone area.

Soda Butte Creek, one of the sites in this study, is contaminated by the tailings of the McLaren mine, an open-pit gold and copper mine located near

Cooke City, MT, which operated from 1870 to 1953. The past mining activities have contaminated the creek, particularly due to a large flood event in June 23, 1950 (Glidden, 2001; Marcus et al., 2001). Riparian vegetation in general has been mainly studied for its role in river bank stability, water quality protection, vegetation succession and nutrient dynamics, with emphasis in N and P (Cushman and Gaffney 2010; Hubble et al., 2010; Osterkamp and Hupp, 2010). A few studies can be found on metal uptake by riparian vegetation. Therefore, the objective of this study was to examine the multi-element concentrations in plant species of riparian zones, along contaminated and un-contaminated streams in the Greater Yellowstone area.

The choice of study sites was based on the considerations that the main aim of the study was to investigate multi-element concentrations in plants in areas affected by past mining activities, and compared with suitable nearby control sites with the same species but along streams not affected by past mining activities. Another consideration was to compare sites within Yellowstone National Park, and thus possibly impacted by volcanic or geothermal activities with a site of similar habitat and elevation (Pebble Creek, PEB), but outside the Park, in this case in Grand Teton National Park (Pacific Creek, PC). The study included *Juncus balticus*, *Salix exigua*, *S. boothii* and *S. wolfii*. Past mining activities had contaminated Cooke City (CC) and Soda Butte Creek (SB). Soda Butte was impacted by tailings impoundment from the McLaren gold mine. Gold mining activities and the flooding of 1950 contaminated Soda Butte Creek downstream from the mining site. Thus we expected that the highest element concentrations

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would be in root-zone soils and plant tissues collected at CC and SB compared to PEB and PC.

3.3. Materials and Methods

3.3.1. Site description

The study was conducted at three locations within Yellowstone National Park (YNP) and one in Grand Teton National Park. The sites in YNP were 1) a site covered with mine tailings at Cooke City (N 45° 01.346 W109° 55. 443); 2) Soda Butte Creek (N 44° 54.753 W110° 06. 549), which is known to be contaminated by the past mining activity at Cooke City, and 3) Pebble Creek (N 44° 54.698 W110° 06.666) a tributary to Soda Butte Creek with no known history of impacts from mining. Pacific Creek (N 43° 51.920 W110° 30. 395) in Grand Teton National Park also has no known mining history, and is also outside Yellowstone National Park and so less impacted by geothermal activities (Fig. 3.1).

3.3.2. Plant species

Four plant species, *Juncus balticus* Willd, *Salix exigua* Nutt., *Salix boothii* Dorn, and *Salix wolfii* were studied because *J. balticus* and *S. exigua* were common in both the contaminated and un-contaminated sites, which would make possible to compare the element concentrations of these species between two sites. Similarly, *S. exigua*, *S. boothii*, and *S. wolfii* were growing together at Pacific Creek due to which multi-element concentrations were comparable among the species from the same site.

Juncus balticus (Baltic rush) belongs to the Juncaceae family. The species is common and found in a variety of habitat and community types. It is often found

with *Salix* species in mixed and deciduous forests. The species prefers alkaline soils with pH ranging from 7.8 to 9.1, and it is an important forage species for elk (Baker and Hobbs, 1982).

Salix exigua Nutt. is commonly known as narrow-leaved or sand bar willow, and it belongs to family of the Salicaceae. It has long, thin leaves with a short petiole, and serrated leaf edge, and is a perennial shrub in habitat. *S. exigua* is an important riparian species, which commonly grows well in sandy soils along streams, rivers, and ditches (USDA-NRCS, 2010). The plants grow well in pH of 6.0 - 8.5 and a coarse to medium soil texture (Foster and Smith, 1991). It stabilizes stream banks and is therefore used in re-vegetation and restoration of riparian wetlands. The species provides important habitat for birds and is a food source for wildlife such as deer and beaver (Beyer et al., 2007; USDA-NRCS, 2010).

Salix boothii Dorn is a perennial shrub. It is adapted to coarse, medium, and fine textured soil. The species prefers pH range of 5.5-8.0 (USDA-NRCS, 2010). It is often found in wetter areas such as swamps or directly in streams. The leaves are equally green on both sides. The species is important forage for deer, elk, moose, and small mammals (USDA-NRCS, 2010). *Salix wolfii* is also a perennial shrub. The species grows better in drier conditions compared to the other two *Salix* species. It is typically found near the edges of upslope wetlands (USDA NRCS, 2010).

3.3.3. Sample collection and plant species identification

Field sampling was carried out during July 2008. At Cooke City, *Juncus balticus* and *Salix wolfii* grew only in a fringe of about 30 meters long at the base

of the tailings along Soda Butte Creek, and the number of plants available limited sampling to three replicates.

Figure 3.1. Map showing sampling sites in the Greater Yellowstone Area (Cooke City, Soda Butte, Pebble Creek, and Pacific Creek). (Map generated in ArcGIS Desktop 9.3.1 Student Evaluation Edition)

The Soda Butte Creek site was further downstream just above the confluence with Pebble Creek. There too, *J. balticus* and *Salix exigua* were largely confined to a narrow fringe along the creeks, but here it was possible to sample more plants (*n* = 10) along a stretch of 250 m at 25 m intervals upstream of confluence between SB and PEB. If concentrations of certain elements were elevated due to the mining activities in Soda Butte Creek, but not Pebble Creek, then a clear

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difference in their concentrations should be discernible, at least at some distance away from the confluence.

At Pacific Creek, *S. exigua*, *S. boothii* and *S. wolfii* grew in a riparian floodplain of about 150 meters with several meters of elevation difference. In contrast to the other sampling sites, this made it possible to discern variation within the sampling area by sampling along transects from the creek to the upland areas. The three *Salix* species were collected from four to five sites along transects depending on the presence of the species, each site contained three replicate samples ($n = 3$). At all sampling locations, the root-zone soils associated with each plant sample were also collected. Here we defined root-zone soils as the soils that were in contact with the roots of plant. Samples were stored in plastic bags, kept cool inside a portable cooler and transported to the laboratory. Plant species identification was done at the North Dakota State University Herbarium.

3.3.4. Sample preparation for analysis

The leaf samples were washed thoroughly in distilled water to remove dust and soil particles. The root-zone soils and plant leaves were oven-dried at 60 °C until constant weight. Leaves were pulverized using a mortar and pestle and homogenized in liquid nitrogen. Leaf and soil samples were digested using microwave digestion (CEM Mars Xpress, 230V/60HZ), using Express 55 ml PFA venting vessels. A known weight (approximately 250 mg) of ground leaf material was pre-digested in 5 ml concentrated nitric acid, 5 ml of distilled water were then added and the samples placed in a MARS Xpress Microwave Digester (16 vessels, 1600 W, 100% Power, ramped to 200 °C over 15 minutes, and held at

this temperature for 5 minutes). The digested samples were transferred to culture tubes. Each vessel was rinsed with 1 ml aliquot of distilled water three times. Similarly, a known weight of approximately 500 mg of soil was pre-digested in 10 ml concentrated nitric acid overnight. The samples were then digested as above. The digests were filtered through Whatman® 1 filter paper into culture tubes, and the vessels rinsed as described above. Both the plant and soil samples were diluted 1:100.

3.3.5. Multi-element analyses

Analyses of 32 elements in the samples were carried out by a Spectro Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) with Crossflow nebulizer, Side-On-Plasma (SOP), with Smart Analyzer Vision v. 3.013.0752. Operating conditions for the ICP-OES instrument were: plasma power 1400 W, coolant gas flow rate 13.5 L min^{-1}, auxiliary gas flow rate was 1.2 and nebulizer flow 0.9 and 21 second sample integration time. The ICP was calibrated using an internal calibration (ICAL solution) and a 4-point standard curve for the elements using individual or combination standards in a five percent HNO_3 matrix. A continuing control verification (CCV) standard was checked after every 10 samples for quality control. The accuracy of elemental analysis was monitored by using a certified reference material (NCS DC 73350 leaves of Poplar and NCS DC 73384 soil from the China National Analysis of Center for Iron & Steel 2004). Instrument detection limits in mg L^{-1} of the plants and root-zone soils are presented in Appendix 1. In the remainder of this chapter element concentrations

will be presented in molar units based on the biological system of the elements (BSE) of Markert (1994).

3.3.6. Loss-on-ignition

Loss-on-Ignition (LOI) was performed to estimate the organic matter content of root-zone soil in the Soil Testing Laboratory, North Dakota State University. Five grams of root-zone soil were weighed into ceramic crucibles. The soils were oven-dried at 120 °C overnight. The oven-dried soils were weighed and kept in furnace for 2 hours at 360 °C. The OM content of soil samples was determined by calculating the difference in weight before and after being placed in the furnace, and were presented as OM g 100 g^{-1}.

3.3.7. Data analysis

Element concentrations and LOI data were log transformed to obtain normality and homogeneity of variance before statistical analysis. Statistical significance of differences were determined by General Linear Model (One-way ANOVA, $P < 0.05$) in Minitab statistical software (Minitab [®] 15 ©2006 Minitab Inc.). We employed student's t-test (two tailed, equal variance) to compare the mean element concentrations of SB and PEB transects towards the confluence. Pearson correlation coefficients and P-values were calculated using Minitab. Only correlations that were $r > 0.446$, explaining (r^2) $> 19\%$ variation were considered important.

3.4 Results

3.4.1. Loss-on-ignition

Loss-on-ignition (LOI), an estimate of organic matter content (OM), varied significantly ($P < 0.001$) between sites (Fig. 3.2A). Cooke City mine tailings (CC)

contained the highest LOI. LOI of CC and Soda Butte (SB) were similar. The lowest mean value was observed at Pacific Creek (PC), which was five times lower than CC. LOI of root-zone soils varied significantly ($P < 0.001$) along the transect at PC (Fig. 3.2B). The OM content was higher at 86, 108, and 126 m distance away from the river compared to 15 m and 150 m, and lowest nearest the river at 15 m.
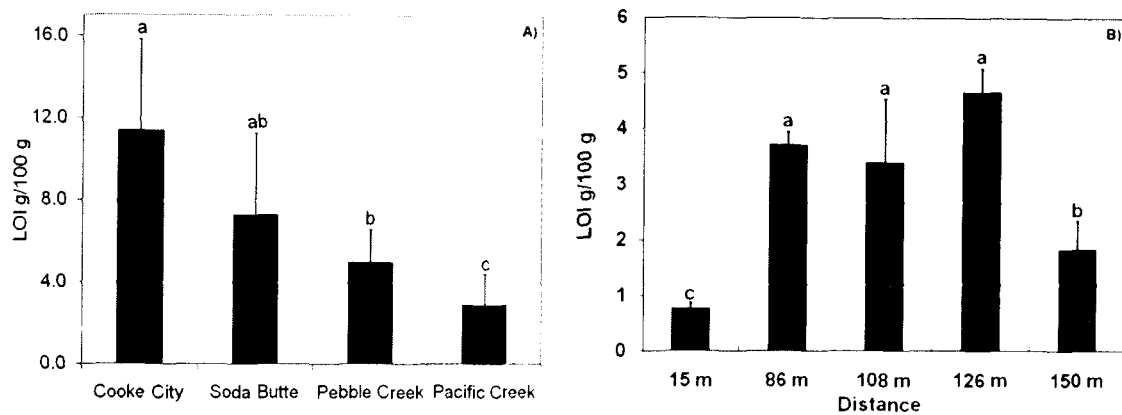
Figure 3.2. Mean Loss-on-Ignition (LOI) in root-zone soil samples, pooled values for all samples at A) Cooke City (n = 6), Soda Butte Creek (n = 10), Pebble Creek (n = 10), and Pacific Creek (n = 15) and B) within the transect at Pacific Creek (n = 3, x-axis shows the distance at transects, which was perpendicular to river). Bar with the same lower case letters do not differ significantly at $P < 0.05$, error bars indicate ± standard deviations,

3.4.2. Root–zone soil element concentrations

The mean element concentrations of root-zone soils among the study sites differed significantly ($P < 0.01$). The mean and standard deviation values for each area are presented in Table 3.1. Cooke City mine tailings root-zone soils contained significantly higher concentrations of S, Cu, Zn Li, Pb, and Ag compared to the other sites. P and As were higher at CC and SB compared to PEB and PC.

Ca and Al concentrations were higher at CC, SB and PEB compared to PC. SB contained high concentrations of V, Co, and Sr compared to other sites.

The comparison of mean element concentrations using student's t-test showed significantly higher ($P < 0.01$) concentrations of Cu, Zn, Ag, Pb, Mn, Fe, Co, Sr, P, S, Ce, and B in SB compared to PEB. These elements did not show any clear patterns along the stretch of 250 m at 25 m intervals upstream of confluence between SB and PEB (Fig. 3.3). K concentrations was significantly higher ($P < 0.01$) at PEB, whereas As and Si were below the detection in PEB. Many other elements such as Ca, Na, Mg, V, Cr, Ni, Li, Al, Ti, and Zr did not show significant differences ($P < 0.01$) between SB and PEB. LOI and elements in root-zone soils from pooled samples showed positive correlations for the majority of elements as follows; Al ($r = 0.666$; $P < 0.000$), As ($r = 0.779$; $P < 0.000$), Ba ($r = 0.625$; $P < 0.000$), Ca ($r = 0.631$; $P < 0.000$), Cr ($r = 0.599$; $P < 0.000$), Cu ($r = 0.672$; $P < 0.000$), Fe ($r = 0.877$; $P < 0.000$), Mn ($r = 0.804$; $P < 0.000$), Mo ($r = 0.638$; $P < 0.000$), Ni ($r = 0.558$; $P < 0.000$), P ($r = 0.917$; $P < 0.000$), S ($r = 0.861$; $P < 0.000$), Sr ($r = 0.557$; $P < 0.000$), Ti ($r = 0.585$; $P < 0.006$), and Zn ($r = 0.706$; $P < 0.000$).

At Pacific Creek, element concentrations of root-zone soils showed significant differences ($P < 0.05$) along transects. The mean and standard deviation of element concentrations are presented in Table 3.2. The majority of element concentrations (P, S, V, Cr, Fe, Co, Cu, Zn, B, Li, Ba, Pb, Zr and Ce) of root-zone soils were low at 15 m from the stream. Similarly, many element concentrations were low at 150 m from the stream.

Table 3.1. Mean element concentrations (μmol g^{-1} and or nmol g^{-1}dry soil) in root-zone soils at mine impacted and un-contaminated sampling sites in 2008 (mean ± standard deviation for pooled values, n = sampling numbers, ns = not significant, <dl = below the detection limit, significant differences among the sites at P < 0.05). Mean values followed by the same letters in rows do not differ significantly at P < 0.01).

Sites	Unit	Cook City (n = 6)	Soda Butte (n = 10)	Pebble Creek (n = 10)	Pacific Creek (n = 15)
Structural					
Ca	μmol g^{-1}	168±45a	142±13a	166±26a	83±40b
P	μmol g^{-1}	16±2a	15±1.2ab	13±1b	11±2c
S	μmol g^{-1}	18±13a	6.2±2.2b	3.9±1.2bc	3.7±1.6c
Si	μmol g^{-1}	0.29±0.29	0.31±0.27	<dl	0.25±0.27
Electrolytic					
K	μmol g^{-1}	46±12a	3.1±8.7b	30±4a	31±7a
Na	μmol g^{-1}	5.1±1.2b	17±1a	15±2a	3.7±1.0b
Mg	μmol g^{-1}	180±25b	772±94a	726±130a	110±30c
Enzymatic					
V	μmol g^{-1}	0.33±0.02c	0.66±0.03a	0.6±0.05b	0.22±0.03d
Cr	μmol g^{-1}	0.42±0.03b	0.54±0.04a	0.56±0.03a	0.21±0.03c
Mn	μmol g^{-1}	15±5a	13±1.9ab	8.2±1.2bc	7.1±3.6c
Fe	μmol g^{-1}	837±289a	645±58ab	485±73b	273±99c
Co	μmol g^{-1}	0.1±0.02c	0.14±0.01a	0.13±0.01b	0.05±0.01d
Ni	nmol g^{-1}	226±34b	363±25a	354±24a	94±22c
Cu	μmol g^{-1}	4.2±5.3a	1.2±0.33b	0.23±0.03c	0.1±0.04c
Zn	μmol g^{-1}	1.7±0.24a	0.66±0.13b	0.29±0.03c	0.33±0.1c
B	μmol g^{-1}	0.8±0.22a	0.56±0.13ab	0.4±0.11b	0.45±0.15b
Main/sub group					
Li	μmol g^{-1}	1.4±0.2a	0.82±0.08b	0.78±0.13b	1.2±0.3.0a
Sr	μmol g^{-1}	0.29±0.03bc	0.41±0.02a	0.34±0.03b	0.26±0.08c
Ba	nmol g^{-1}	895±149ab	1117±140a	949±53a	745±200b
Al	μmol g^{-1}	500±116a	344±50a	373±113a	87±105b
Pb	μmol g^{-1}	0.59±0.94a	0.05±0.01b	0.02±0.0b	0.02±0.01b
As	nmol g^{-1}	70±42a	34±11a	<dl	10±9b
Ti	μmol g^{-1}	14.3±1.7b	26.3±2.3a	23.4±3.7a	2.0±0.4c
Zr	nmol g^{-1}	93±19b	246±11a	259±27a	71±11c
Ag	nmol g^{-1}	23.3±14.6a	7.0±1.7b	2.5±0.4c	0.56±0.59d
Ce	nmol g^{-1}	209±25a	184±7a	167±12b	193±26a
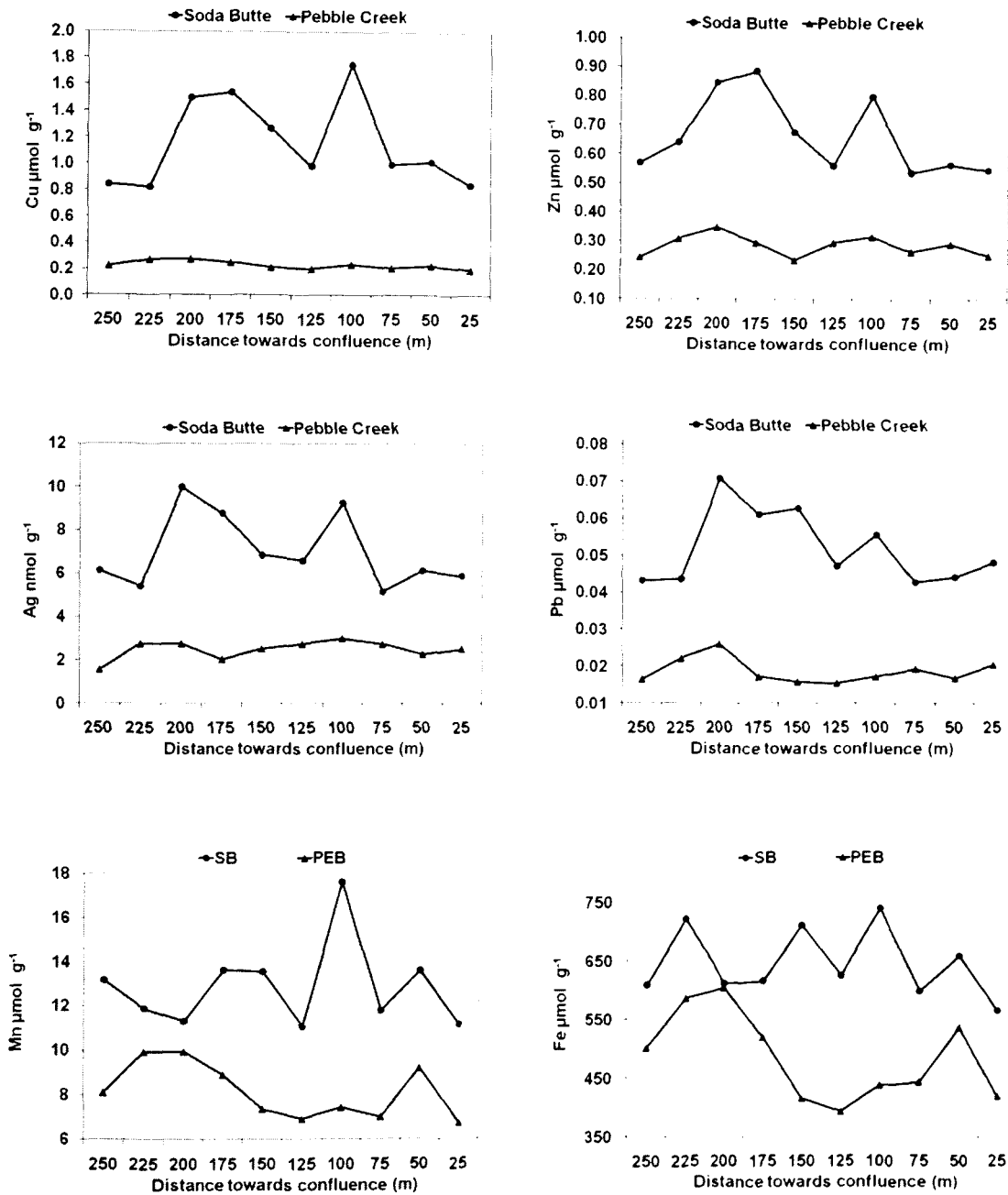
Figure 3.3. Mean concentrations of Cu, Zn, Ag, Pb, Mn, Fe, Co, Sr, P, S, Ce, and B in root-zone soils of Soda Butte and Pebble Creek as a function of distance (m) towards their confluence. Log transformed mean element concentrations are significantly different at $P < 0.01$ probability level by using student's t-test.

Figure 3.3. Continued...
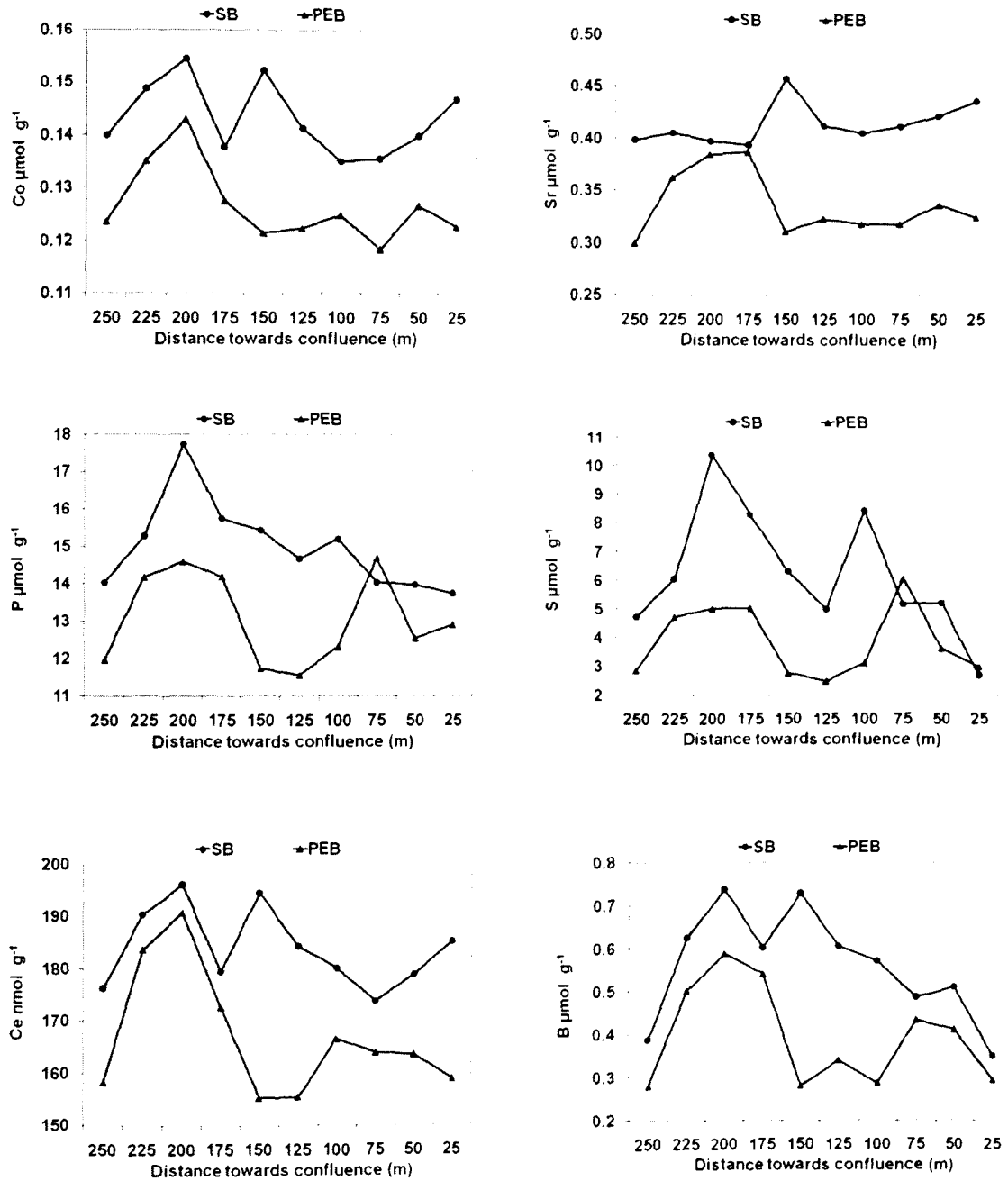
Table 3.2. Mean element concentrations (μmol g^{-1} or nmol g^{-1} dry soil) in root-zone soils along a transect away from river distances at Pacific Creek in 2008 (mean ± standard deviation, n = 3)

Elements	Unit	Sampling gradient distances away from river at PC				
		15 m	86 m	108 m	126 m	150 m
Structural						
Ca	μmol g^{-1}	58±10	125±3	120±42	79±12	31±3.4
P	μmol g^{-1}	8.7±0.52	13±0.52	12±1.5	12±0.35	11±0.24
S	μmol g^{-1}	1.7±0.50	5.4±0.57	4.0±1.4	4.8±0.37	2.2±0.44
Si	μmol g^{-1}	0.630.11	0.1±0.11	0.07±0.11	0.2±0.38	0.25±0.24
Electrolytic						
K	μmol g^{-1}	20±2.0	32±8.3	34±4.0	38±0.77	29±1.3
Na	μmol g^{-1}	3.5±1.1	4.8±0.7	3.9±1.3	3.3±0.29	2.7±0.37
Mg	μmol g^{-1}	77±6.8	135±9.3	139±25.0	123±9.9	77±1.6
Enzymatic						
V	nmol g^{-1}	178±8.9	229±14	236±30	245±16	22±16
Cr	nmol g^{-1}	169±5.4	219±11	228±26	224±13	203±12
Mn	μmol g^{-1}	2.2±0.21	9.6±1.4	10.4±3.7	8.3±0.97	4.6±1.6
Fe	μmol g^{-1}	101±3.9	339±42	343±78	321±31	259±21
Co	nmol g^{-1}	35±3.3	53±4.2	57±7.9	51±4.0	41±2.2
Cu	nmol g^{-1}	52±15	142±9.5	131±37	123±7.2	75±2.9
Zn	nmol g^{-1}	198±85	399±52	372±55	406±94	250±37
B	μmol g^{-1}	0.21±0.06	0.56±0.08	0.5±0.15	0.55±0.02	0.42±0.07
Main/sub group						
Li	μmol g^{-1}	0.720.10	1.4±0.10	1.4±0.31	1.3±0.13	0.91±0.05
Sr	nmol g^{-1}	183±23	355±33	323±74	293±29	166±10
Ba	nmol g^{-1}	487±71	884±36	936±177	849±71	570±30
Al	μmol g^{-1}	186±15	4.0±0.81	4.1±1.8	3.9±0.53	237±12
Pb	nmol g^{-1}	12±2.0	21±2.7	26±8.8	23±3.4	17±3.7
Ti	μmol g^{-1}	2.3±0.31	1.70.21	1.6±0.15	1.8±0.26	2.5±0.27
Zr	nmol g^{-1}	55±4.4	76±3.3	79±9.8	77±4.2	65±3.5
Ag	nmol g^{-1}	0.0±0.0	0.90±0.61	1.0±0.42	0.82±0.64	0.0±0.0
Ce	nmol g^{-1}	151±3.7	204±7.9	218±24	210±11	183±9.7

OM content and elements of root-zone soils along the transect away from the river showed positive correlations for the majority of elements, except Al and Ti (Table 3.3).

Table 3.3. Pearson correlation coefficients and probabilities of significance (in parentheses) for LOI and element concentrations in root-zone soils along the transects at Pacific Creek. Only correlations with $r \geq 0.612$ (explain 0.37 % or more variation) are shown.

	Al	Ba	Ca	Ce	Co	Cu	Li	Mg	Mn
LOI	-0.876	0.814	0.914	0.612	0.745	0.809	0.783	0.888	0.689
	(0.000)	(0.000)	(0.000)	(0.015)	(0.001)	(0.000)	(0.001)	(0.000)	(0.005)
	Na	Ni	P	Pb	S	Sr	Ti	Zn	Zr
LOI	0.666	0.774	0.640	0.634	0.815	0.898	-0.817	0.699	0.709
	(0.007)	(0.001)	(0.010)	(0.011)	(0.000)	(0.000)	(0.000)	(0.004)	(0.003)

3.4.3. Element concentrations in plants

3.4.3.1 Element concentration in *J. balticus*

The majority of element concentrations in *J. balticus* varied significantly ($P < 0.05$) between CC, SB, and PEB (Table 3.4). At CC, concentrations of K, Mn, Fe, Cu, Al, As, and Cd were significantly higher in *J. balticus* leaves compared to the other two sites. Elements such as S, Zn, B, and Sr were similar at CC and SB. These elements were lower at PEB compared to CC and SB. The concentrations of K, Mn, Fe, Al, and As were similar in *J. balticus* at SB and PEB. Copper concentrations varied significantly in *J. balticus* among the three sites, with the highest value in the sample collected at CC. Mean concentrations of Ca, P, Si, Li, and Ba were similar in the leaves of *J. balticus* across the sites. Correlation analysis between root-zone soils and *J. balticus* did not show significant relationships with the majority of elements except with Cu ($r = 0.842$; $P < 0.000$) and Zn ($r = 0.710$; $P < 0.000$). Similarly, positive correlations were observed between the LOI and concentrations of Cu ($r = 0.501$; $P < 0.015$) and Zn ($r = 0.446$; $P < 0.033$) in *J. balticus*.

Table 3.4. Mean element concentrations (μmol g^{-1} or nmol g^{-1} dry leaf weight) in *Juncus balticus* at Cooke City, Soda Butte, and Pebble Creek (mean ± standard deviation, n = sample size, significant differences at P < 0.05, ns = not significant, <dl = below the detection limit, - = not determined due to <dl, Mean values followed by the same letters in rows do not differ significantly at P < 0.01).

Element	Unit	P-value	Cooke City n = 3	Soda Butte n = 10	Pebble Creek n = 10
Structural					
Ca	μmol g^{-1}	ns	99±21	91±10	83±12
P	μmol g^{-1}	ns	68±14	54±7	60±7
S	μmol g^{-1}	0.001	81±6a	77±11a	63±3b
Si	μmol g^{-1}	ns	25±11	18±3	19±4
Electrolytic					
K	μmol g^{-1}	0.040	592±77a	486±56b	480±47b
Mg	μmol g^{-1}	0.015	56±13b	68±6a	59±4ab
Enzymatic					
Mn	μmol g^{-1}	0.000	3.2±1.3a	0.9±0.5b	1.4±0.5b
Fe	μmol g^{-1}	0.003	2.2±1.7a	0.4±0.1b	0.9±0.6b
Ni	nmol g^{-1}	0.025	17±15a	8.7±6.6b	26±14a
Cu	nmol g^{-1}	0.000	348±132a	211±70b	61±24c
Zn	μmol g^{-1}	0.000	1±0.1a	0.9±0.1a	0.4±0.1b
B	μmol g^{-1}	0.026	0.8±0.2a	0.9±0.1a	0.7±0.1b
Main/sub group					
Li	nmol g^{-1}	ns	77±117	40±49	88±92
Sr	nmol g^{-1}	0.002	105±42a	88±17a	60±9b
Ba	nmol g^{-1}	ns	129±31	91±40	105±24
Al	μmol g^{-1}	0.005	1.7±1.4a	0.3±0.1b	0.9±0.6b
As	nmol g^{-1}	0.047	9.2±11.2a	2.2±3b	0.9±2.1b
Cd	nmol g^{-1}	-	8.3±7.1	1.4±1.1	<dl

3.4.3.2. Element concentration in *Salix exigua*

The mean, standard deviations, and significance of differences of element concentrations are presented in Table 3.5. The multi-element fingerprint of *S. exigua* showed significant differences between sampling sites only for a few elements. *S. exigua* contained higher concentrations of P, K, Ni, and Ti at SB and PEB compared to PC. The highest concentration of Sr was found at PC followed

Table 3.5. Mean element concentrations (μmol g^{-1} or nmol g^{-1}dry leaf weight) in *S. exigua* in Yellowstone (Soda Butte and Pebble Creek) and Grand Teton (Pacific Creek) National Parks (mean ± standard deviation, n = sampling numbers, Mean values followed by the same letters in rows do not differ significantly at $P < 0.01$)

Element	Unit	Soda Butte (n = 10)	Pebble Creek (n = 10)	Pacific Creek (n = 15)
Structural				
Ca	μmol g^{-1}	362±69a	328±46a	411±85a
P	μmol g^{-1}	97±10a	105±13a	81±23b
S	μmol g^{-1}	141±22a	147±32a	122±36a
Si	μmol g^{-1}	6.5±4.5a	5.8±1a	6.9±2.3a
Electrolytic				
K	μmol g^{-1}	368±42a	358±49a	287±44b
Mg	μmol g^{-1}	101±9a	122±18a	118±28a
Enzymatic				
Mn	μmol g^{-1}	1.1±0.3a	0.9±0.3a	1.5±0.7a
Fe	μmol g^{-1}	1.8±2.5a	1.1±0.2a	0.7±0.5a
Ni	nmol g^{-1}	20±17a	28±11a	8.5±6.3b
Cu	nmol g^{-1}	111±54a	87±42a	80±32a
Zn	μmol g^{-1}	0.9±0.5a	0.6±0.2a	0.6±0.5a
B	μmol g^{-1}	2.3±0.4ab	2.7±0.7a	1.8±0.8b
Main/sub group				
Li	μmol g^{-1}	0.1±0.2a	0.1±0.2a	0.1±0.1a
Sr	μmol g^{-1}	0.3±0.1b	0.2±0c	0.7±0.2a
Ba	nmol g^{-1}	45±9b	59±15b	177±49a
Al	μmol g^{-1}	1.8±1.8a	1.1±0.2ac	0.7±0.5bc
Ti	nmol g^{-1}	48±65a	25±7a	4.7±6.7b
Cd	nmol g^{-1}	3.2±2.7a	1.7±1.3a	2.2±3.1a
Ce	nmol g^{-1}	3.2±2.7a	1.7±1.3a	2.2±3.1a

by SB and PEB. Correlation analysis of elements between root-zone soils and *S. exigua* did not show significant relationships for the majority of elements except positive correlations were found with Al (r = 0.617; $P < 0.000$) and Ni (r = 0.562; P

< 0.001) and negative correlations were found with Ba (r = -0.590; P < 0.000) and Sr (r = -0.502; P < 0.003). LOI in root-zone soils and element concentrations in leaves of *S. exigua* showed negative correlations with Ba (r = -0.585; P = < 0.000), and Sr (r = -0.529; P = < 0.002).

3.4.3.3. Element concentration in *S. exigua*, *S. boothii*, and *S. wolfii*

S. exigua, *S. wolfii*, and *S. boothii* varied significantly (P < 0.05) in element concentrations in their leaf tissues from the same habitat at PC (Table 3.6).

Table 3.6. Mean element concentrations (μmol g^{-1} or nmol g^{-1}dry leaf weight) in three *Salix* species at Pacific Creek (mean ± standard deviation, pooled values along transects, n = sampling numbers, Mean values followed by the same letters in rows do not differ significantly at P< 0.01).

Element	Unit	P-value	S. exigua (n = 15)	S. wolfii (n = 12)	S. boothii (n = 12)
Structural					
Ca	μmol g^{-1}	0.002	411±82	276±74	288±90
P	μmol g^{-1}	ns	81±21	67±13	74±21
S	μmol g^{-1}	0.000	122±35a	55±26b	91±26a
Si	μmol g^{-1}	0.000	6.9±2.3b	21±11a	8±4b
Electrolytic					
K	μmol g^{-1}	0.031	287±43a	353±77a	341±128a
Mg	μmol g^{-1}	ns	118±28a	110±20a	106±28a
Enzymatic					
Mn	μmol g^{-1}	0.000	1.5±0.76b	1.5±0.63b	3.4±2.8a
Fe	μmol g^{-1}	0.003	0.75±0.48b	0.93±0.43b	1.5±0.8a
Ni	nmol g^{-1}	0.012	8.5±6.5b	14±12bc	24±15ac
Cu	nmol g^{-1}	ns	80±38a	108±55a	108±48a
Zn	μmol g^{-1}	0.000	0.63±0.58b	2.2±1.0a	2.0±1.0a
B	μmol g^{-1}	ns	1.8±0.7a	1.1±0.4a	2.0±1.6a
Main/sub group					
Li	μmol g^{-1}	ns	0.11±0.08a	0.2±0.22a	1.8±5.8a
Sr	μmol g^{-1}	0.000	0.74±0.15a	0.4±0.14b	0.51±0.2b
Ba	μmol g^{-1}	0.000	0.18±0.05a	0.1±0.03b	0.15±0.05a
Al	μmol g^{-1}	0.000	0.67±0.5b	0.5±0.1b	1.8±1.3a
Ti	nmol g^{-1}	0.001	4.6±7.7b	4.2±3.1b	35.1±34.1a

S. exigua contained high concentrations of Sr compared to other two species. *S. wolfii* contained high Si compared to *S. boothii*. *S. boothii* contained higher concentrations of Mn, Fe, Al, and Ti compared to *S. exigua* and *S. wolfii*. Concentrations of S and Ba were similar in *S. exigua* and *S. boothii*, and higher than *S. wolfii*. Concentrations of Zn and Sr were higher in *S. wolfii* and *S. boothii* compared to *S. exigua*. Concentrations of P, Mg, Cu, B, and Li were similar among the three species. The mean element concentrations in leaves for these three species along transect from the river to upland areas in the Pacific Creek riparian zone are presented (Tables 3.7, 3.8, and 3.9).

Table 3.7. Mean element concentrations (μmol g^{-1} dry weight) in leaf of *S. exigua* at different sampling sites along transects away from river distances at Pacific Creek in 2008 (mean ± standard deviation, $n = 3$, < dl = below the detection)

Elements	Unit	Distances away from river at Pacific Creek				
		15 m	**86 m**	**108 m**	**126 m**	**150 m**
Structural						
Ca	μ mol g^{-1}	415±24	452±102	357±221	379±78	350±118
P	μ mol g^{-1}	78±19	78±16	59±37	94±44	75±12
S	μ mol g^{-1}	133±31	146±26	103±67	70±20	124±28
Si	μ mol g^{-1}	5.8±2.9	6.4±0.36	7.1±5.9	5.3±2.4	7.7±0.57
Electrolytic						
K	μmol g^{-1}	312±51	288±28	202±155	252±45	297±55
Na	μmol g^{-1}	<dl	5.4±9.4	4.7±4.4	1.8±3.0	3.7±6.4
Mg	μmol g^{-1}	112±43	122±14	110±84	102±26	108±18
Enzymatic						
Mn	μmol g^{-1}	1.9±1.3	1.0±0.23	1.5±1.2	1.2±0.39	1.31±0.63
Fe	μmol g^{-1}	0.81±0.17	0.46±0.06	1.2±1.03	0.44±0.24	0.60±0.04
Cu	μmol g^{-1}	0.11±0.08	0.09±0.02	0.06±0.03	0.08±0.02	0.06±0.02
Zn	μmol g^{-1}	1.3±0.94	0.53±0.14	0.60±0.67	0.22±0.03	0.37±0.11
B	μmol g^{-1}	2.0±0.79	1.6±0.38	1.3±0.8	1.27±0.52	2.3±1.3
Main/sub group						
Li	μmol g^{-1}	0.07±0.09	0.15±0.01	0.14±0.09	0.09±0.04	0.11±0.07
Sr	μmol g^{-1}	0.73±0.05	0.87±0.18	0.59±0.36	0.73±0.17	0.62±0.20
Ba	nmol g^{-1}	178±10	201±55	159±91	166±53	141±68
Al	μmol g^{-1}	1.2±0.25	0.70±0.15	0.07±0.08	0.28±0.26	0.96±0.34
Ti	nmol g^{-1}	12.7±13.6	<dl	0.98±1.7	<dl	8.0±5.1

In *S. exigua*, some element concentrations (Mn, Cu, Zn, Al, and Ti) tended to be higher at the site nearest to the river but no clear patterns could be discerned for any of the elements (Table 3.7).

S. *boothii* was present at all sites along the transect except at 150 m (Table 3.8). In *S. boothii*, the concentrations of K, Mn, B, and Li tended to be high at 15 m and again no clear patterns could be discerned for these elements.

Table 3.8. Mean element concentrations (μmol g^{-1} dry leaf weight) in leaves of *S. boothii* at different sampling sites along transects away from river at Pacific Creek (PC) in 2008 (mean ± standard deviation, *n* = 3, < dl = below the detection limit, - = samples absent at given distance along transect).

Element	Unit	Distances away from river at PC (*S. boothii*)				
		15 m	86 m	108 m	126 m	150 m
Structural						
Ca	μmol g^{-1}	186±103	368±37	293±27	290±16	-
P	μmol g^{-1}	70±22	95±10	68±12	68±11	-
S	μmol g^{-1}	82±39	108±15	86±9	91±30	-
Si	μmol g^{-1}	6.7±0.5	9.4±3.4	9.7±3.1	5.6±2.9	-
Electrolytic						
K	μmol g^{-1}	440±239	289±17	361±25	287±38	-
Na	μmol g^{-1}	<dl	<dl	<dl	<dl	-
Mg	μmol g^{-1}	95±15	121±27	92±8	104±33	-
Enzymatic						
Mn	μmol g^{-1}	5.5±5.7	2.4±0.3	3.7±1.1	1.9±0.1	-
Fe	μmol g^{-1}	1.6±0.5	1.3±0.6	2.4±1	0.8±0.2	-
Cu	μmol g^{-1}	0.1±0.05	0.12±0.04	0.1±0.05	0.13±0.1	-
Zn	μmol g^{-1}	1.24±0.92	3.05±0.58	2.51±1.02	1.02±0.32	-
B	μmol g^{-1}	3.0±3.6	1.6±0.1	1.4±0.8	1.7±0.3	-
Main/sub group						
Li	μmol g^{-1}	7.1±12.3	0.03±0.04	0.08±0.07	0.01±0.00	-
Sr	μmol g^{-1}	0.37±0.28	0.69±0.07	0.4±0.15	0.53±0.04	-
Ba	nmol g^{-1}	125±73	186±31	130±53	134±8	-
Al	μmol g^{-1}	1.3±0.6	2.5±2	2.3±1.5	0.9±0.2	-
Ti	nmol g^{-1}	26±21	38±28	66±55	10±9	-

S. wolfii was absent at 15 m and there were no clear pattern for the elements in the rest of the sampling points (Table 3.9). The LOI showed significant correlations with few elements in the *Salix* species

. The LOI in root-zone soils and *S. boothii* leaves showed positive correlations for Ca (r = 0.628; P < 0.029). In *S. wolfii*, the LOI in root-zone soil, however, showed negative correlations for B (r = -0.720; P < 0.009). In *S. exigua* leaves, concentrations of Al (r = -0.629; P = < 0.016), Ni (r = -0.671; P = < 0.009), Ti (r = -0.652; P = < 0.012), Zn (r = -0.612; P = < 0.020) correlated negatively with LOI.

Table 3.9. Mean element concentrations (μmol g^{-1} dry leaf weight) in leaves of *S. wolfii* at different sampling sites along transects away from river distances at Pacific Creek (PC) in 2008 (mean ± standard deviation, n = 3, < dl = below the detection limit, - = samples absent at given distance along transect).

Element			Distances away from river at PC (*S. wolfii*)				
	Unit	*P*-value	15 m	86 m	108 m	126 m	150 m
Structural							
Ca	μmol g^{-1}	0.000	-	345±34	250±39	291±50	217±44
P	μmol g^{-1}	0.000	-	78±12	64±3	60±10	69±7
S	μmol g^{-1}	0.000	-	59±10	49±5	71±46	41±3
Si	μmol g^{-1}	ns	-	22.2±6.8	29.2±8.1	19.6±19.6	13.7±3.5
Electrolytic							
K	μmol g^{-1}	0.004	-	337±22	325±41	411±51.4	342±42
Na	μmol g^{-1}	0.001	-	1.08±1.9	6.3±9.3	0.5±0.87	0.28±0.41
Mg	μmol g^{-1}	0.000	-	121±3	103±16	107±8	107±19
Enzymatic							
Mn	μmol g^{-1}	0.000	-	1.36±0.35	2.1±0.96	1.1±0.55	1.5±0.31
Fe	μmol g^{-1}	0.000	-	1.25±0.55	1.3±0.33	0.7±0.01	0.52±0.04
Cu	μmol g^{-1}	0.001	-	0.13±0.03	0.06±0.02	0.17±0.06	0.07±0.01
Zn	μmol g^{-1}	0.009	-	3.5±0.84	2.3±0.44	1.8±1.1	1.4±0.04
B	μmol g^{-1}	0.002	-	0.76±0.22	1.2±0.28	0.89±0.17	1.7±0.08
Main/sub group							
Li	μmol g^{-1}	0.001	-	0.04±0.04	0.47±0.17	0.02±0.02	0.27±0.25
Sr	μmol g^{-1}	0.000	-	0.53±0.02	0.33±0.09	0.43±0.13	0.31±0.08
Ba	nmol g^{-1}	0.000	-	125±7	91±22	103±34	82±21
Al	μmol g^{-1}	0.000	-	0.4±0.15	0.62±0.03	0.55±0.04	0.58±0.08
Ti	nmol g^{-1}	0.000	-	4.02±4.7	6.92±1.6	3.0±3.8	2.9±1.9

3.5. Discussion

3.5.1. Root-zone soils

The majority of element concentrations in root-zone soils followed the order CC>SB≥PEB>PC. One reason explaining this order is that CC and SB are impacted by mining activities. The direct input of metals from past mining activity and headwater mining disturbance contributed to elevated concentrations of elements at CC and SB. Among the elements, concentrations of Fe, Cu, Zn, Pb, As, and Ag were the highest at CC compared to all other sites. This suggests that CC is contaminated with these elements by mining activities.

We expected that SB would contain higher element concentrations compared to PEB. Gold mining activities and the flooding of 1950 contaminated Soda Butte Creek (Marcus et al., 2001) downstream from the mining site. The sediments and soil analyses at Soda Butte (SB) confirmed the contamination and their impacts on plants diversity (Stoughton and Marcus, 2000). Marcus et al. (2001) reported higher concentrations of As, Cu, Fe, Pb, and Zn in flood plain tailings deposits in Soda Butte Creek. Our study also showed higher concentrations of V, Co, Cu, Zn, Sr, As, Ag and Ce in the root-zone soils of Soda Butte compared to relatively un-contaminated sites PEB and PC. Pebble Creek has similar lithology and geomorphic character to Soda Butte Creek (Marcus et al., 2001), but has no evidence of mining activity. The geology of CC, SB, and PEB can be described as mixed minerals rich in silicate, iron, magma, and rocks, which are rich in Al, Na, and K (McCarthy et al., 1998). In contrast, Pacific Creek, in Grand Teton National Park, is both unimpacted by mining activities and not influenced by geothermal

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activity. There is no additional input mining of metals in this system and PC reflects this with the lowest concentrations of the majority of metals. Except K, Li, and Ce, all elements at PC were either lower or similar to PEB.

Previous studies reported that mining activities and the flooding of June 1950 contaminated sediments downstream in Soda Butte Creek. Meyer (1993) reported that metals along Soda Butte were elevated up to 28 km downstream from the mining site. The majority of previous studies in Soda Butte were confined to the McLaren impoundment and flood plain tailings near the impoundments (Nimmo et al. 1998; Stoughton and Marcus, 2000; Marcus et al. 2001). Some of these studies have examined the element concentrations from the mining site through to the confluence of the Lamar River (Marcus et al. 2001). However, the majority of these studies have examined the metal concentrations in stream water and stream sediments as opposed to root-zone soils and plants (Nimmo et al., 1998; Marcus et al., 2001). Erickson and Norton (1994) found greater concentrations of Cu, Pb, and Zn in riparian vegetation such as grass, horsetail and willow immediately downstream from the McLaren tailings compared to upstream populations. Furthermore, they reported that the concentrations of these metals in riparian vegetation decreased further downstream towards the park boundary.

Marcus et al. (2001) studied the element concentrations from the tailings deposits at Cooke city, along Soda Butte and through to the confluence of Lamar River, which is approximately 28 km downstream from McLaren tailings. These authors reported concentration ranges for Cu (1.5 - 19.2), As (0.1 - 3.6), Fe (627 - 2865), Pb (0.2 - 3.6), and Zn (1.1 - 9.9) μmol g^{-1}.

In the current study, we have studied the element concentrations within 250 m upstream from the confluence of Soda Butte and Pebble Creek (8 km upstream from the Lamar River) and compared them with the lower ranges of element concentrations reported by Marcus et al. (2001). Our results showed similar concentration of Cu, but Fe and Zn concentrations were higher compared to the Marcus et al. (2001). However, Pb and As concentrations were lower in current study compared to Marcus et al. (2001). These elements were higher at CC compared to Marcus et al. (2001), except As. Stoughton and Marcus, (2000) studied the impact of mining on grass communities in the flood plain along Soda Butte Creek. One of the study sites closest to ours, Round Prairie near the SB and PEB confluence, recorded Cu 0.9, As 0.04, Fe 537, Pb 0.1, and Zn 1.1 μmol g^{-1} in the sediment. Among these elements, Cu and Fe were 1.3 and 1.2 times higher in current study compared to Stoughton and Marcus (2000). In contrast, Zn (1.6 times), Pb (2 times), and As (1.2 times) higher in their study compared to ours. Nimmo et al. (1998) studied the effect of mining on metal concentrations and their impact in micro-invertebrates and fish along Soda Butte Creek. These authors collected samples from approximately 3 km upstream from the North East Yellowstone entrance downstream to the Lamar River confluence. They reported that Cu concentrations in the river decreased downstream. At the sampling site nearest to ours, approximately 5 km upstream from Soda Butte and Pebble Creek confluence, they reported concentrations of Cu at 0.9 μmol g^{-1}, which is lower compared to our result. The Cu concentration of current study was similar to Nimmo et al. (1998) at Pebble Creek. Our data on element concentrations

especially Cu, Fe, Zn, Pb, and As at SB and previous data reported by Marcus et al (2001), Stoughton and Marcus (2000) and Nimmo et al. (1998) did not show wide variations. There are various reasons for low variation in metal concentration in these studies compared to ours. First, all these studies were conducted downstream of the McLaren tailings. Studies showed that metal concentrations in Soda Butte decrease exponentially downstream (Marcus et al., 2001). Second, we compared the lower range of metal concentrations reported by Marcus et al. (2001) because their studies encompass a long transect downstream of Soda Butte. Third, these study sites are within 5 km of SB and PEB confluence and are less likely to show wider variation in metal concentrations.

As expected, at least for some of these metals, higher mean concentrations of V, Co, Cu, Zn, Sr, Pb, As, Ag, and Ce were observed in root-zone soils at SB compared to PEB. Furthermore, we have shown that Zn, Cu, Pb, Fe, Co, Sr, Mn, Ce, Ag, and B were always higher at SB compared to PEB along transects towards confluence. Therefore, the contamination of Soda Butte from McLaren impoundments for Cu, Zn, Pb, Fe, and As is in agreement with previous studies. Marcus et al. (2001) argued that the exponential decrease in Cu at Soda Butte was due to dilution effects from various Cu-poor tributaries that are mixed together with Soda Butte. Our data, after comparison of element concentrations in SB and PEB upstream from the confluence, also support this result. First, Cu, Zn, Pb, Fe, and As were always higher in SB compared to PEB. Interestingly, As was below the detection limit in PEB whereas it was 33.6 nmol g^{-1} of root zone soils at SB. We found 2.8 times higher Ag concentration in SB compared to PEB. Our data

indicate SB is contaminated with Ag from mining activities. Therefore, our results showed that SB has not contaminated PEB, because should SB have contaminated PEB, there would have been no significant differences in concentrations of these metals along transects toward the confluence between these two sites. The higher concentrations of elements in SB were maintained due to regular mixing of sediments from contaminated source McLaren tailings (Marcus et al. 2001).

3.5.2. Element concentrations in plants

3.5.2.1. *Juncus balticus*

In this study, we reported multi-element concentrations of plant species from riparian zones influenced by contaminated and un-contaminated sources. Copper concentrations varied significantly in *J. balticus* among the sites. Cu concentration in *J. balticus* at CC was significantly higher than SB (1.6 times) and PEB (5.7 times). Similarly, Fe was higher in CC compared to SB (5-5 times) and PEB 92.4 times). As concentrations were four times higher at CC compared to SB, and was 10 times higher compared to PEB. Concentrations of Al were 5.6 and 1.8 times higher in CC compared to SB and PEB. Similarly, Cd was 5.9 times higher in CC compared to SB, whereas it was below the detection limit at PEB. The concentrations of S, B, Zn, and Sr were significantly higher in *J. balticus* collected from contaminated sites CC and SB. K, Mn, Fe, Al, and As were similar between SB and PEB. Plant species varies in their metal concentrations at polluted and un-polluted sites even though they are from the same family (Franco-Hernández et al., 2010). Freitas et al. (2004) reported that *Juncus efusus* and *J. conglomerates*

showed high concentrations of Pb and As compared to *Helichrysum stoechas* (L.) Moench. (Asteraceae), *Agrostis castellana* Boiss. (Poaceae) and *Erica australis* L (Ericaceae) at the São Domingos gold mine, Portugal. These authors reported As concentrations in *J. conglomerates* and *J. efusus* of 0.31 and 0.11 μmol g^{-1}, respectively, which are higher than the concentrations in *J. balticus* in this study. The differences in element concentrations between *J. balticus* and other *Juncus* species could be due to difference in species, as well as geographical variations and the sources of metal concentrations (Ernst, 1995; Marschner, 1995). *Rumex crispus* showed higher concentrations of elements in flooded treatments compared to non-flooded treatments (Kissoon et al., 2009). In different habitats, soil heterogeneity, local environment, rhizosphere environment, and presence of mycorrhizae also affect the ability of plant species to accumulate elements (Kabata-Pendias, 2001).

In this study *J. balticus* reflected the variation for the majority of element concentrations (except Ca, P, Si Li, and Ba) in the soils of the different study sites. This suggests that *J. balticus* may be useful in studying the element composition in soils for selected elements. *J. balticus* leaves showed significant positive correlations for Cu ($r = 0.842$; $P = 0.000$), Zn ($r = 0.710$; $P = 0.000$), and As ($r = 0.473$; $P = 0.023$) with the root-zone soils, while Ba showed a negative concentration ($r = -0.432$; $P = 0.040$). Stoughton and Marcus (2000) found higher density of *J. balticus* in soils contaminated with > 3.9 μmol g^{-1} Cu compared to other plant species such as *Phleum pretense* and *Poa pretense* along Soda Butte Creek. From the species diversity at Cu concentration > 3.9 μmolg^{-1} in soils of

Soda Butte, Stoughton and Marcus (2000) suggested that *J. balticus* from Soda Butte might have developed tolerance to Cu. In the current study, uptake of Cu by *J. balticus* from CC was 1.6 and 6.3 times higher than SB and PEB. Similarly, *J. balticus* had Zn 1.1 and 2.5 times and As 4.1 and 10.2 times higher uptake than SB and PEB. Current results also suggest that *J. balticus* might have higher level of tolerance to Cu, Zn and As. Furthermore, the positive correlations of LOI with Cu ($r = 0.501$; $P = 0.015$), and Zn ($r = 0.446$; $P = 0.033$) concentrations in *J. balticus* indicated the plants' ability to uptake Cu and Zn under higher LOI in soil. Mobility of Cu, Zn, and As in plants depends on the level of concentration in the soil (Meharg and Macnair, 1990; Carbonellu et al., 1998; Kabata-Pendias, 2001), and this pattern is reflected in *J. balticus*.

Effort has been made to study metal uptake in *Salix* species. Studies have reported that *Salix* species have potential for phytoremediation of metal contaminated soils (Landberg and Greger, 1996; Pulford et al., 2002; Vandecasteele et al., 2004). Meers et al. (2007) showed that ability of *Salix* clones to accumulate Cd and Zn was high in heavily polluted sediment and in moderately elevated concentrations in sandy soils compared to moderately contaminated sediment. Kuzovkina et al. (2004) reported that *S. exigua* accumulated Cu concentrations in the range of 0.22 - 0.30 $\mu mol\ g^{-1}$ in leaves of plants treated with 25 μM of Cu, which was lower compared to the current result in *S. exigua*. Vandecasteele et al. (2004) reported that *Salix* species growing in dredged soils accumulated higher concentrations of Cd and Zn compared to control sites. In current study, *S. exigua* was compared for multi-element uptake from

contaminated (SB) and un-contaminated sites (PEB and PC). *S. exigua* showed similar element concentrations for the majority of elements in leaf tissues when compared between contaminated site (SB) and un-contaminated sites (PEB and PC). The species reflected the element concentrations in soil for Al (r = 0.617; P = 0.000), Cu (r = 0.345; P = 0.049), Fe (r = 0.387; P = 0.026), Ba (r = -0.590; P = 0.000), Ni (r = 0.562; P = 0.001) and Sr (r = -0.520; P = 0.003). Higher concentrations of Mn, Sr, and Ba were found in leaf tissues at PC compared to other two sites. *S. exigua* tends to uptake higher amount of Mn, Sr, and Ba even though the corresponding concentrations in soil are the least among all sites. Furthermore, these elements have negative correlations (Ba, r = -0.585, P = 0.000 and Sr, r = -0.529, P = 0.002) with LOI. These results suggest that both Ba and Sr are readily available in the soil with low organic matter, thus become more available to plants. Kabata-Pendias (2001) also reported that higher organic matter tends to fix these elements in the soil, and thus decrease the potential for uptake. All three study sites, SB, PEB, and PC have significantly lower LOI than CC, which affected the availability of the elements to the plants in these sites.

3.5.2.2. *S. exigua*, *S. wolfii*, and *S. boothii*

To our knowledge, the multi-element fingerprinting reported in current study are the very first reports for *S. wolfii* and *S. boothii*. Many studies have compared *Salix* species and/or clones from contaminated and un-contaminated sites comparing a few elements at a time. In the current study, we compared *S. exigua*, *S. wolfii*, and *S. boothii* from an un-contaminated site at PC. These three species showed variations in the majority of element concentrations. Plant species varies

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in the metal concentration even though they were collected at the same site and from the same family (Franco-Hernández et al., 2010). In general, *S. boothii* was found to accumulate more elements followed by *S. wolfii* and *S. exigua. S. boothii* accumulated higher concentration of Mn, Fe, Ni, B, Li, Al, and Ti compared to *S. exigua* and *S. wolfii*. Nissen and Lepp (1997) studied the baseline concentration of Cu and Zn in shoots of different *Salix* spp. and reported distinct variations between species. The highest concentration was found for Cu (0.02) and Zn (0.11) μmol g^{-1} in the leaf of *S. purpurea*. The accumulation pattern of Cu, Cd and Zn vary varies between *Salix* spp (Punshon and Dikension, 1997). Our results showed that Cu concentration was similar among the species but Zn concentrations were higher in *S. boothii*, and S. *wolfii* compared to *S. exigua*. The differences in metal concentrations among *Salix* species in the current study can be explained by genetic differences at the species level. Previous study has showed significant correlations between accumulation of elements in *Salix* species and metal availabitlity in the medium. Vysloužilová et al. (2003) studied the elements (Cd, Cu, Pb, As, and Zn) uptake in clones of *Salix spp.* in pot experiment. These authors reported that *Salix spp.* grown under high metal pollutilled soils had high concentration of Zn (77.3 μmol g^{-1}) and in moderate polluted soils contained high Cd (1.01 μmol g^{-1}) concentration in leaves of *Salix* spp. In current study, the *Salix* species did not reflect the majority of root-zone soil element concentrations in plant tissues.

To explore the variation in element concentrations within the study site, *Salix* species (*S. exigua, S. boothii*, and S. wolfii) were studied along transects from the

river to upland at PC. First, we found a distinct habitat preferences of *Salix* species along the transects. *Salix exigua* was found from the river banks up to 150 m away from the river. This indicates that the species has wide adaption from higher moisture regime to dry upland conditions. *S. bothii* was present in gradients closer to river banks and was absent at 150 m distance. In contrast S. *wolfii* was absent at 15 m (the closest gradient to Pacific Creek river) and present through to 150 m. USDA-NRCS (2010) reported that *S. bothii* inhabits soils with higher moisture regimes, whereas, *S. wolfii* is found in soils with drier soils. Our observations from Pacific Creek agree with the findings of the USDA-NRCS (2010).

Second, multi-element concentrations varied significantly along the transects within each species, *S. exigua*, *S. boothi*, and *S. wolfii*. In the field, the variations in the element concentrations within species can be atributed to genotypic variations and are affected by various factors such as soil properties and chemistry, soil biota. Vandecasteele et al. (2004) and Landberg and Greger (1996) reported that *Salix* speices varied in element uptake pattern when grown under polluted compared with un-polluted soils. These authors reported that soil chemistry influenced the Mn, Cu, K, and S uptake in *Salix* species whereas genetic varition played a role in Cd, Cu and Zn uptake. Marshner (1995) suggested that genotypic differences could lead to selective uptake of elments from the medium. In the current study, the variation in Ca, Ni, Zn, B, and Al concentrations within *Salix* species can be partially attributed to LOI of the root-zone soils. Whether the genotypic differences in plant samples within each species along the transects led to multi-element uptake could not be determined in current study.

In summary, to our knowedge, this is the first study of multi-element concentrations in plant species compareing the mine impacted SB and the un-contaminated PEB in YNP. As expected, root-zone soils at contaminated sites contained higher metal concentrations compared to un-contaminated sites. We have found that SB has no and/or less imapcts on major contamination of Cu, Zn, Pb, Fe, and Ag into PEB, because if it had been contaminated the PEB, the concentrations of those elments would have been similar between SB and PEB.. *Jucus balticus* showed marked variations in element concentrations between contaminated and un-contaminated sites suggesting that this species can be used for assessing soil contamination. Unlike *J. balticus*, *S. exigua* showed less variations in element concentration between different study sites. Although the element contents of many *Salix* species have been reported previously, to our knowledge, the multi-element fingerprints of *S. wolfii* and *S. boothii* are reported for the first time. The *Salix* species *S. exigua*, *S. wolfii*, and *S. boothii* showed different multi-element figngerprints erprints. *S. boothii* has the potential to be used in phytoremediation applications for Mn, Fe, Al, and Ti.

CHAPTER 4. EFFECTS OF TEMPERATURE AND SOIL BIOTA (MYCORRHIZAE) ON ELEMENT CONTENT OF *TRIGLOCHIN MARITIMA* L.

4.1. Abstract

It has been shown that for some plant species, thermotolerance is achieved though a symbiotic relationship between the roots and a fungus. The current study examined the role of temperature and soil biota on multi-element concentrations and contents in *Triglochin maritima* L. In a greenhouse experiment *T. maritima* was grown at 20, 30, and 40 °C in non-autoclaved or autoclaved soils for eight weeks. To examine the presence of mycorrhizae in *T. maritima*, roots were stained using trypan blue. Multi-element concentrations and contents were analyzed in roots and leaves of *T. maritima* using inductively coupled plasma mass spectrometry (ICP-MS). Mycorrhizae were found in both soil treatments and temperature regime, however the highest counts of hyphae and vesicles of mycorrhizae were found at 30 °C on non-autoclaved soil, which confirmed the presence of mycorrhizae in *T. maritima*. Mycorrhizae were also confirmed in *T. maritima* collected from the field. To our knowledge, this is the first report of mycorrhizae in *T. maritima*. Temperature and soil treatment affected root and leaf biomass, and a significant interaction of soil and temperature was found for total biomass of *T. maritima*. The highest root and leaf biomass were found at 20 and 30 °C compared to 40 °C. Temperature significantly affected uptake of the majority of elements (Ca, P, S, Na, Mg, B, Mn, Mo, Cu, Zn, Se, Ag, Ba, Hg, Li, Sr, and Ti) in *T. maritima*. Against our expectations, the majority of element contents were

lower at 40 °C compared to 20 and 30 °C, which could be due to reduced physiological and metabolic activities in the plants. Temperature also affected the translocation of several elements from roots to leaves. Higher contents of P, B, Cr, and Cs, were found in roots at 20 and 30 °C, compared to 40 °C. In contrast, the contents of these elements were higher in leaves compared to roots at 40 °C. Lower contents of K, Na, Mo, Li, Rb, and Sr were found in the leaves at 20 and 30 °C, compared to 40 °C however contents of these elements were always higher in leaves compared to roots in all temperature regimes.

4.2. Introduction

Abiotic factors, including temperature, salinity, pH, clay content, cation exchange capacity, oxide mineral content, organic matter content, nutrient balance and concentrations of elements in the soil affect element mobility in soils and their bioavailability to plants (Goldberg, 1997; Kabata-Pendias, 2001). However, little is known about the effects of temperature on element content in plant tissues.

Biotic factors affect element mobility and accumulation in plants as well, and include plant species (Jacob and Otte, 2003; Marseille et. al., 2000; Quan et al., 2007) and mycorrhizal fungi. The latter play an important role in nutrient cycling (Martin, 2001). Mycorrhizal fungi are probably the most abundant and widespread fungi in soil. Olsson et al. (1999) estimated that these fungi account for 5 - 50% of the total biomass of soil microorganisms. The role of associations between mycorrhizal fungi and plants (known as mycorrhizae) in plant nutrient cycling has been well documented for many plants, including agricultural crops (Lambert et al., 1979; Marschner and Dell, 1994). Furthermore, mycorrhizae have been shown to

be beneficial for both plant and fungus under biotic and abiotic stresses (Bradley et al., 1981; Jones and Hutchinson, 1986; Clark et al., 1999; Redman et al. 2002; Márquez et al. 2007; Chen and Zhao, 2009). For example, mycorrhizae have been shown to reduce metal toxicity in plants (Jones and Hutchinson, 1986; Hegg and Angle, 1990), regulate the mobility of elements in plant organs at different stages of plant development (Pongrac et al., 2007), and improve the micro-environments of rhizosphere soils resulting in increased nutrient availability and uptake in plants (Chen and Zhao, 2009).

Metal contents in plants are affected by mycorrhizae (Jones and Hutchinson, 1986; Hegg and Angle, 1990; Pongrac et al., 2007; Chen and Zhao, 2009). Many studies indicate that mycorrhizae result in higher tolerance to metals (Christie et al. 2004; Bradley et al. 1981; Carvalho et al., 2006; Andrade et al. 2010; Janoušková and Pavlíková, 2010; Klugh-Stewart and Cummings, 2009). The effects of mycorrhizae on metal tolerance in plants have been primarily researched for Al, Cd, Cr, Fe, Mn, Ni, and Zn. These studies show that higher tolerance to metals is related to their distribution in plant tissues. In the presence of mycorrhizae, higher metal contents are frequently reported in roots, while their translocation to shoots is limited (Bradley et al., 1981; Andrade et al., 2010; Bissonnette et al., 2010).

Fungal plant association in plants has been reported in also to be involved in heat tolerances. *Dichanthelium lanuginosum* is widely distributed at temperatures of above 40 °C in hot springs of Yellowstone National Park (Stout and Al-Niemi, 2002). The thermotolerance in *D. lanuginosum* is associated with a plant fungal symbiosis (Redman et al., 2002). A fungus of the genus *Curvularia* is symbiotic

with *D. lanuginosum*, and helps to tolerate soil temperatures of 50 °C to 65 °C compared to non-symbiotic plants. Not only had that, but Márquez et al. (2007) reported that the thermotolerance is generated by a mutualistic interaction of a virus with the fungus. Only *Curvularia protuberate* infected with the virus imparts heat tolerance in *D. lanuginosum,* which therefore is a three-way symbiosis between fungus, virus and a plant. Other plants adapted to the 'chemical soup' of hot spring environments have hardly been studied for multi-element uptake. In addition to that there are no studies which have investigated the role of temperature and mycorrhizal association in element uptake in *Triglochin maritima.*

T. maritima is adapted to a wide range of saline/alkaline habitats such as salt marshes, inland saline depressions, and hot springs (Davy and Bishop, 1991; Channing, 2009). Fungal association with angiosperms is widespread, but *T. maritima*-mycorrhizae have not been reported (Mason, 1928; Davy and Bishop, 1991). In a field survey of *T. maritima* in wetlands influenced by hot springs in Yellowstone National Park (YNP) in 2007, it was found that *T. maritima* grew at temperatures greater than 40 °C (Chapter 2). However, in an initial experiment to investigate the effects of temperature on multi-element content of *T. maritima* using hydroponic culture, it was found that *T. maritima* did not survive at 40 °C under greenhouse conditions. These observations and previous reports on involvement of fungi in heat tolerance in *Dicanthelium lanuginosum* (Redman et al., 2002; Márquez et al., 2007) led to the questions (1) if there is any fungal association with *T. maritima*, and, if so, (2) does this association aid in the survival of *T. maritima* growing in soils with elevated temperature?

There are many possible reasons explaining why *T. maritima* did not survive at 40 °C in hydroponic culture, including the need for the plants to grow in association with micro-organisms, and therefore in a solid medium, in order to tolerate relatively high temperatures. Therefore, the experiment reported here used soil and was designed to examine how temperature and mycorrhizal association affect multi-element uptake in *T. maritima*.

It was expected that mycorrhizae in *T. maritima* would enhance survival at high temperatures. It was further hypothesized that high temperatures in combination with mycorrhizae would increase element contents in *T. maritima* tissue compared to low temperatures and/or low rates of mycorrhizal association.

4.3. Materials and Methods

4.3.1. Seed sources and germination

Seeds of *T. maritima* were collected from Rabbit Creek, Yellowstone National Park (N 44° 31.341, W 110° 48.783), in 2007, surface-sterilized with 5% sodium hypochlorite for one minute, and rinsed three times for one minute with sterile distilled water to prevent fungus attack during germination. To break dormancy, seeds were kept moist in sterile distilled water, and maintained at 4 °C for 6 weeks in petri dishes containing Whatman ® 1 filter paper. The seeds were then transferred to new petri dishes containing filter paper, and germinated in a growth chamber for 12 days. The temperature was set to 25/5 °C day/night, the photoperiod was set to 12 h day/night with light intensity of 3.87 log LUM m^{-2}. Germinated seeds were transferred to sterilized sand (autoclaved at 121°C, 15 psi for 2 hrs) and watered (distilled water) as needed.

4.3.2. Stock plant growth conditions

Seedlings were grown in sterile sand culture for four weeks in the greenhouse in 2008. Three seedlings were uprooted carefully, cleaned with distilled water, and transplanted to individual plastic pots (731.3 ml) containing sterile sand. The average temperature of the greenhouse was 25.5 °C with 1.24 log LUM m^{-2}light intensity. From two weeks transplanting, each individual plant received 5 ml of nutrient solution once a week. Nutrient solution was modified from Harmens et al. (1993):KNO$_3$ KNO$_3$ (1.5 mM), NH$_4$H$_2$PO$_4$ (0.5 mM), MgSO$_4$.7H$_2$O (0.25 mM), Ca(NO$_3$)$_2$.4H$_2$O (1 mM), TES (1 mM) NaCl (1%), H$_3$BO$_3$ (25 µM), MnSO$_4$.H$_2$O (2 µM), ZnSO$_4$.7H$_2$O (2 µM), CuSO$_4$.5H$_2$O (0.5 µM), (NH$_4$)6Mo$_7$O$_{24}$.4H$_2$O (0.5 µM), CoSO$_4$.7H$_2$O (0.05 µM), FeEDDHA (10 µM). The pH of the solution was adjusted to approximately 7.5 with KOH. This relatively high pH more closely reflected the neutral to alkaline growth conditions of the natural habitat of *T. maritima*.

4.3.3. Soil preparation

Soils were collected from two sites from natural habitats of *T. maritima*, that were included in the field studies (Chapter 2): 'Landfill'(Grand Forks, North Dakota, N 47° 56' 29.1, W 97° 07' 53.8, elevation 261 m) and Rabbit Creek (Yellowstone National Park, Wyoming, N 47° 56' 29.1, W 97° 07' 53.8, elevation 261 m). The soils were kept in the greenhouse for 3 - 4 days to drain all the excess moisture and plant debris and roots were removed, then the soils were packed in plastic ziploc bags and stored in a fridge at 4 °C until further use. In YNP, the soils were collected from nearby hot springs because it was possible that mycorrhizae of *T.*

maritima from hot spring environments could be very specific to that environment. To increase the porosity of the soil, we added sand to the soil (3 parts soil: 1 part play sand (Quikrete Premium Play Sand)), because the soils collected from Landfill were rich in clay. Individual plants were grown in 480 g of soils in greenhouse in plastic pots (563 ml). To prepare the medium, 290 g of soil from Landfill and 120 g of play sand were mixed thoroughly for each pot. All 48 soil mixtures were individually wrapped in aluminum foil and autoclaved at 121 °C for 2 hrs. Then 70 g of soil from Rabbit Creek was added to each of the 48 mixtures, to get a final mixture of 480 g soil per pot. The purpose of this was to ensure that mycorrhizae from the hot spring environment, if present at all, were inoculated in the soil medium for the experiment. Then half of the soil mixtures (n = 24) were again autoclaved, the other half was not. Each individually wrapped soil was transferred to a plastic pot (563 ml) and flooded with distilled water overnight to enhance soil moisture content before transplanting the *T. maritima*.

Studies have shown that autoclaving soils have an effect on soil physical and chemical properties (Alphei and Scheu, 1993; Bank et al., 2008; Miransari et al., 2009). To minimize this effect, Alphei and Scheu, (1993) suggested to re-inoculating autoclaved soils as a non-sterile treatment, which may reduce the effect of autoclaving. Therefore, in current study, the effects of autoclaved soil were minimized as much as possible as by using the major proportion (410 g) of autoclaved soils in both treatments, and then re-inoculating the 70 g to autoclaved soils for the purpose of non-autoclaved treatment.

4.3.4. Experiment set-up

In order to examine the effects of temperature and the role of soil biota (mycorrhizae) in element uptake by *T. maritima*, the plants were grown at three temperature regimes (20, 30, and 40 °C) and two soil conditions (inoculated non-autoclaved, or autoclaved) (Table 4.1). Stock plants, which were grown in sterilized sand, were uprooted, and washed thoroughly in distilled water. To reduce the effects of differences in growth, similar sized plants were used. The fresh weight and root and leaf length were recorded prior to assignment to an experimental unit and used as covariates in the data analysis. The experiment was conducted in a randomized complete block design with factorial arrangements of treatments during June - September, 2009. The main block (temperature) was replicated eight times (*n* = 8) and consisted of three cooler boxes filled with tap water. In each cooler box, temperatures were maintained at 20, 30, and 40 °C. Biotherm heaters 250 W (Won Brothers Inc., VA, U.S.A.) with PRO-HEAT-D 58 thermostat controllers (40 °C ± 1°C) were used to maintain the desired temperature regimes. Each main block contained two plastic pots of 950 ml. Plants were grown in 563 ml pots, which fitted tightly into the 950 ml plot. The holes in the bottom of the small 563 ml plastic pots containing the soils and plants were covered with nylon mesh of 2 mm mesh to prevent soil loss from pot. The larger 950 ml pots were closed at the bottom, so that, after these were filled with 500 ml solution and became equilibrated with the cooler water temperature, as described below, the smaller pots with the plants would be submerged while no contact existed with the water in the heating baths.

Table 4.1. Layout of experimental units with three levels of temperatures (20, 30, and 40 °C), two levels of soil treatments (autoclaved and non-autoclaved) and eight replicates.

Replicate	Experimental units		
# 1	30 °C Autoclaved Non-autoclaved	20 °C Non-autoclaved Autoclaved	40 °C Non-autoclaved Autoclaved
# 2	20 °C Autoclaved Non-autoclaved	40 °C Non-autoclaved Autoclaved	30 °C Non-autoclaved Autoclaved
# 3	30 °C Non-autoclaved Autoclaved	20 °C Autoclaved Non-autoclaved	40 °C Non-autoclaved Autoclaved
# 4	20 °C Non-autoclaved Autoclaved	40 °C Autoclaved Non-autoclaved	30 °C Non-autoclaved Autoclaved
# 5	40 °C Autoclaved Non-autoclaved	20 °C Non-autoclaved Autoclaved	30 °C Autoclaved Non-autoclaved
# 6	30 °C Non-autoclaved Autoclaved	40 °C Non-autoclaved Autoclaved	20 °C Autoclaved Non-autoclaved
# 7	20 °C Autoclaved Non-autoclaved	30 °C Autoclaved Non-autoclaved	40 °C Non-autoclaved Autoclaved
# 8	20 °C Autoclaved Non-autoclaved	40 °C Non-autoclaved Autoclaved	30 °C Non-autoclaved Autoclaved

At the beginning of the experiment, the temperatures were increased slowly, to allow the seedlings to acclimate. The temperatures were set to 20 °C for all the treatments for the first two days and then increased by 5 °C every two days until the temperatures appropriate to the treatment were reached. The temperatures were maintained at the final desired levels for next eight weeks. The solution in larger plastic pots was changed twice in a week with 500 ml nutrient solution and distilled water alternating, in order to avoid excess of nutrient supply if replaced twice a week. The pH of the nutrient solutions was maintained to approximately 7.5. The soil temperatures were measured by thermometer and recorded twice a

week to monitor temperature fluctuations. Plants were grown under 16 hour photoperiod and ambient greenhouse conditions (mean temperature 25.6°C, mean light intensity 1.24 log LUM m^{-2} (HOBO-LI [C] 1993 ONSET data logger).

4.3.5. Multi-element analyses

The plant samples were harvested, washed with distilled water, blotted to remove surface moisture, and plant height and fresh biomass recorded. The samples were then separated into roots and leaves, and oven-dried (60 °C) until constant weights were observed, then crushed and homogenized in liquid nitrogen using a mortar and pestle. Leaf and root samples were analyzed separately for total element concentrations (Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Ge, Hf, Hg, In, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Pd, Pt, Rb, Re, S, Sb, Sc, Se, Sn, Sr, Ta, Te, Th, Ti, Tl, U, V, W, Y, Zn, Zr) using Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) by ACME Analytical Laboratories, Ltd.(Aqua Regia digestion ICP-MS and 4-acid digestion ICP-ES Finish). Method detection limits in mg kg^{-1} are presented in appendix 2. Au, Be, Bi, Hf, In, Nb, Pd, Pt, Re, Sc, Ta, Te, Th and V concentrations were always below the detection limits of the instrument and will not be discussed further.

In the remainder of this chapter element concentrations will be presented in molar units following the biological system of the elements (BSE) of Markert (1994). In the BSE, elements are grouped in a fashion similar to the Periodic System of the Elements, but according to correlations between and among the elements, their possible physiological and biological roles, and their toxicity in organisms. According to Markert (1994), structural elements (C, H, O, N, P, S, Si,

and Ca) are important in the function and structure of cell metabolism, electrolytic elements (K, Mg, and Na) are vital in maintaining osmotic conditions in cell metabolism, while enzymatic elements (V, Cr, Mo, Mn, Fe, Co, Ni, Cu, Zn, B, Sn, and Se) enhance the catalytic functions in cell metabolisms, whereas main / sub group elements (Li, Rb, Cs, Be, Sr, Ba, Al, Ge, Pb, As, Sb, Y, Ti, Zr, W, Ag, Au, Cd, and Hg) including the lanthanoids / actinoids group (La, Ce, and U) have no known biological functions.

4.3.6. Preparation of roots for staining and mycorrhizal fungi counts

Of the *T. maritima* roots collected from hot spring influenced wetlands in 2008, a few rootlets were examined for the presence of mycorrhizae using Trypan blue staining method modified from Grace and Stribley (1991). Similarly, roots of greenhouse experiments were examined for presence of mycorrhizae using same Trypan blue staining method. Approximately 1.3 g (fresh weight) of washed root samples was separated for mycorrhizal counts (hyphae colonization and number of vesicles). Washed roots from each pot were randomly selected and cut into pieces of approximately 2 cm length. Root samples were cleared in 10% KOH solution for 10 minutes at 94 -100 °C, immediately soaked in 2% (v/v) fresh HCl for 5 minutes, transferred into Trypan blue (0.5 g L^{-1} deionized water for 25 - 30 minutes, and then rinsed in running water for 10 minutes. For each treatment, eight stained root pieces of 2 cm were observed under a light microscope (Olympus) at 100x magnification. The colonization of fungus was estimated by using the modified intersection method of McGonigle et al. (1990). The roots were aligned parallel to the long axis of the slide and the eyepiece was rotated to

ensure that the roots were perpendicular to the vertical crosshair (Fig. 4.1). The number of vesicles was within the field of view was counted, as well as the number of fungal hypha crossing each intersection at 100x. For each sample, eight pieces of root were scored and the values averaged. All observations of fungal colonization were done in a random order.

4.3.7. Statistical analysis

Data on element concentrations, element content, fresh weight (FW), dry weight (DW), number of leaves, and leaf length were log_{10} transformed to obtain normality and homogeneity of variance. Statistical analysis was carried out using GLM procedures in MINITAB (Minitab[®] 15 ©2006 Minitab Inc.). To examine the effects of initial fresh weight and plant height on total biomass yield, fresh weight and plant height of stock plants were used as covariates in the analysis. It was found that the covariates were non-significant at $P < 0.05$ level and therefore data were further analyzed by two way ANOVA ($P < 0.05$) without covariates. Two-way ANOVA for element concentrations ($\mu mol\ g^{-1}$) in roots and leaves were first performed with root and leaf dry weight biomass as covariates. For those element concentrations where the covariates were non-significant at $P < 0.05$, data were further analyzed by two way analysis of variance (ANOVA, $P < 0.05$) without covariate.

Element concentrations in plants do not give good measures of element uptake. Generally, small plants have higher concentrations of elements than large plants (Ernst, 1995), because the differences in biomass affect concentrations of elements due to dilution effects. Therefore, element contents, rather than

concentrations, in root and leaf (μmol root^{-1} or leaf^{-1}) were obtained by multiplying the concentrations in roots and leaf by their respective dry weight biomass. The element contents were subjected to further two way analysis of variance (ANOVA, $P < 0.05$). To examine the uptake and translocations of elements in leaf tissues of *T. maritima*, ratios of leaf element content to the sum of element contents in roots and leaves content were also calculated. The ratios were arcsine transformed for two way analysis of variance (ANOVA, $P < 0.05$).
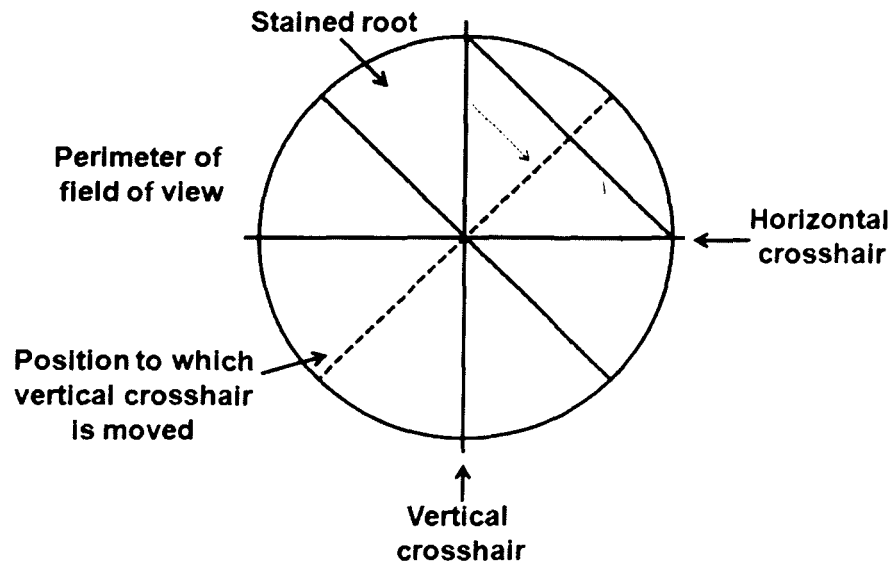
Figure 4.1. Diagram of the magnified intersection of a piece of root. The ocular lens is then turned so that the root piece is aligned parallel to the horizontal crosshair of the glass slide and perpendicular to the vertical crosshair of eyepiece (McGonigle et al., 1990).

The hypha colonization and vesicle count data were root-squared transformed before performing two-way ANOVA. Pearson correlation coefficients and P-values were calculated for element content in leaves using Minitab.

Correlation coefficients (r) $\geq$ 0.700, representing more than 50% variation were deemed important enough to be discussed further.

4.4. Results

4.4.1. Fungal association in *T. maritima*

Mycorrhizae were observed in roots of *T. maritima* collected from Elk Park and Rabbit Creek in Yellowstone National Park during 2008 (Fig. 4.2a). The presence of mycorrhizae in *T. maritima* roots was also verified in the greenhouse experiment (Fig. 4.2b). Hyphal colonization was found in both non-autoclaved and autoclaved soil treatments at 20, 30, and 40 °C. All samples showed the presence of an aseptate fungus. The hyphal colonizations and vesicles were clearly visible in root tissues of *T. maritima* after staining by Trypan blue. Temperature (P = 0.027) and soil treatment (P = 0.046) significantly affected hyphal counts whereas vesicle counts were not significantly different at $P < 0.05$. The hyphal count was 1.5 - 2 times higher in non-autoclaved soil treatment compared to autoclaved soils in 20 and 40 °C. At 30 °C, the hyphal count was 8.7 which was significantly (P = 0.021) higher than observed at 40 °C (4.6), but not compared to the value of 5.6 observed at 20 °C (Table 4.2). The interaction effect of temperature-by-soil treatment was not significant. Therefore, the presence of hypha and vesicle in both autoclave and non-autoclave soil treatments did not allow to discern the effect of mycorrhizae in element uptake and other parameters; hence the effect of mycorrhizae will not be discussed further.
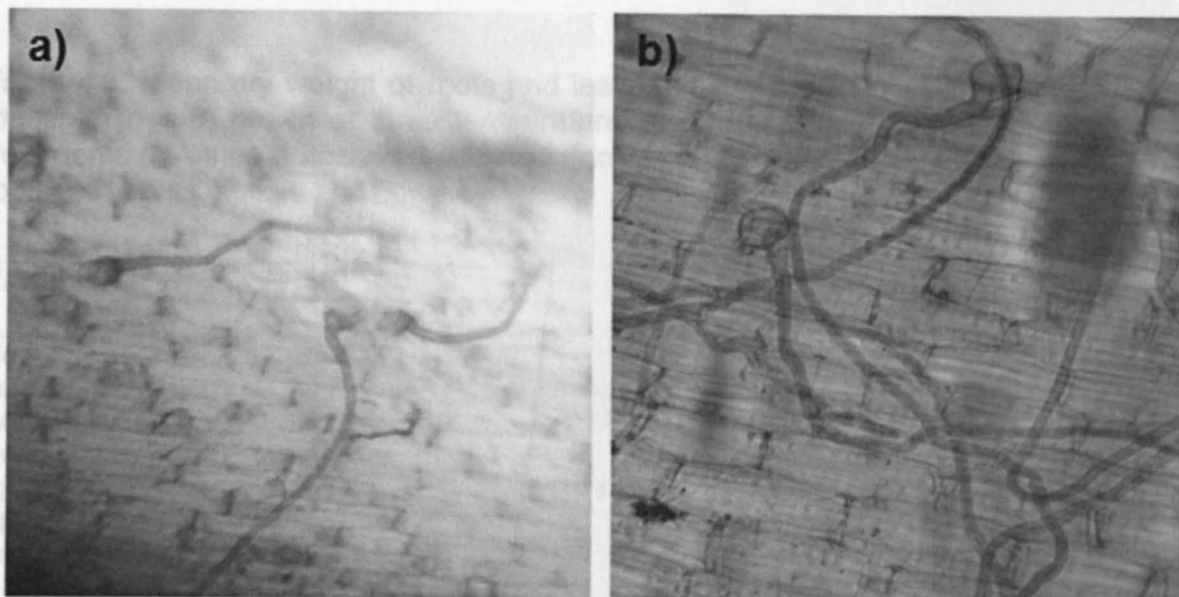
Figure 4.2. Arbuscular mycorrhizae (hypha and vesicles) in *T. maritima* roots after Trypan blue staining; a) collected from the field, Yellowstone National Park, 2008; b) in the greenhouse experiment in 2009.

Table 4.2. Mean counts of hypha and vesicles in temperature treatments (20, 30, and 40 °C) and the soil treatments (non-autoclaved and autoclaved) in *T. maritima* (mean ± standard deviation; $n = 8$).

Fungal colonization	20 °C		30 °C		40 °C	
	Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved
Hypha	5.8±2	5.2±1.5	8.7±2.9	4.8±1.7	4.6±3.2	4.2±2.6
Vesicles	2.7±1.2	2±0.7	3.1±1.5	2.3±1	1.8±1.1	2±1

4.4.2. Effects of temperature and soil biota on plant biomass

The total numbers of leaves and leaf biomass were generally higher at 30 °C. Similarly, root biomass and total biomass were higher at 20 and 30 °C, whereas higher dead leaf biomass was found at 40 °C (Table 4.3). In general, higher root, leaf and total biomass, and longer leaf lengths were found in the autoclaved compared to the non-autoclaved soil (Table 4.3).

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Table 4.3. Mean dry weight of roots and leaves biomass and shoot counts of *T. maritima* (n = 8) grown at three temperatures (20, 30, and 40 °C) and two soil treatments (NA=non-autoclaved and A=autoclaved). †Total biomass includes both roots, all leaves, spikes and flowers)

Variables	20 °C		30 °C		40 °C	
	NA	**A**	**NA**	**A**	**NA**	**A**
No. of Spikes	2.4±0.5	2.5±0.8	2.4±0.9	2.4±1.1	2.6±1.1	2.5±0.8
Leaf length (cm)	24.8±2.2	26.5±0.7	24.9±1.4	27.1±1.8	25.5±2.5	26.1±1.7
No. of leaves	35.5±5.2	37.7±5.5	39.1±7.6	42.9±5.5	23.3±3.9	23.9±7.6
Dead leaf biomass (g)	0.3±0.1	0.3±0.1	0.3±0.1	0.4±0.1	0.5±0.2	0.6±0.2
Spike biomass (g)	0.9±0.3	1.1±0.3	1.0±0.5	1.4±0.5	1.4±0.5	1.1±0.4
Leaf biomass (g)	3.2±0.3	3.4±0.8	3.4±0.4	4.3±0.3	2.9±0.5	3.1±0.8
Root biomass (g)	4.7±0.6	5.4±0.6	3.7±0.5	4.7±0.8	1.6±0.3	1.9±0.6
Total biomass†(g)	9.1±0.8	10.3±1.4	8.4±0.6	10.7±1.2	6.5±0.7	6.7±1.0

The effects of temperature, soil treatments and their interactions on plant biomass are presented in Table 4.4. The weight of dead leaves (dry) was always higher in 40 followed by 30 and 20 °C (Table 4.3).The temperature x soil interaction was significant for total biomass only, indicating the effect of temperature was not consistent for non-autoclaved and autoclaved treatments (Table 4.4).

Table 4.4. Significance of differences (probability) for growth related parameters of *T. maritima* at three levels of temperature (20, 30, and 40 °C) and two soil treatments (non-autoclaved and autoclaved), two-way ANOVA (ns = not significant at probability P < 0.05, n = 8).

Variables	Temperature (T)	Soil treatment (ST)	Interaction (T x ST)
No. of Spikes	ns	ns	ns
Leaf length	ns	0.007	ns
No. of leaves	0.000	ns	ns
Dead leaf biomass	0.000	ns	ns
Spike biomass	ns	ns	ns
Leaf biomass	0.001	0.008	ns
Root biomass	0.000	0.000	ns
Total biomass	0.000	0.000	0.017

4.4.3. Element concentrations in *T. maritima*

The mean element concentrations in roots and leaves of *T. maritima* in different temperatures and soil treatments are shown in Tables 4.5 and 4.6, and the results of the statistical analysis in Table 4.7.

Table 4.5. Mean element concentrations in roots (µmol or nmol g^{-1} dry biomass) at three temperature levels (20, 30, and 40 °C), and two soil treatments (non-autoclaved and autoclaved) in *T. maritima* (mean ± standard deviation, n = 8).

Element	Unit	20 °C		30 °C		40 °C	
		Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved	Non-Autoclaved	Autoclaved
Dry Wt.	g	4.7±0.5	5.4±0.6	3.7±0.5	4.7±0.8	1.6±0.3	1 9±0.6
Structural							
Ca	µmol g^{-1}	129±15	143±26	154±22	167±34	223±99	222±81
P	µmol g^{-1}	86±6.1	86±6.7	112±14	109±10	76±12	73±8.6
S	µmol g^{-1}	35±2.8	33±3.2	44±3.9	39±4.8	66±8.9	62±10
Electrolytic							
K	µmol g^{-1}	223±24	222±24	308±32	302±41	315±132	307±105
Na	µmol g^{-1}	331±89	284±57	332±34	350±57	248±67	286±65
Mg	µmol g^{-1}	91±11	96±7.9	98±7.5	106±22	134±7.1	165±47
Enzymatic							
Cr	µmol g^{-1}	0.05±0.01	0.05±0.01	0.05±0.02	0.06±0 02	0.07±0.04	0 09±0.04
Mo	µmol g^{-1}	0.07±0.02	0.08±0.01	0.1±0.01	0.09±0.02	0.09±0.01	0.07±0.02
Mn	µmol g^{-1}	6.1±0.8	5.4±0.9	5.5±0.6	4.9±1.2	6.7±2.4	5±0.8
Fe	µmol g^{-1}	35±7.5	25±8.4	62±13	35±10	119±72	61±23
Co	µmol g^{-1}	0.1±0.01	0.08±0.02	0.09±0.01	0.08±0.02	0.08±0.01	0.09±0.02
Ni	µmol g^{-1}	0.08±0.01	0.07±0.02	0.09±0.02	0.08±0.02	0.12±0.05	0.12±0.04
Cu	µmol g^{-1}	0.33±0.04	0.4±0.11	0.41±0.04	0.46±0.06	0.41±0.12	0.50±0.12
Zn	µmol g^{-1}	0.57±0.09	0.69±0.17	0.76±0 12	0.86±0.16	0.83±0.12	0.84±0.13
B	µmol g^{-1}	3.3±0.4	2.7±0.2	3.4±0.3	2.9±0.3	4.6±0.9	4.1±1.1
Sn	µmol g^{-1}	0.04±0.01	0.03±0.02	0.03±0.02	0.03±0.01	0.03±0.03	0.04±0.02
Se	µmol g^{-1}	0.01±0	0.01±0	0.01±0	0.02±0	0.02±0	0.03±0.01
Main/sub group							
Li	nmol g^{-1}	206±56	243±37	263±59	237±38	373±98	271±69
Rb	nmol g^{-1}	208±30	179±21	269±34	203±14	338±106	243±62
Cs	nmol g^{-1}	101±17	122±45	169±36	146±42	218±53	154±40
Sr	nmol g^{-1}	469±41	562±38	540±67	631±55	699±82	881±271

Table 4.5. Continued...

Table 4.5. Continued...

Element	Unit	20 °C		30 °C		40 °C	
		Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved	Non-Autoclaved	Autoclaved
Ba	nmol g^{-1}	266±73	498±86	309±97	476±76	566±281	1228±851
Al	nmol g^{-1}	14362±6183	9531±4038	17142±6330	12046±5328	21775±17483	17049±6348
Ge	nmol g^{-1}	20±4.8	21±6.5	20±6.5	27±21	37±22	33±16
Pb	nmol g^{-1}	18±5.1	20±2.6	21±5	24±3.2	29±5.9	28±9.2
As	nmol g^{-1}	397±127	483±118	558±168	933±398	1373±350	2185±1335
Sb	nmol g^{-1}	37±10	31±8.4	40±15	41±24	73±27	57±31
Y	nmol g^{-1}	5.3±2.4	3.8±0.8	6.9±2.4	5.6±2	11±5.9	7.2±3.8
Ti	nmol g^{-1}	441±46	384±54	520±57	460±31	405±51	404±137
Zr	nmol g^{-1}	6.1±3.5	5.1±0.8	4.2±1.7	3.6±1.5	5±5	4.5±2.8
W	nmol g^{-1}	13±3.1	18±4.3	14±4.8	32±38	25±8	30±17
Ag	nmol g^{-1}	1628±265	1139±259	1730±377	1129±140	1896±317	970±366
Au	nmol g^{-1}	44±59	10±6	15±10	7±2.3	29±12	7.4±1.9
Cd	nmol g^{-1}	6.1±1.3	3.7±0.6	7.5±1.8	4±0.6	8±1.5	3.9±0.5
Hg	nmol g^{-1}	86±17	54±10	90±20	70±17	145±20	101±38
La	nmol g^{-1}	4.3±1.8	3.4±0.7	6.8±2.9	5.2±2.2	9.1±5.4	6.1±2.9
Ce	nmol g^{-1}	9.3±3.7	7.4±1.3	14±5.4	11±4.5	18±10	12±5.6
U	nmol g^{-1}	2±0.6	1.7±0.2	2.3±0.6	1.8±0.5	3.5±0.8	2.5±1
Ga	nmol g^{-1}	22±13	49±30	29±11	48±30	40±25	44±45

The majority of element concentrations in the roots, except for P and Na, were higher at 40 °C followed by 30 and 20°C. Soil treatments also resulted in different element concentrations. In general, the concentrations of Fe, Al, Cd, Hg, Au, and Y were higher in roots of non-autoclaved compared to autoclaved soils at all temperature treatments. In contrast, Mg, Ba, Sr, As, and W were higher in autoclaved compared to non-autoclaved soils.

Temperatures and soil treatments significantly ($P < 0.05$) affected several elements in roots except Co, Ni, Sn, Ge, As, and W concentrations in roots. Temperature-by-soil treatment interaction effects on element concentrations were not significant in roots and leaves, except for Se and Zr in roots.

Table 4.6. Mean element concentrations in leaves (μmol or nmol g^{-1} dry biomass) at three temperature levels (20, 30, and 40 °C), and two soil treatments (non-autoclaved and autoclaved) in *T. maritima* (mean ± standard deviation, n = 8).

Element	Unit	20 °C		30 °C		40 °C	
		Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved
Dry Wt.	g	3.2±0.3	3.4±0.7	3.4±0.4	4.3±0.3	2.9±0.5	3.1±0.7
Structural							
Ca	μmol g^{-1}	318±35	352±25	341±22	348±24	193±27	214±30
P	μmol g^{-1}	66±4	69±5	77±5	78±7	57±6	62±4
S	μmol g^{-1}	85±12	85±8	95±11	92±8	64±13	64±9
Electrolytic							
K	μmol g^{-1}	600±121	606±84	551±66	528±88	608±106	567±112
Na	μmol g^{-1}	1623±133	2075±115	1855±171	2250±181	1783±212	2131±259
Mg	μmol g^{-1}	206±21	183±14	200±14	180±16	131±17	132±9
Enzymatic							
Cr	μmol g^{-1}	0.05±0.02	0.05±0.02	0.04±0.02	0.05±0.02	0.05±0.01	0.05±0.02
Mo	μmol g^{-1}	0.18±0.02	0.21±0.04	0.19±0.02	0.23±0.03	0.1±0.02	0.1±0.02
Mn	μmol g^{-1}	3.9±0.5	3.9±0.7	4.4±0.5	3.5±0.7	2.9±0.4	3±0.6
Fe	μmol g^{-1}	1.3±0.3	1±0.2	1.5±0.5	1.1±0.3	1.7±0.8	1.2±0.8
Ni	μmol g^{-1}	0.02±0.01	0.03±0.01	0.02±0.01	0.03±0.01	0.02±0.01	0.02±0.01
Cu	μmol g^{-1}	0.23±0.02	0.25±0.04	0.27±0.02	0.3±0.04	0.12±0.01	0.19±0.02
Zn	μmol g^{-1}	0.15±0.02	0.25±0.08	0.15±0.01	0.19±0.03	0.11±0.01	0.16±0.04
B	μmol g^{-1}	2.9±0.2	2.3±0.2	2.6±0.3	2.3±0.2	2.5±0.2	2.1±0.2
Main/sub group							
Se	nmol g^{-1}	9.5±1.7	4.9±0.8	10.6±1.3	5.5±1.3	11.2±2.2	5.5±1.1
Li	nmol g^{-1}	1899±216	1937±452	1931±406	1693±297	2002±359	1435±134
Rb	nmol g^{-1}	399±63	318±66	356±82	265±38	522±105	393±70
Cs	nmol g^{-1}	99±6.6	87±6.9	138±17	112±13	181±25	126±16
Sr	nmol g^{-1}	873±89	788±84	853±56	833±62	665±93	549±82
Ba	nmol g^{-1}	386±60	469±50	399±54	510±53	261±51	344±49
Pb	nmol g^{-1}	0.6±0.3	0.4±0.3	0.5±0.1	0.4±0.1	0.6±0.3	0.5±0.2
Y	nmol g^{-1}	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.1	0.2±0.2	0.2±0.1
Ti	nmol g^{-1}	196±10	146±12	217±32	159±15	175±28	125±11
Zr	nmol g^{-1}	0.5±0.1	0.7±0.3	0.5±0.2	0.5±0.2	1.3±1.9	0.6±0.3
Ag	nmol g^{-1}	545±129	566±170	483±127	460±90	375±55	232±66
Hg	nmol g^{-1}	29±10	29±10	30±10	28±15	21±6.2	27±11
La	nmol g^{-1}	0.2±0	0.3±0.1	0.2±0.1	0.2±0.2	0.2±0.2	0.2±0.1
Ce	nmol g^{-1}	0.3±0.1	0.5±0.2	0.3±0.2	0.4±0.3	0.4±0.4	0.4±0.2

Root dry weight was a significant covariate with the majority of root element concentrations, emphasizing the inverse relationship between biomass and concentrations, which suggest dilution played a role in determining plant concentrations.

Table 4.7. Significance of differences (probability) for element concentrations in root and leaf biomass at three temperature levels (20, 30, and 40 °C) and two soil treatments (non-autoclaved and autoclaved) in *T. maritima* as determined by two-way ANOVA (ns indicates non-significance; - = below the detection limits; $P <$ 0.05, n = 8; T x ST = Temperature-by-soil treatment interaction).

Element	Roots (Probability)				Leaves (Probability)			
	Covariate (Dry wt.)	Temperature (T)	Soil treatment (ST)	T x ST	Covariate (Dry wt.)	Temperature (T)	Soil treatment (ST)	T x ST
Structural								
Ca	ns	0.000	ns	ns	ns	0.000	0.019	ns
P	0.038	0.000	ns	ns	ns	0.000	0.045	ns
S	0.000	0.020	ns	ns	ns	0.000	ns	ns
Electrolytic								
K	0.036	0.001	ns	ns	ns	0.000	ns	ns
Na	ns	0.005	ns	ns	ns	0.000	0.000	ns
Mg	ns	0.000	ns	ns	ns	0.000	0.023	ns
Enzymatic								
B	0.000	0.013	ns	ns	ns	0.004	0.000	ns
Cr	0.012	ns	0.025	ns	0.010	ns	ns	ns
Mn	ns	ns	0.009	ns	ns	0.000	ns	ns
Fe	ns	0.000	0.000	ns	ns	ns	0.006	ns
Mo	ns	0.001	ns	ns	ns	0.000	0.003	ns
Ni	0.006	ns	ns	ns	0.035	ns	0.001	ns
Cu	0.000	0.001	0.000	ns	ns	0.000	0.000	ns
Zn	0.001	0.001	0.001	ns	ns	0.000	0.000	ns
Main/sub group								
Sn	0.001	ns	ns	ns	-	-	-	-
Se	0.001	ns	0.001	0.001	ns	ns	0.000	ns
Ag	0.018	ns	0.000	ns	ns	0.000	ns	ns
Al	ns	0.033	ns	ns	-	-	-	-
As	0.028	ns	ns	ns	-	-	-	-
Au	ns	ns	0.009	ns	-	-	-	-
Ba	0.002	ns	0.014	ns	ns	0.000	0.000	ns
Cd	ns	ns	0.000	ns	-	-	-	-

Table 4.7. Continued...

Table 4.7. Continued...

Element	Roots (Probability)				Leaves (Probability)			
	Covariate (Dry wt.)	Temperature (T)	Soil treatment (ST)	T x ST	Covariate (Dry wt.)	Temperature (T)	Soil treatment (ST)	T x ST
Ce	ns	0.01	0.037	ns	ns	ns	ns	ns
Co	ns	ns	ns	ns	ns	ns	0.008	ns
Cs	ns	0.000	ns	ns	ns	0.000	0.000	0.004
Ga	ns	ns	0.048	ns	-	-	-	-
Ge	0.030	ns	ns	ns	-	-	-	-
Hg	ns	0.000	0.000	ns	ns	ns	ns	ns
La	ns	0.009	ns	ns	0.044	ns	ns	ns
Li	ns	0.001	ns	ns	ns	ns	0.009	ns
Pb	ns	0.000	ns	ns	ns	ns	ns	ns
Rb	ns	0.000	0.000	ns	ns	0.000	0.000	ns
Sb	0.009	ns	0.024	ns	-	-	-	-
Sn	0.011	ns	ns	ns	-	-	-	-
Sr	0.001	ns	0.035	ns	ns	0.000	0.003	ns
Ti	ns	0.002	0.000	ns	ns	0.000	0.000	ns
U	ns	0.000	0.006	ns	-	-	-	-
W	ns	ns	ns	ns	-	-	-	-
Y	ns	0.004	0.039	ns	0.046	ns	ns	ns
Zr	0.001	0.001	0.001	0.017	ns	ns	ns	ns

Several elements (Sn, Al, Ge, As, Sb, W, Au, Cd, U, and Ga) that were present in detectable concentrations in the roots were below the detectable limits in the leaves. Temperature had a significant effect ($P < 0.05$) on the concentrations of the majority of elements in the leaves, except Fe, Co, Ni, Se, and Li. Similarly, soil treatments showed a significant ($P < 0.05$) effect on the majority of elements, except for S and Mn. The majority of element concentrations were higher at 30°C compared to 20 and 40 °C. Generally, the concentrations of elements in leaves were low at 40 °C, except for Rb and Cs. The concentrations of Fe, Se, Rb, Cs, Sr, Pb, and Ti were higher in leaves of plants grown on non-autoclaved soils compared to autoclaved in all temperature treatments.

Plant leaves of autoclaved soils however generally contained high concentrations of Na, Zn, and Ba in all temperature treatments. Dry leaf biomass was not significant covariate for the majority of elements, except for Cr, Ni, Y, and La. A temperature-by-soil treatment interaction was found significant ($P < 0.004$) only for Cs concentrations in leaves.

4.4.4. Element content in *T. maritima*

Differences in biomass can affect concentrations of elements due to dilution effects. In this experiment, the significant covariate of biomass with some element concentrations suggests this was the case. Therefore, element uptake in *T. maritima* was measured as the total amount of element per plant part. Mean element contents of roots and leaves are presented in tables 4.8 and 4.9, while the results of the statistical analysis are presented in Table 4.10.

Generally, element contents in roots were higher at 20 and 30 °C compared to 40 °C. The effect of temperature was significant ($P < 0.05$) for all element contents in roots, except for As. The mean element contents also showed significant differences between non-autoclaved and autoclaved soils for the majority of element contents, except for S, K, Mn, Fe, Co, Ni, Zn, Sn, Sr, Ge, Pb, Ag, As, Th, and Ga. Several element contents in roots were generally high in autoclaved soils in all temperatures. In contrasts, Fe, Sn, Ba, As, Co, and Ga contents were usually higher in non-autoclaved soils compared to autoclaved soils in all temperature treatments. The temperature-by-soil treatment interactions were not significant in roots.

Table 4.8. Mean element content in roots (µmol or nmol root^{-1} dry biomass) at three temperature levels (20, 30, and 40 °C), and two soil treatments (non-autoclaved and autoclaved) in *T. maritima* (mean ± standard deviation, *n* = 8).

Element	Unit	20 °C Non-autoclaved	20 °C Autoclaved	30 °C Non-autoclaved	30 °C Autoclaved	40 °C Non-autoclaved	40 °C Autoclaved
Structural							
Ca	µ mol	603±108	785±184	569±109	770±143	340±111	434±247
P	µ mol	402±56	467±44	410±57	509±57	120±15	137±53
S	µ mol	165±24	179±29	164±22	180±25	105±17	110±21
Electrolytic							
K	µ mol	1036±149	1208±178	1127±99	1394±81	537±281	614±361
Na	µ mol	1510±284	1565±455	1218±139	1621±227	415±166	531±185
Mg	µ mol	424±71	524±84	362±43	495±108	214±34	326±171
Enzymatic							
Cr	µ mol	0.25±0.05	0.26±0.06	0.18±0.06	0.28±0.05	0.11±0.07	0.17±0.06
Mo	µ mol	0.33±0.08	0.43±0.08	0.37±0.07	0.43±0.08	0.15±0.04	0.13±0.06
Mn	µ mol	28±2.1	29.5±5.8	20.4±3.4	22.5±5.7	10.4±2.7	9.4±3.4
Fe	µ mol	164±39	135±49	232±62	161±46	177±82	110±39
Co	µ mol	0.45±0.04	0.41±0.11	0.33±0.05	0.36±0.09	0.12±0.04	0.18±0.07
Ni	µ mol	0.37±0.08	0.37±0.09	0.35±0.07	0.38±0.06	0.18±0.06	0.22±0.07
Cu	µ mol	1.53±0.15	2.13±0.52	1.51±0.16	2.12±0.31	0.64±0.13	0.89±0.2
Zn	µ mol	2.67±0.55	3.7±0.78	2.79±0.35	3.94±0.54	1.32±0.29	1.52±0.32
B	µ mol	15.2±1.3	14.9±2.3	12.5±1.5	13.7±2.1	7.2±0.5	7.1±0.9
Main/sub group							
Sn	n mol	161±61	147±90	130±79	142±56	45±46	61±14
Se	n mol	48±6.5	68±13	47±12	77±16	25±5.8	50±9
Li	µ mol	1.13±0.2	1.13±0.37	0.88±0.21	1.22±0.26	0.43±0.1	0.73±0.37
Rb	n mol	835±148	1130±175	747±80	1246±145	405±153	651±330
Cs	n mol	584±264	548±116	546±196	784±162	240±32	425±206
Sr	µ mol	2.6±0.21	2.56±0.38	2.32±0.3	2.5±0.24	1.36±0.25	1.28±0.37
Ba	µ mol	2.31±0.45	1.44±0.39	1.77±0.42	1.41±0.33	1.8±0.96	0.96±0.28
Al	µ mol	46±22	79±36	45±21	81±34	26±6	47±43
Ge	n mol	97±32	109±29	97±74	95±34	52±24	60±30
Pb	n mol	93±12	98±31	89±17	98±25	43±12	55±18
As	µ mol	2.25±0.63	2.16±0.75	3.46±1.54	2.6±0.87	3.25±1.53	2.42±0.54
Sb	n mol	146±42	203±63	152±87	188±79	87±38	125±36
Y	n mol	18±4.4	29±14	21±8.8	32±11	11±4.9	22±16
Ti	µ mol	1.8±0.37	2.4±0.34	1.69±0.14	2.43±0.36	0.62±0.09	0.78±0.34
Zr	n mol	24±4.4	33±17	13±5.3	20±10	6.7±3.1	7.1±5
W	n mol	82±22	70±19	115±128	65±23	47±24	44±12
Ag	µ mol	5.29±1.27	8.87±1.77	4.2±0.95	7.95±1.41	1.48±0.36	3.46±0.9
Au	n mol	47±32	223±291	25±10	66±41	12±4.1	49±18

Table 4.8. Continued...

Table 4.8. Continued...

Element	Unit	20 °C		30 °C		40 °C	
		Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved
Cd	n mol	17±4	34±9.5	15±2.7	35±7.2	6.3±1.2	15±5.2
Hg	n mol	252±67	471±115	265±89	419±86	154±39	267±74
La	n mol	16±4	24±11	19±10	32±14	9.3±3.8	18.8±15
Ce	n mol	34±7	51±22	40±19	65±26	19±7	38±28
U	n mol	7.9±1.3	11±3.3	6.8±2.2	11±2.9	4±1.5	6.5±2.6
Ga	n mol	239±157	122±74	183±123	135±58	60±46	80±63

Significant ($P < 0.05$) effects of temperatures were observed for the majority of element contents of leaves, except for Ni, Rb, and Pb. Soil treatments showed significant effects ($P < 0.05$) on the majority of element contents, except S, Mg, Hg, and Se. The majority of leaf element contents were higher at 30 °C compared to 20 and 40 °C (Tables 4.9 and 4.10). Soil treatments resulted in differences in mean element contents in leaves. In general, the contents in the leaves for the majority of elements tended to be were higher in autoclaved soils compared to non-autoclaved in all temperature combinations. Only contents of Ba and As were higher in leaves from non-autoclaved soils compared to autoclaved in all temperature combinations. Temperature and soil treatments were significant factors ($P < 0.05$) for the majority of element contents in leaves, except for K, Cr, Fe, Ce, Co, La, Y, and Zr. The temperature-by-soil treatment interactions were significant ($P < 0.05$) for contents of B, Cs, and Li (Table 4.10).

Translocation of elements from roots to leaves was assessed by calculating the ratio of leaf element content relative to total element content (roots plus leaves). Analysis of variance on arcsine transformed data is presented on Table 4.11.

Table 4.9. Mean element content in leaves (µmol or nmol leaf^{-1} dry biomass) at three temperature levels (20, 30, and 40 °C), and two soil treatments (non-autoclaved and autoclaved) in *T. maritima* (mean ± standard deviation, n = 8).

Element	Unit	20 °C		30 °C		40 °C	
		Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved	Non-autoclaved	Autoclaved
Structural							
Ca	µ mol	1045±169	1206±274	1152±188	1482±118	566±137	659±156
P	µ mol	216±24	237±49	260±41	332±40	166±33	193±48
S	µ mol	278±38	294±84	323±70	389±21	191±68	200±56
Electrolytic							
K	µ mol	1948±320	2089±607	1837±232	2261±448	1765±398	1764±515
Na	µ mol	5325±684	7130±1702	6267±1175	9547±436	5243±1266	6538±1464
Mg	µ mol	675±97	620±93	674±113	768±82	379±67	409±95
Enzymatic							
Cr	µ mol	0.16±0.06	0.16±0.05	0.15±0.05	0.2±0.06	0.13±0.04	0.15±0.04
Mo	µ mol	0.58±0.07	0.73±0.23	0.64±0.12	0.97±0.11	0.29±0.08	0.32±0.1
Mn	µ mol	12.8±1.9	13±2.3	14.8±2.9	14.9±2.9	8.6±1.9	9.1±2.4
Fe	µ mol	4.3±0.9	3.3±0.8	5±1.9	4.7±1.5	4.9±2.2	4.1±3.8
Ni	µ mol	0.06±0.02	0.08±0.02	0.06±0.02	0.11±0.04	0.05±0.02	0.07±0.02
Cu	µ mol	0.74±0.11	0.85±0.14	0.91±0.18	1.28±0.19	0.35±0.06	0.59±0.17
Zn	µ mol	0.51±0.07	0.82±0.23	0.52±0.08	0.81±0.17	0.32±0.06	0.48±0.18
B	µ mol	9.3±1	7.9±1.9	8.7±0.8	9.9±1.1	7.3±1.2	6.3±1.2
Main/sub group							
Se	n mol	16±2.2	32±6.8	19±6	45±7.2	16±3.7	35±10
Li	n mol	6319±1396	6404±1015	5705±1340	8187±1602	4226±1023	6061±1164
Rb	n mol	1038±223	1374±383	892±169	1520±373	1142±270	1592±347
Cs	n mol	284±32	339±67	380±78	585±58	372±91	555±116
Sr	n mol	2588±391	2943±419	2817±509	3632±307	1605±375	2051±527
Ba	n mol	1543±246	1287±114	1732±362	1702±260	1000±209	812±266
Pb	n mol	1.4±0.9	1.9±0.9	1.5±0.5	2±0.4	1.3±0.5	1.9±1.1
Y	n mol	0.9±0.5	0.7±0.3	0.7±0.5	0.7±0.5	0.7±0.2	0.7±1
Ti	n mol	479±59	670±142	538±96	926±162	363±57	552±186
Zr	n mol	2.4±1.1	1.9±0.4	1.6±0.8	2±0.8	1.9±1.1	4.1±5.6
Ag	n mol	1860±610	1844±490	1578±473	2062±558	663±170	1175±364
Hg	n mol	96±34	97±42	95±54	128±47	76±28	66±25
La	n mol	0.9±0.4	0.6±0.2	0.7±0.6	0.7±0.4	0.6±0.3	0.8±0.9
Ce	n mol	1.8±0.7	1.1±0.3	1.3±1.0	1.1±0.8	1.3±0.5	1.4±1.7

Table 4.10. Significance of differences (probability) in root and leaf biomass element content at three temperature levels (20, 30, and 40 °C) and two soil treatments (non-autoclaved and autoclaved) in *T. maritima* as determined by two-way ANOVA (ns indicates non-significance; - = below the detection limits; $P < 0.05$, $n = 8$; T x ST = Temperature-by-soil treatment interaction).

Element	Root (Probability)			Leaf (Probability)		
	Temperature (T)	Soil treatment (ST)	T x ST	Temperature (T)	Soil treatment (ST)	T x ST
Structural						
Ca	0.001	0.035	ns	0.000	0.004	ns
P	0.000	0.046	ns	0.000	0.009	ns
S	0.002	ns	ns	0.000	ns	ns
Electrolytic						
K	0.000	ns	ns	ns	ns	ns
Na	0.000	0.047	ns	0.000	0.000	ns
Mg	0.001	0.024	ns	0.000	ns	ns
Enzymatic						
Cr	0.000	0.001	ns	ns	ns	ns
Mo	0.000	0.008	ns	0.000	0.000	ns
Mn	0.000	ns	ns	0.000	ns	ns
Co	0.000	ns	ns	ns	ns	ns
Fe	0.022	ns	ns	ns	ns	ns
Ni	0.000	ns	ns	ns	0.000	ns
Cu	0.000	0.000	ns	0.000	0.000	ns
Zn	0.000	0.000	ns	0.000	0.000	ns
B	0.000	ns	ns	0.000	ns	0.027
Main/sub group						
Sn	0.000	ns	ns	-	-	-
Se	0.000	0.000	ns	0.004	ns	ns
Li	0.000	0.003	ns	0.001	0.000	0.049
Rb	0.000	0.000	ns	ns	0.000	ns
Cs	0.000	0.004	ns	0.000	0.000	0.047
Sr	0.000	ns	ns	0.000	0.000	ns
Ba	0.002	0.028	ns	0.000	0.037	ns
Al	0.002	0.002	ns	-	-	-
Ge	0.001	ns	ns	-	-	-
Pb	0.000	ns	ns	ns	0.033	ns
As	ns	ns	ns	-	-	-
Sb	0.001	0.007	ns	-	-	-
Y	0.021	0.001	ns	ns	ns	ns
Ti	0.000	0.000	ns	0.000	0.000	ns
Zr	0.000	0.027	ns	ns	ns	ns

Table 4.10. continued...

Element	Root (Probability)			Leaf (Probability)		
	Temperature (T)	Soil treatment (ST)	T x ST	Temperature (T)	Soil treatment (ST)	T x ST
W	0.030	ns	ns	-	-	-
Ag	0.000	0.000	ns	0.000	0.008	ns
Au	0.031	0.011	ns	-	-	-
Cd	0.000	0.000	ns	-	-	-
Hg	0.000	0.000	ns	0.028	ns	ns
La	0.008	0.000	ns	ns	ns	ns
Ce	0.003	0.001	ns	ns	ns	ns
U	0.000	0.000	ns	-	-	-
Ga	0.002	ns	ns	-	-	-
Nb	0.005	0.004	ns	-	-	-

Analysis of variance on arcsine transformed data showed that neither temperature nor soil treatments significantly affected the ratios of Ca, S, Fe, Co, Zn, Ba, Ge, Pb, As, Sb, Y, La, and Ce, while a significant effect for temperature only was observed for most of the other elements (Table 4.11). The distribution of Mg was affected by soil treatment only, while Ag, and Hg ratios were significantly affected by both temperature and soil treatments (Table 4.11). The distribution of element contents in roots and leaves in which ratios were significantly affected (Table 4.11) by temperature and/or soil treatment and their interaction effect are presented in Fig 4.3 and 4.4.

The uptake of P, Na, Mn, B, Cu, Mo, Li, Sr, Ti, Se, and Cs at 40 °C were significantly decreased in both roots and leaves (Fig. 4.3 and Table 4.10) compared to 20 and 30 °C, but translocation of these elements from root to leaves was increased (Fig 4.3 and Table 4.11). Similarly, uptake of K, Cr, and Ni contents were significantly decreased in roots at 40 °C (Table 4.11) but the differences in leaves were not significant. Rb uptake in roots and translocation to the leaves

were significantly different (Fig. 4.3; Tables 4.10 and 4.11). The translocation of

Hg was affected by temperature and by soil treatment (Fig. 4.4a)

Table 4.11. Significance of differences (probability) in ratio of element contents in leaves / total element contents in leaves and roots at three levels of temperature (20, 30, and 40 °C) and two soil treatments (non-autoclaved and autoclaved) in *T. maritima* as determined by two-way ANOVA (ns indicates non-significance; - = below the detection limits; $P < 0.05$, $n = 8$; T x ST = Temperature-by-soil treatment interaction).

Elements	Temperature (T)	Soil treatment (ST)	T x S
Ca	ns	ns	ns
P	0.000	ns	ns
S	ns	ns	ns
K	0.000	ns	ns
Na	0.000	ns	ns
Mg	ns	0.017	ns
Cr	0.002	ns	ns
Mo	0.029	ns	ns
Mn	0.001	ns	ns
Fe	ns	ns	ns
Co	ns	ns	ns
Ni	0.026	ns	ns
Cu	0.039	ns	ns
Zn	ns	ns	ns
B	0.002	ns	ns
Se	0.007	ns	ns
Li	0.001	ns	ns
Rb	0.000	ns	ns
Cs	0.000	ns	ns
Sr	0.036	ns	ns
Ba	ns	ns	ns
Y	ns	ns	ns
Ti	0.000	ns	ns
Zr	0.001	ns	ns
Ag	0.023	0.021	ns
Hg	0.037	0.009	0.03
La	ns	ns	ns
Ce	ns	ns	ns
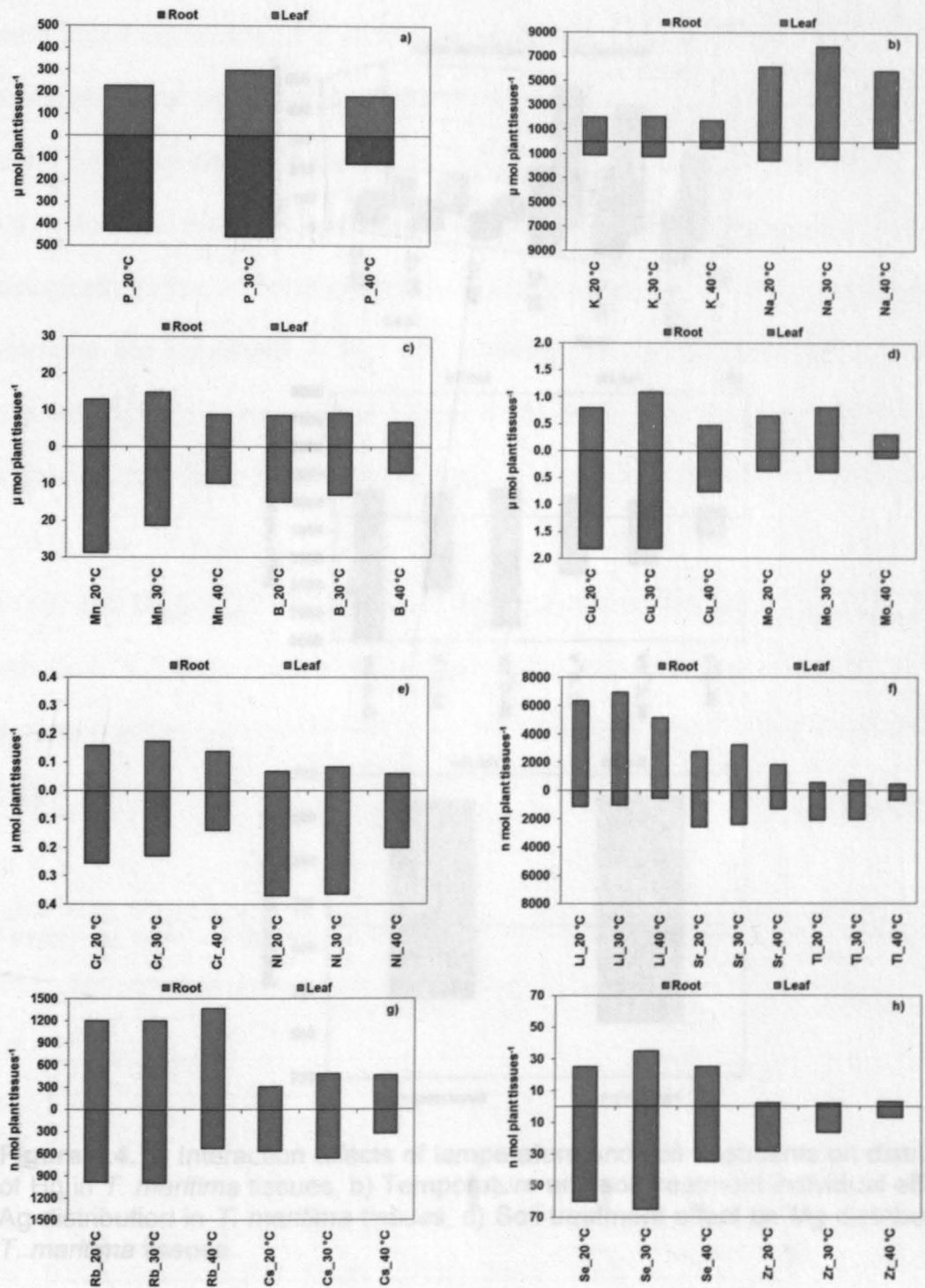
Figure 4.3. Effects of temperature on distribution of multi-element in *T. maritima* tissues at three temperature regimes, 20, 30, and 40 °C; a) P, b) K and Na, c) Mn and B, d) Cu and Mo, e) Cr and Ni, f) Li, Sr, and Ti, g) Rb and Cs, h) Se and Zr.
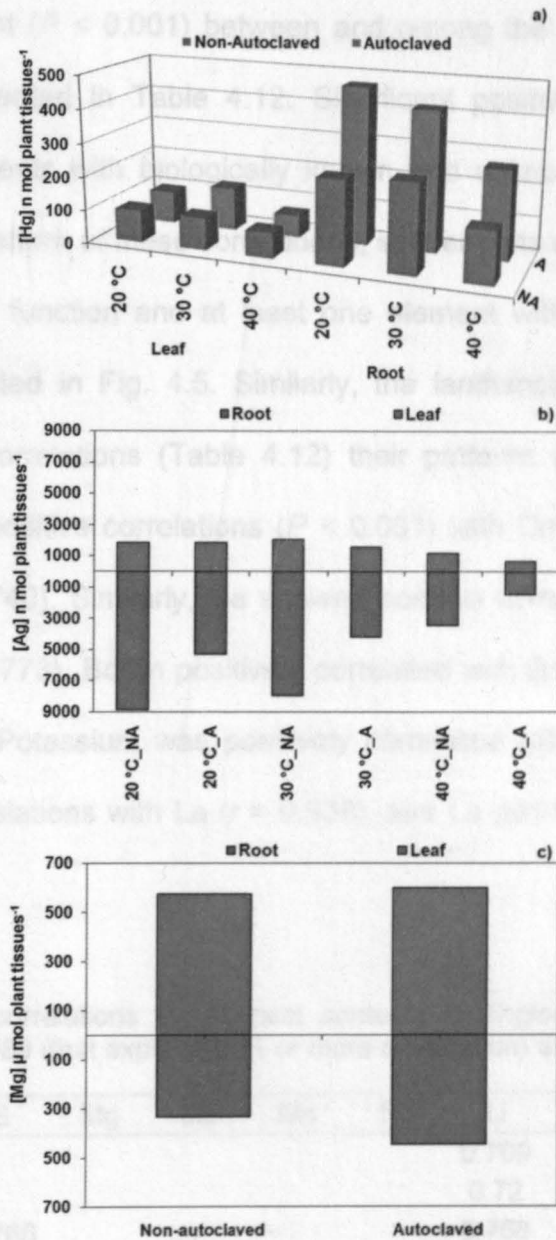
Figure 4.4. a) Interaction effects of temperature and soil treatments on distribution of Hg in *T. maritima* tissues, b) Temperature and soil treatment individual effect on Ag distribution in *T. maritima* tissues, c) Soil treatment effect on Mg distribution in *T. maritima* tissues.

4.4.5. Correlation analyses

The correlation analyses of element contents were performed to study the interaction effects of elements in *T. maritima* leaves. Only positive correlations

were found significant ($P < 0.001$) between and among the elements and these elements were presented in Table 4.12. Significant positive correlations were found between elements with biologically known and unknown functions (Table 4.12). To show the pattern of these correlations, scatter plots of each element with biologically unknown function and at least one element with biologically known functions are presented in Fig. 4.5. Similarly, the lanthanoids (La, Y, and Ce) showed significant correlations (Table 4.12) their patterns were also shown in figure 4.5. Iron has positive correlations ($P < 0.001$) with Ce ($r = 0.745$), La ($r = 0.761$), and Y($r = 0.740$). Similarly, Na showed positive correlations with Cs ($r = 0.749$) and Se ($r = 0.773$). Boron positively correlated with Ba ($r = 0.717$), and Ni with Cr ($r = 0.841$). Potassium was positively correlated with Rb ($r = 0.689$). Y showed positive correlations with La ($r = 0.938$), and La positively correlated with Ce ($r = 0.976$).

Table 4.12. Pearson correlations for element contents in *Triglochin maritima* leaves. Correlations with r ≥ 0.689 (that explain 47 % or more of variation) are shown ($P < 0.001$).

	Ca	P	S	Mg	Mo	Mn	Na	Li	Cu	Sr	Ti
P	0.866							0.709			
S	0.915	0.873						0.72			
Na	0.733	0.805	0.766					0.766			
Mg	0.942	0.873	0.863								
Mo	0.921	0.804	0.858	0.858							
Mn	0.898	0.739	0.831	0.878	0.764						
B	0.728	0.764	0.757	0.773		0.691					
Cu	0.903	0.889	0.839	0.885	0.862	0.815	0.749	0.710			
Sr	0.967	0.875	0.889	0.912	0.871	0.876	0.803	0.748	0.918		
Ba	0.85	0.742	0.808	0.889	0.75	0.86			0.758	0.834	
Ti	0.745	0.873	0.741		0.714		0.847	0.758	0.834	0.804	
Ag	0.799	0.703	0.705	0.815	0.727	0.722			0.843	0.809	
Zn	0.724				0.733				0.701	0.726	0.734
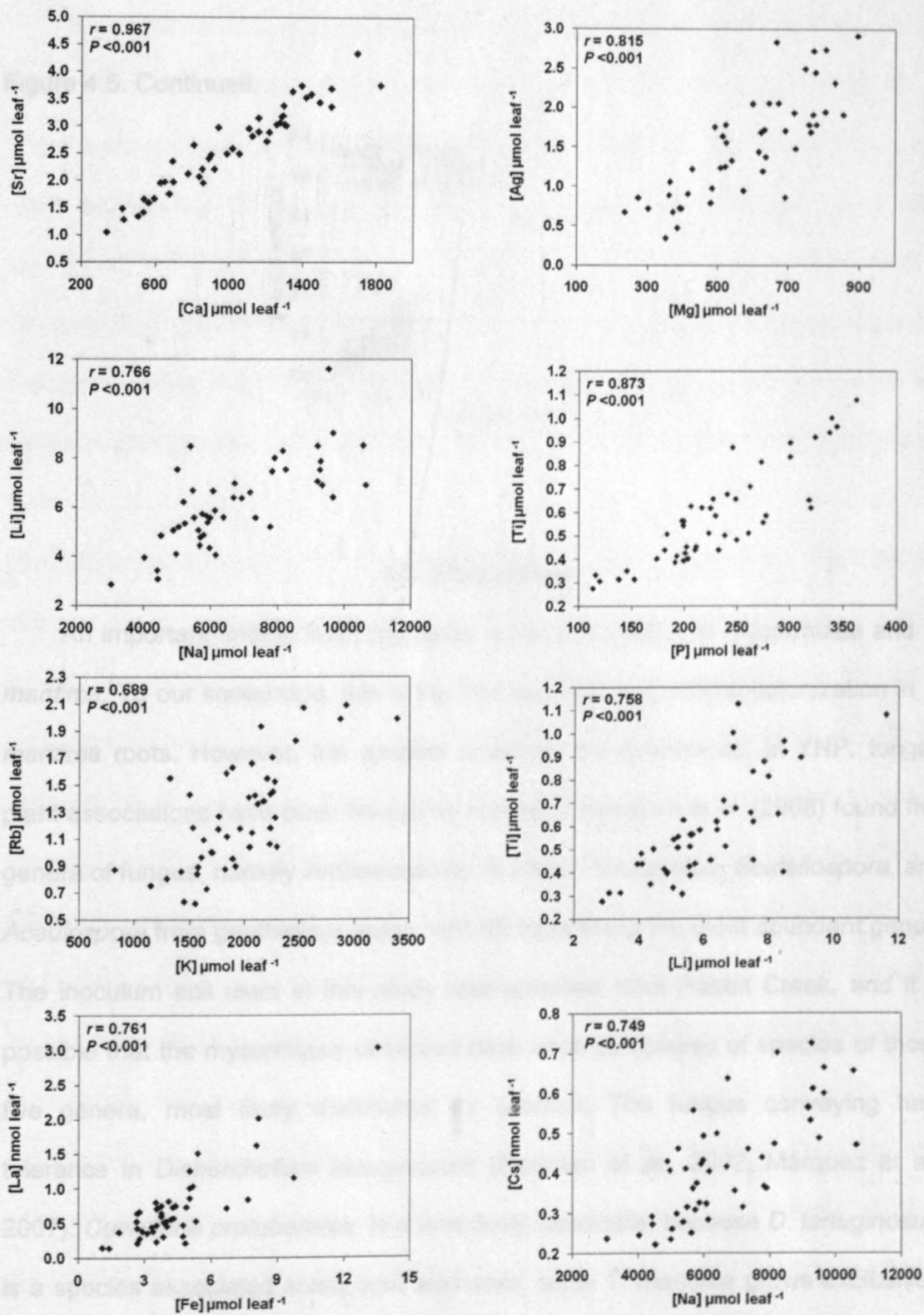
Figure 4.5. Correlations between the elements with known and no known biological functions' and between La and Y, and Ce in leaves of *T. maritima*.

Figure 4.5. Continued...
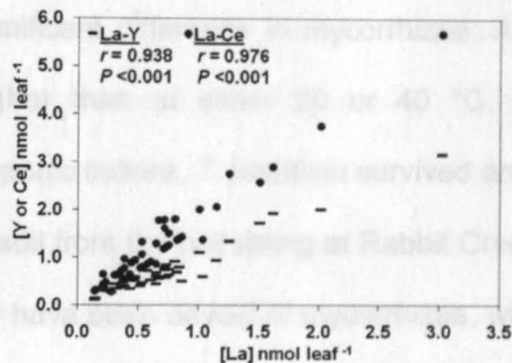
4.5. Discussion

An important finding from this study is the association of mycorrhizae and *T. maritima*. To our knowledge, this is the first report of mycorrhizal colonization in *T. maritima* roots. However, the species could not be determined. In YNP, fungal-plant associations have been frequently reported. Appoloni et al. (2008) found five genera of fungus, namely *Archaeospora, Glomus, Paraglomus, Scutellospora, and Acaulospora* from geothermal areas, with *Glomus* being the most abundant genus. The inoculum soil used in this study was obtained from Rabbit Creek, and it is possible that the mycorrhizae observed here were complexes of species of those five genera, most likely dominated by *Glomus*. The fungus conveying heat tolerance in *Dichanthelium lanuginosum* (Redman et al., 2002, Márquez et al., 2007), *Curvularia protuberates*, is a less likely candidate, because *D. lanuginosum* is a species associated solely with acid soils, while *T. maritima* grows exclusively in neutral to alkaline soils.

Temperature and soil treatments were significant in root and leaf biomass of *T. maritima*. The highest root and leaf biomass yield was found at 20 and 30 °C. There was also a significant difference in mycorrhizae. At 30 °C hyphal counts were significantly higher than at either 20 or 40 °C. In contrast to a pilot experiment with hydroponic culture, *T. maritima* survived and produced biomass at 40 °C when grown in soil from the hot spring at Rabbit Creek, YNP. In hydroponic culture the plants may have been devoid of mycorrhizae, which may be necessary for survival of this species, as it is for many other species (Jones and Hutchinson, 1986; Bradley et al., 1981; Clark et al., 1999; Redman et al. 2002; Márquez et al. 2007; Chen and Zhao, 2009). Decreased biomass at 40 °C may be explained by reduced physiological and metabolic activities at elevated temperatures. Treshow (1970) suggested that temperature has effects on overall growth and development, as well as the physiological and chemical reactions within the plants. Different plant species have different lower and upper limits of temperature in terms of growth and development. In this study at 40 °C, biomass production in roots and leaves as well as fungal colonization were the least, which suggests that 40 °C was near the higher temperatures of the normal range for *T. maritima* and its mycorrhizae. Similarly, higher biomass of roots and leaves were observed in autoclaved compared to non-autoclaved soils. Previous research has shown that autoclaving soil releases N and P (Alphei and Scheu, 1993), which are major and essential elements in plants growth, and so the availability of those and other nutrients to the plants may have been greater in autoclaved soil, leading to greater biomass production. Temperature had significant effects on element

concentrations and contents in *T. maritima*. In roots, temperature effect was significant except for As. Mean element contents were low in both roots and leaves at 40 °C compared to 20 and 30 °C. Both decreased biomass and element contents at 40 °C may be explained by reduced physiological and metabolic activities at elevated temperatures (Treshow, 1970). Turner and Lahav (1985) also reported that when growth of plants is reduced due to change in environmental conditions such as temperature, the absorption of elements in the plants will be reduced.

The uptake rate of elements and their partition in plants parts are affected by temperatures. Turner and Lahav (1985) reported that temperature effects on element uptake in different parts are element specific. Adebooye et al. (2010) studied the effect of temperature on element uptake in *Trichosanthes cucumerina* L. These authors reported that concentrations of Ca and P were higher at roots at 20 °C but the concentrations of these elements increased in leaves at 25 and 30 °C compared to 20 °C. Gadallah (1996) reported that temperature significantly affected element uptake in *Carthamus fincforius* with marked effects on K, Mg, P, and S in shoots, and P and S in roots. He showed that P content was increased in shoots, but there was decreased content in roots as temperature was raised from 15 to 35 °C. This effect was prominent in P when other stresses such as increased osmotic potential and exogenous ABA application were combined with temperature. Mengel, (2007) described that K ion in plants cycles from roots to leaves via xylem and from leaves to roots via phloem depending on the physiological demand.

In this study, the ratio of element content in leaves and roots, an indicator of translocation, showed marked differences at different levels of temperatures, which suggest that plants redistribute elements under different environmental conditions. The distributions of elements in roots and leaves changed from lower to higher temperature regimes. Both elements with biologically known (P, K, Na, Mn, B, Cu Mo, Cr, Ni, and Se) and unknown functions (Li, Sr, Rb, Ti, Cs and Zr) in plants were redistributed to either roots or leaves at increased temperatures. Among these elements, contents of Cu, Mn, Ni, Se, Zr, and Ti were higher in roots compared to leaves in all levels of temperatures. Cu, Mn and Ni are known for their physiological and biochemical roles. Cu is important in plants' biochemical functions such as photosynthesis, respiration, protein metabolism, and Cu also influences water permeability of xylem vessels (Kabata-Pendias, 2001). Manganese is important in formation of chlorophyll and carbon assimilation, and is involved in photosynthetic electron transport system in plants (Marschner, 1995; Humpries et al., 2007). Ni is essential in plants as a component of urease, an enzyme, which catalyzes the hydrolysis of urea into carbon dioxide and ammonia (Brown, 2007). Ti is considered beneficial to plants due to its positive effects on plants physiology (Lopez-Moreno et al., 1996; Carvajal and Alcaraz, 1998) and its increased overall performance including post-harvest quality traits (Alcarez-Lopez et al., 2003).

Plants remobilize elements during ontogenesis or morphogenesis during their life cycle (Marschner, 1995). It is unknown if *T. maritima* has similar mechanisms for remobilization of elements in elevated temperature. For example, P, B, Cr, and

Cs distribution changed according to elevated temperatures in *T. maritima*. At 20 and 30 °C, these elements were distributed into the roots in greater amounts. As temperature is further increased to 40 °C, the uptake and translocation of these elements changed, resulting in higher amounts in leaves compared to roots. These findings suggest that *T. maritima* regulates the distribution of elements upon different temperature regimes. In addition, the translocation of K, Na, Mo, Li, Rb, and Sr were higher at 40 °C compared to 20 and 30 °C. *T. maritima* is a halophytic plant (Cooper, 1982; Davy and Bishop, 1991; Piernik, 2003) and it accumulates higher amounts of K and Na in the leaves to maintain osmotic conditions and water potential (Albert, 1975; Unger, 1991). Our result is in agreement with Albert (1975), and indicates a higher requirement for these elements in leaves to maintain physiological activity. At higher temperatures, evapotranspiration is increased in plants compared to the lower temperature. Therefore, the elevated translocation of K, Na, Li, Sr, and Rb from roots to leaves could be a response to higher evapotranspiration at 40 °C. Several other reasons may explain the higher translocation of Li, Sr, Rb, and Cs in leaves at 40 °C, 1) these elements, being in the same column in the Periodic Table as Na and K, may mimic the pattern in osmoregulation (Kabata-Pendias, 2001), 2) they were simply unable to prevent uptake and translocation of these elements. Similarly, Sr and Ca compete with each other for uptake and translocation into the leaves (Kabata-Pendias, 2001), but in this study these two elements showed positive correlations.

Other elements may have shown strong correlations in terms of concentrations and contents for similar reasons. Particularly strong positive

correlations were found for La, Ce and Y, which were not yet known for their physiological functions, but, being lanthanoids, have very similar chemistry. Hu et al. (2002) found that *Triticum aestivum* (wheat) showed no selective uptake for La and Ce. He and Loh (2000) studied the effect of Ce and La on reproductive growth of *Arabidopsis thaliana* in controlled conditions, these authors reported that these two elements promoted the floral initiation and reproductive growth in *A. thaliana.* Similarly, Yajia et al. (2008) reported effectiveness of La on disease control caused by *Rhizoctonia solani* in *Isatis indigotica* Fort., *Festuca arundinucea* and in Oryza sativa (rice) in a pot culture experiment. The positive correlations of elements between biologically known and unknown functions indicate the possibility of synergistic effects among these elements.

For many elements showing significant correlations, uptake was also affected by temperature and soil treatments. For example Ca and P were positively correlated with Ti. The temperature and soil treatment effects in the uptake of these elements were similar, which indicated that when temperature is increased/decreased then plants will take up Ti together with Ca and/or P. However, only the temperature was significant in the translocation of these elements. Similar patterns were observed for other elements as well.

The effects of mycorrhizae were not discernable in autoclaved and non-autoclaved soils on the majority of element content because of the presence of fungal colonization in both soil treatments. The colonization of fungi in autoclaved soils suggests that growth of fungi re-germinated during the experiment. Trevors (1996) suggested that re-germination of fungus spores may occur after sterilization

of the soil. Fungi may also have grown from the plants themselves. Soil treatment was significant for several element concentrations and contents. However, S, K, Mg, Se, and Hg were not. The two way interaction effects of temperature and soil treatments on B, Li, and Cs content in leaves indicate the different behaviors of these elements in temperature and soil treatments. Similarly, temperature and soil treatments interaction effect on the Cs concentration of leaves and the ratio of Hg in leaves and roots indicate unpredictable uptake pattern with temperature and soil treatments. Another explanation may be that this is simply due to a Type II error, a false negative. The majority of element contents were affected by soil treatment only, with higher values in the roots and leaves of autoclaved compared to non-autoclaved soils. Previous studies (Alphei and Scheu, 1993; Bank et al., 2008; Miransari et al., 2009) have shown that autoclave methods of sterilization in soils affect the chemical and physical properties of soils. The possible explanation for higher element contents in autoclaved compared to non-autoclaved soils could be partially explained by increased solubility of elements in autoclaved soils. Autoclave and γ-sterilization are two frequently used methods in soil sterilization. Shaw et al. (1999) reported that autoclaving of soil affects physical and chemical properties of soils by decreasing soil pH and increase the soluble organic carbon. Alphei and Scheu (1993) reported increased content of N and P after autoclave process in the soil. Decreased in CEC and increased sorption of U was reported by Bank et al. (2008). Therefore the autoclave process may have contributed to elevated concentrations and contents of elements in soils and thereby increased availability to *T. maritima*.

To our knowledge, this is the first report on effects of high temperature on element uptake in *T. maritima* and presence of mycorrhizae in the plant. Temperature and soil treatment effect was apparent in multi-element uptake in *T. maritima,* and only the temperature was significant in translocation of the majority of elements from roots to leaves. Elements such as Li, Sr, Rb, and Cs were highly translocated into the leaf at 40 °C, which indicate their possible role in osmolytic maintenance in *T. maritima* in elevated temperature or these elements had passive movement in the leaves regardless of their physiological roles in plant tissue. The positive correlations of elements between biologically-unknown functions (Li, Sr, Rb, Cs, Ag, and Ti) and biologically-known functions (Ca, P, Mg, K, Na) suggest their synergistic effect as well as their possible physiological and biological roles in plants, but further study is necessary to elucidated this effect.

5. GENERAL DISCUSSION

In the research presented in this dissertation, the multi-element composition of root-zone soils and various plant species was studied. Many factors affect element concentrations and their uptake pattern in plants, including abiotic (soil chemical and physical properties and environmental factors such as temperature and salinity) and biotic factors (plant species, soil biota) (Kabata-Pendias, 2001). Understanding the effects of these factors on multi-element composition of plants is important because we lack understanding of environmental cycling of many elements, a great number of which are entering the environment due to anthropogenic activities. For example, the use of chemical fertilizer enriched with rare earth elements (REEs) has increased in agriculture production in recent years, however, only a little is known about their behavior in plants as well as impacts on the environment (Hu et al., 2002; Xu et al., 2002). Studies have shown that significant uptake and the possible transfer of these REEs into the food chain have occurred in agricultural crops (Wen et al. 2001; Kucera et al., 2007). In addition, the emerging invention of nanotechnology may release more elements into the environment (Lin and Xing, 2007). Similarly, the use of platinum group elements (Pd, Pt, Rh) in automobile catalysts has increased the risk of accumulation of these elements in the environment and in organisms (Shafer et al., 1998; Ek et al., 2004). Therefore, the study of multiple elements in plants and their interactions in ecosystems is important for our understanding of their behavior and biological roles in plants and the environment.

Triglochin maritima L. was used as a model plant to study the multi-element uptake in current studies because of its wide distribution in different habitats such as inland and coastal salt marshes, hot spring influenced wetlands, and temporary wetlands throughout America and Europe (Davy and Bishop, 1991; Otte and Jacob, 2005). Other plant species, *Eleocharis rostellata, Juncus balticus, Salix exigua, Salix boothii,* and *Salix wolfii* were also included in the study. Multi-element composition in these plant species was studied in samples from contrasting habitats and compared with different species from the same habitat. The patterns of element composition in *T. maritima* were further compared between greenhouse experiments and field studies, and the effects of soil micro-biota and temperature on multi-element uptakes were also investigated.

The overall patterns of the multi-element contents in *T. maritima* are summarized in Fig. 5.1. It depicts elements that showed significant differences in four contrasts across this study: Plant Species (*T. maritima* vs. *E. rostellata*, and *J. balticus*), Habitat Differences (Hot spring influenced wetlands vs. Temporary wetlands), Growth Conditions (field experiment vs. greenhouse conditions), and Temperature (greenhouse experiment). Only those elements that showed detectable concentrations in the plants in all four contrasts have been included. For example, Ca varied significantly for all four contrasts and so is located in the center overlap area of all four. Na on the other hand, was detectable in all four contrasts, but showed significant variation only due to variation in temperatures and species. Elements that were included in the studies described in this dissertation, but those were not detectable in all four contrasts, were not included.

The observed patterns in element contents in plants (Fig. 5.1) will be discussed following the Biological System of the Elements of Markert (1994).
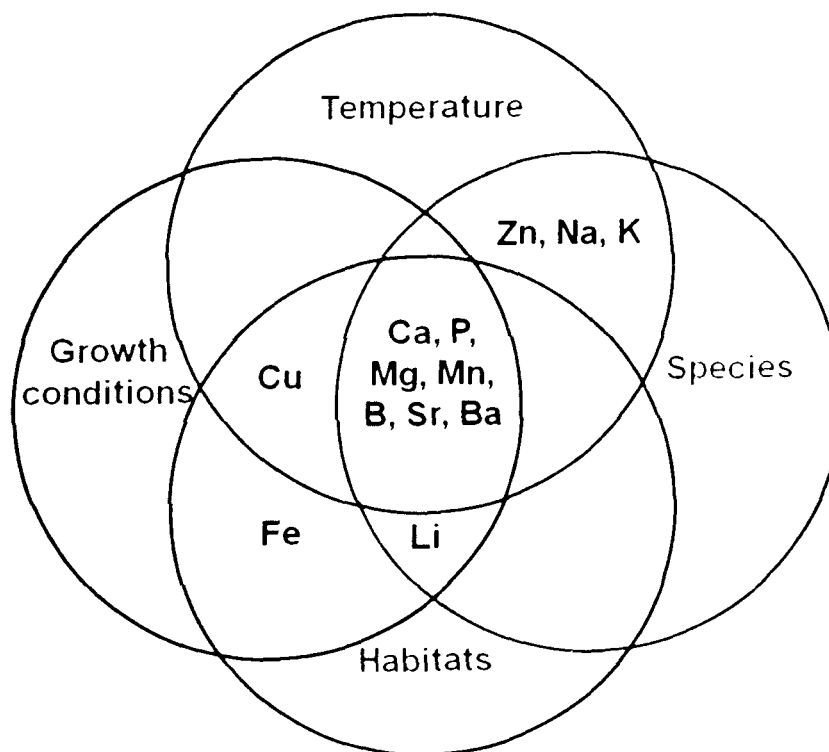
Figure 5.1. Venn diagram for multi-element concentrations (detectable and present in all four comparisons) in *T. maritima* influenced by Plant Species (*T. maritima* vs. *E. rostellata*, and *J. balticus*), Habitat Differences (Hot spring influenced wetlands vs. temporary wetlands), Growth Conditions (field experiment vs. greenhouse conditions), and Temperature (greenhouse experiment and field).

5.1. Structural Elements

Plant species, habitat differences, growth conditions and temperature all influenced the concentration patterns of Ca and P. These elements were grouped together in structural elements in BSE due to their important role in cell structure (Markert, 1994).

5.1.1. Calcium

Ca is an important element in cell structure due to its role in maintaining cell membranes and cell walls in plants (Pilbeam, 2007). In general, *T. maritima* showed higher Ca concentration patterns compared to other species; almost double compared to *E. rostellata* and *J. balticus* within the hot spring influenced wetlands. Ca and salinity are antagonistic with each other where salinity decreases the uptake of Ca in plants (Kabata-Pendias, 2001), and Gorai et al. (2010) reported that Ca concentrations were reduced in *Phragmites australis* (Cav.) Trin. Ex Steudel at high salinity (200 mM) in hydroponic culture. Fogel et al. (2004) reported that *T. maritima* produces ring like structures of root mass, which lowers salinity and increases redox potential of soils in its vicinity. Therefore, the lower salinity in the root-zone soils of *T. maritima* compared to two other species from same location may be one of the possible mechanisms leading to higher Ca concentrations in *T. maritima*. Another explanation for the variation of Ca concentrations among these plants could be due to their selective preferences for the ions from the soils.

Variations in Ca concentrations were also observed in *T. maritima* from contrasting habitats. The variation may be attributed to differences in soil properties. The temporary wetland soils in this study were mainly silt clay. The silt clay holds higher CEC compared to sandy soils (Kabata-Pendias, 2001), whereas hot spring influenced wetlands in Yellowstone National Parks were sandy textured and aquic inceptisols. These soils are young and formed through leaching of Al, Fe, and bases (Ca, K, and Mg) compared to other soil types. The differences in Ca

concentrations in *T. maritima* between temporary and hot spring wetlands may be due to the variation in availability of these elements in the soils. Pilbeam (2007) reported that Ca concentrations in plants depend on the rate of Ca supply in the external medium.

Ca concentration patterns in *T. maritima* further varied between greenhouse and field studies, which may be due to differences in environmental and soil factors. The environmental factors were different between these two experiments. In the greenhouse many factors were controlled, for instance temperature, pH, and light were constant. In contrast, in the field, there is fluctuation in day and night temperature and differences may have occurred in salinity of root-zone soils. The differences in Ca concentrations between the two growing conditions may further be attributed to differences in soil properties (pH, OM).

Temperature certainly affected Ca concentrations in *T. maritima* in the controlled greenhouse experiment, with higher Ca concentrations at 20 and 30 °C compared to 40 °C. Ca movement in the roots from the soil solution is mainly through mass flow, and transpiration affects the Ca uptake in plants (Marschner, 1995). High temperature increases transpiration and thereby accelerates the flow of Ca into the leaves of plants. In contrast to this, the lower concentrations of Ca at 40 °C in this study may have been due to slower metabolic activity and thereby lowered uptake of Ca.

5.1.2. Phosphorus

Phosphorus is important in phospholipids, nucleic acids (nucleotides), co-enzymes and phosphoproteins (Marschner, 1995; Schachtman et al. 1998;

Sanchez, 2007). The movement of P in plants is through the diffusion process (Marschner, 1995). Concentrations of P showed variation in all four contrasts in current study. P concentrations were higher in *T. maritima* compared to *E. rostellata* and *J. balticus*. The variation in P contents could be due to the differences in the plants' abilities to take up P in their tissues. Fraser et al. (2004) reported the differential uptake of P in wetland plant species. These authors studied the ability of four wetland plants (*Scirpus validus, Carex lacustris, Phalaris arundinacea,* and *Typha latifolia*) in the removal of P in a microcosm experiment. They reported that *S. validus* contained higher concentrations of P compared to the other three species. Römer and Schenk (1998) suggested that the uptake efficiency of P in agricultural crops like rice (*Oryza sativa* L.) and faba bean (*Vicia faba* L.) were attributed to genetic variability and root parameters. In spring Hordeum vulgare L. (barley), P content of shoots was attributed to the root length when P supply was low (Römer and Schenk, 1998).

Temperature was one of the significant factors for the variation in concentrations of P in *T. maritima* (Chapter 4). A high concentration pattern of P was observed at 30° C. P is immobile due to its low solubility in soils (Kabata-Pendias, 2001), however at elevated temperatures, P becomes more soluble and its availability in plants increases (Grant et al., 2001). In this study, temperature influenced the translocation of P in *T. maritima*, as more P was translocated from the roots to the leaves at 40 °C compared to the lower temperatures.

Habitat differences and growth conditions also influenced the concentrations patterns of P in *T. maritima*. In general *T. maritima* showed higher concentrations

of P in temporary wetlands compared to hot spring influenced wetlands. Similarly, variation in P concentrations was observed between field and greenhouse grown plants. The differences in P between these two growing condition may be attributed to variation in soil characteristics (pH, salinity, temperature, redox)

5.2. Electrolytic Elements

K, Na, and Mg fall under the electrolytic elements due to their role in electrolytic functions, which are important in osmoregulation in plants (Markert, 1994). K and Na share similar chemical properties and are monovalent whereas Mg is divalent. These elements too were influenced by two of the four contrasts in different ways.

5.2.1. Sodium

Na plays an important role in cell expansion and the water balance in plants (Marschner, 1995). It stimulates the growth of halophytes and C4 plants (Ball and O' Leary, 2003; Gorham, 2007). Species and temperature effects were apparent for Na in *T. maritima* in this study, but habitat differences and growth conditions did not influence the concentrations of Na in *T. maritima*. The translocation of Na from roots to leaves was much higher at 40 °C, which may be attributed to increased transpiration rates.

In general, Na concentrations patterns were higher in *T. maritima* compared to *E. rostellata* and *J. balticus* showing differences in uptake of Na among the plant species. The uptake of Na in plants varies between species as well as between the genotypes of species (Clark, 1983; Marschner, 1995).

High concentrations of Na in leaf tissues were observed in *T. maritima* irrespective of habitat. Otte and Jacob (2005) also reported that *T. maritima* collected from two geographically distant populations (Soda Dam, New Mexico, USA vs. North Bull Island, Ireland) showed similarly high concentrations of Na. Other researchers have reported that *T. maritima* is an exceptional monocotyledonous plants species, which accumulates high sodium levels in the leaves (Albert, 1975; Davy and Bishop, 1991).

5.2.2. Potassium

K is important in activation of various enzymes in plants, protein synthesis, and it maintains electrochemical potential in plant cells (Marschner, 1995; Mengel, 2007). K is a highly mobile element within individual cells, within in tissues and plant parts, for instance from roots to leaves, and from leaves to roots (Marschner, 1995). The movement of K from soil solution to roots is by diffusion and the uptake of K in plants is mainly by concentration gradients (Marschner, 1995). K is abundant in the cytoplasm of plant cells and maintains the osmotic potential and water balances in plant cells. In this study, concentrations of K were higher in *E. rostellata* compared to *T. maritima* and *J. balticus* (Chapter 2). Plant species and genotypes vary in element uptake (Clark, 1983; Marschner, 1995; Kabata-Pendias,). For example, Jin-Tao et al. (2011) reported on the genotypic differences in the uptake and use efficiency of K in barley varieties in a pot experiment. Marschner (1995) described that root length and root hairs are important factors and showed positive correlations for the uptake rate of K in per unit of root length for *Allium cepa* L. (onion), *Zea mays* L. (maize), *Lolium spp.*

(ryegrass), *Lycopersicon esculentum* (tomato), and *Brassica rapa* (rape). He reported that rape, which had the highest root length of the investigated species, contained higher uptake rate of K compared to the onion, which had very short roots. In current study, the differences between K concentrations between *T. maritima* and *E. rostellata* may be possibly due to differences in root parameters, which need further study for explanation.

Irrespective of habitat differences, K concentrations in *T. maritima* were similar, even though significant differences in K concentrations were observed between soils in the field. This suggests that the differences in soil properties had little effect on K concentrations in plants. This is supported by a study by Rocca and Vallejo (1995), who reported that soil types did not affect K uptake in spinach.

The effect of temperature on K concentrations followed the similar pattern of Na concentrations, and may again be explained by differences in evapotranspiration and other factors affecting the electrochemical characteristics of the plants. The translocation of K was influenced by temperature in *T. maritima*, where more K was translocated from roots to leaves at 40 °C compared to 20 and 30°C. Mengel (2007) has described that K ion in plants cycles from roots to leaves via xylem and from leaves to roots via phloem depending on the physiological demand in the plant.

5.2.3. Magnesium

Mg plays a significant role in physiology and molecular mechanisms in plants, and is an important component of chlorophyll and enzymatic processes (Marschner, 1995; Merhaut, 2007). Mg concentrations varied among all for

contrasts in *T. maritima*. Genotypes of plant species affect the Mg concentrations and plants are selective in uptake (Clark, 1983; Marschner, 1995). Mg movements in plants are mainly through mass flow however this mechanism differs between plant species (Marschner, 1995) and therefore uptake in plants depends on the external concentrations in the soil medium. In this study, *T. maritima* showed generally higher concentration patterns of Mg compared to *E. rostellata* and *J. balticus* in hot spring influenced wetlands.

Temperature also affected the concentration of Mg. The concentration of Mg tends to increases in the transpiring organs in plants, for instance in leaves and flowers. Huang and Grunes (1992) reported correlations between elevated temperature and Mg uptake in wheat seedlings, but also showed differential uptake of Mg in young and old seedlings.

5.3. Enzymatic Elements

Concentrations of Mn and B were affected by all four contrasts but one or two of the contrasts only affected Fe, Cu, and Zn in different ways (Fig 5.1). Elements, which are important in catalytic functions in the form of metal complexes in cell metabolism are grouped under enzymatic elements in the BSE classification.

5.3.1. Manganese

Mn plays roles in activating different enzymes that catalyze oxidation-reduction reactions, and it is prominent in photosynthesis in higher plants (Marschner, 1995). Differential uptake for Mn is reported for several plant species (Clark, 1983). Pollard et al. (2009) reported that *Phytolacca americana* L. (Pokeweed) showed hyperaccumulation characteristics for Mn, accumulating

much higher concentrations of Mn in leaves (2000 µg/g dry weight) compared to other species (*Rumex crispus* L., *Solanum carolinense* L.) from the same place. In a hydroponic experiment, the same species accumulated 10,000 µg/g dry of Mn. In this study, Mn concentrations of *T. maritima* showed lower Mn concentrations compared to *E. rostellata* and *J. balticus*. Furthermore, Mn concentrations were higher in *T. maritima* from hot spring influenced wetlands compared to temporary wetlands. Differences in soil properties and environmental conditions between these two sites may have influenced Mn concentrations in plants. Flooded or waterlogged conditions as well as a pH > 8 or < 5.5 are more conducive for Mn uptake in plants (Kabata-Pendias, 2001). In this study, the high soil temperature and waterlogged situation in hot spring influenced wetlands may be more conducive for higher uptake of Mn in *T. maritima* compared to temporary wetlands. Adebooye et al., (2010) reported that Mn uptake at 30 °C was higher compared to 20 and 25 °C in *Trichosanthes cucumerina* L. Similarly, Turner and Lahav (1985) also reported that both concentrations and content of Mn was increased in banana plants at 29 and 37 °C compared to 17 °C. In the current study root-zone soil temperature was one of the distinct differences between hot spring influenced wetland (25 – 45.6 °C) and temporary wetlands (17-21°C) that may explain the differences of Mn concentrations. This result is supported by greenhouse experiment where temperature was significant in Mn concentrations in *T. maritima*.

5.3.2. Iron

Fe is important in biosynthesis of heme enzymes (catalase and peroxidases), that have important roles in synthesis of chlorophyll, photosynthesis, respiration,

cellular protection and nitrogen fixation (Clark, 1983; Marschner, 1995). In general, Fe concentrations were influenced neither by plant species (*T. maritima, E. rostellata*, and *J. balticus*) nor temperature in this study. This contrasts with extensive scientific studies showing that Fe uptake in plants varies between plant species (e.g. Clark, 1983; Marschner, 1995). For example, Mahmoudi et al. (2005) reported that the uptake of Fe was different between lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*), with high accumulation ability in chickpea.

Also, habitat differences and growth conditions showed their influences in Fe concentrations in *T. maritima*, resulting in higher concentrations in temporary wetlands compared to hot springs. The variation in Fe in *T. maritima* could be due to soil factors. The lower concentrations in hot springs may be due to differences in soil properties, because the soils in Yellowstone National Parks were aquic inceptisols, which leach Fe more easily compared to the other habitats. It is difficult to discern the effect to a particular factor on element uptake in plants in field compared to the greenhouse experiment. Temperature was not significant in Fe concentrations in *T. maritima* in this study. However, Adebooye et al. (2010) reported that temperature had effect on the uptake of Fe contents including Ca, Mg, P, K, and Mn in *Trichosanthes cucumerina* L.. In their study Fe uptake was higher in *T. cucumerina* L at 30 °C compared to 20 and 25 °C. In general, *T. maritima* from greenhouse grown plants showed lower concentrations of Fe compared to the plants collected from the field, these differences again may be due to differences in environmental and soil factors.

5.3.3. Copper

Cu is an enzymatic element and essential in plant metabolism. Cu uptake in plants is by an active process (Kopsell and Kopsell, 2007). Cu is essential in redox reactions in plants as a co-enzyme and is important in physiological process (e. g photosynthesis, respiration, protein and cell wall metabolism) and disease resistance mechanisms in plants (Kabata-Pendias, 2001). In this study, Cu concentrations in *T. maritima* were influenced by temperature, habitat differences, and growth conditions. Cu concentration was not affected by plant species differences in this study even though Cu uptake varies greatly between species (Kabata-Pendias, 2001; Kopsell and Kopsell, 2007).

5.3.4. Boron

B has metabolic roles in plants and is important in sugar synthesis and translocation in plants (Kabata-Pendias, 2001). B concentrations in *T. maritima* were lower than *J. balticus* in hot spring influenced wetlands, which showed species specific concentrations for the element. Plant genotypes also vary in the uptake of B from soils (Gupta, 2007). A temperature effect was apparent in B uptake in plants in previous studies. Forno et al. (1979) reported that uptake of B was higher at 28 and 33°C compared to 18 °C in Cassava (*Manihot esculentum Crantz*) in a hydroponic experiment. Turner and Lahave (1985) also reported that both B concentrations and contents in banana were higher at 33 °C, compared to 25 and 37 in a controlled experiment.

Gupta (2007) discussed that at elevated temperatures and moisture conditions the uptake of B increases in plants compared to low temperatures and

dry conditions. The movement of B in plant is via mass flow and the external concentrations in the medium affect the uptake of B in plants (Marschner, 1995). The distribution of B in plants also depends on the rate of transpiration. In elevated temperatures there is a high transpiration rate due to which water and B flows (Gupta, 2007). In this study, the content of B was higher at 20 and 30 °C compared to 40 °C. The lower concentrations of B at 40 °C may be explained by direct contact of roots may have imbalanced the transpiration rate and thus reduced physiological and metabolic activities at 40°C compared to 20 and 30 °C. Lukac et al. (2010) also suggested that at elevated temperature beyond 25.5 °C, oxygen availability is increased at RuBisCo (Ribulose-1, 5-bisphosphate carboxylase oxygenase, an enzyme that catalyzes the Calvin cycle) sites in chloroplasts, which increase photorespiration, however it depends on optimal temperature requirement of individual plant species. Increased photorespiration reduces the efficiency of photosynthesis and thereby reduces the plant growth, which causes low element uptake. Then low element uptake may reduce growth and development of plants, which affect physiological and metabolic activities in the plant thereby reducing growth and development of plant.

The concentrations of B in *T. maritima* were also affected by habitat differences. B concentrations were lower in temporary wetlands compared to hot spring influenced wetlands. This could be due to relatively low temperatures in temporary wetlands, which might have lowered the transpiration and thereby reducing mass flow of B in plant tissue. Therefore, all four contrasts influenced B

concentrations in *T. maritima*. The variation in B in *T. maritima* could also be associated with the soil physical and chemical properties of the study sites.

5.3.5. Zinc

Zn is essential element of some enzymes. It is important in DNA and RNA metabolism where zinc metalloproteins are involved in DNA replication, transcription, and thus in gene expression (Marschner, 1995). For example, zinc finger proteins are complexes containing Zn, which play a significant role in the regulation of transcription factors in plants especially under abiotic (heat, cold, light etc.) and biotic stresses (infections by microorganisms). In this study, temperature was significant in Zn concentration and higher concentrations were found in 20 and 30 °C in *T. maritima* but the concentration of Zn did not vary by habitat differences and growth conditions. In a previous study temperature also significantly affected the concentrations of Zn in *Musa* sp. (Turner and Lahav, 1985). Their study reported that Zn concentrations in *Musa* sp. were higher in 29 °C compared to 25 and 37 °C. As would be expected, Zn concentrations varied between species; *T. maritima* contained lower concentrations of Zn compared to *E. rostellata* and *J. balticus*. This indicates that Zn concentrations in plants are species specific. Ozturk et al. (2003) also reported that Zn hyperaccumulator *Thalspi caerulescens*, and the non-accumulator species *T. arvense* showed variations in Zn concentrations with differences in the soil medium.

5.4. Elements with No Known Biological Functions

Li, Sr, and Ba are not known for any biological functions and are therefore grouped together in the BSE. According to the Periodic Table of the Elements, Li

is an alkali metal and is monovalent from group 1a, whereas Ba and Sr are divalent alkali earth metals from group II a. In this study the concentrations of these elements were easily detectable in *T. maritima* and Sr and Ba were influenced by all four contrasts whereas Li was not affected by temperature but was affected by the other three contrasts.

5.4.1. Lithium

In general, Li concentrations were higher in *T. maritima* compared to the other two species, which supports previous results of the species specific concentrations of Li in plants (Brown 1981; Ernst 1995; Kabata-Pendias, 2001; Lovkova, 2006; Aral and Vecchio-Sadu, 2008; Li et al., 2009). The role of Li in plant growth and development has not yet been defined, but at a low dose it stimulates in plant growth, seed germination rate, root growth and fresh weight (Aral and Vecchio-Sadu, 2008; Li et al., 2009). Li may have a role in halophyte metabolism because *T. maritima* showed higher concentrations of Li compared to the other species from same habitat. Li activates chlorophyllase (an enzyme functional in chlorophyll catabolism) in plants (Shkolnix, 1984; Bargagli, 1998). Both Li and Na were reported to activate poly- β - hydroxybutyric acid depolymerase enzyme in halophytes (Evans and Šorger, 1966). Therefore, elevated uptake of Li and Na might have synergistic effects in *T. maritima* under higher temperature conditions. Li concentrations in plants were also affected by habitat differences. The concentrations of Li were higher in hot spring influenced *T. maritima* compared to temporary wetlands, and the growth condition influenced its concentrations as well. Otte and Jacob (2005) also reported that Li

concentrations were higher in *T. maritima* collected from hot spring in New Mexico, USA compared to the plants from North Bull Island, Ireland.

Field grown plants showed higher concentrations of Li compared to plants in the greenhouse experiment. In general, temperature did not affect Li concentrations in *T. maritima*. However, the content of Li in *T. maritima* leaves was affected by temperature and an interaction effect was observed for soil sterilization and temperature, which indicates that variation in Li contents in *T. maritima* cannot be explained by temperature alone.

5.4.2. Strontium

Sr is not known for any biological roles in plants; however it is taken up in plants (Kabata-Pendias, 2001). Sr is from same column in the Periodic Table as Ca and Mg, which have similar chemical properties. Plant species vary in the concentrations of Sr (Kabata-Pendias, 2001; Sasmaz and Sasmaz, 2009). Veresoglou et al. (1995) also reported that uptake of Sr is plant specific and soil properties affect its uptake. Their study showed that higher uptake of Sr in *Trifolium repens* L. was found in inorganic soils compared to organic soils, and compared with Lolium *perenne* L. Rediske and Selders (1953) also reported species specific uptake and translocation of Sr with maximum translocation of Sr from root to leaf in tomato. Similarly, Fuhrmann et al. (2002) reported that Sr uptake between plant species varied but showed no correlation between Sr uptake and its concentrations in soils. In the current study also, Sr concentration varied between species. *T. maritima* showed higher concentrations of Sr compared to *E. rostellata* and *J. balticus*. In addition, Sr concentrations were higher in *T. maritima*

collected from temporary wetlands compared to the hot spring environments and also Sr concentrations varied in *T. maritima* collected from the field compared to greenhouse plants. Roca and Vallejo (1995) reported that Sr uptake in plants is influenced by soil properties. These authors reported that Sr was higher in lettuce from sandy soils compared to sandy loam. The differences of Sr between greenhouse and field plants in this study may be partly attributed with the soil texture, because the soils in the fields were coarse and sandy whereas greenhouse soils were silty clay loam.

5.4.3. Barium

Ba is an alkali earth metal. Information on concentrations and uptake of Ba in plants is limited (Nogueira et al., 2010) and the biological role of Ba has not been determined yet. However, Debnath and Mukherji (1982) have reported inhibitory effects in germination and root elongation in rice (*Oryza sativa* L.). These authors reported that Ba uptake in plants varies by plant parts and concentrations of Ba were high in cobs of maize. In this study, Ba concentrations were higher in *E. rostellata* and *J. balticus* then in *T. maritima*. The opposite patterns of Sr were observed for Ba concentrations in different growth conditions. Ba showed higher concentrations in the greenhouse compared to field conditions, whereas Sr was higher in the field compared to greenhouse. This may be due to competition for binding sites between the two elements in *T. maritima*, which is likely because both are in the same column as Ca in the Periodic Table and so has similar chemical characteristics.

The above discussed elements were detectable in all four contrasts. However, S, Pb, Hg, Cs, Rb, Ag, Zr, Ti, La, Y, and Ce were below the detection limits in *T. maritima* in field collected samples, but were found in detectable concentrations in the greenhouse experiment. This is most likely due to differences in environmental and soil chemical factors affecting their uptake. In the greenhouse study, the rare earth elements La, Y, and Ce, and other less studied elements, Ag, Ti, Rb, and Cs, were present in detectable concentrations in *T. maritima*. The effect of temperature was significant for these elements and significant positive correlations were found with elements that have known biological functions. For example, Ag concentrations, content, and translocation in *T. maritima* were affected by temperature. Ag showed positive correlations with Ca, P, S, Mg, Mo, Mn, Cu, and Sr. Ag is a transition metal in the Periodic Table of elements and in the same column with Cu. Ag and Cu share similar geochemical characteristics. Ag uptake in plants varies between species (Kabata-Pendias, 2001). Ward et al. (1977) studied Ag uptake in six pasture species growing near Ag mine sites and treatment plants in New Zealand. They reported that Ag concentrations in plants correlated with soil concentrations, but varied between species. Similarly, Handl et al. (2000) reported elevated uptake of radiosilver in *Taraxacum officinale, Lolium perenne* and *Holcus lanatus* and that it was affected by biomass. A few studies exist on Ag uptake in plants and its effect in plants. Xu et al. (2010) reported that Ag affected cell structure, chlorophyll and protein content, enzyme activity and mitochondria in *Potamogeton crispus* L. Silver is used in nanotechnology and the toxicity of nanoparticles containing silver is

reported by a few researches. Kumari et al. (2009) studied the effects of silver nanoparticles in *Allium cepa*, and reported that they affected cells. Nair et al. (2010) discussed the interaction of plant cells and nanoparticles and effects on plant growth and development. Ma et al (2010) showed that at a low dose (< 1ppm), silver nanoparticles had inhibitory effects on seedlings of *Arabidopsis thaliana*.

The rare earth elements Ce, Y, and La were also present in detectable concentrations in *T. maritima* in the greenhouse experiment. A few publications report that these elements have beneficial effects in plants (He and Loh, 2000; Hu et al., 2002; Yajia et al., 2008). Rare earth elements are increasingly entering into environment and plants are the first major components in cycling of these elements in the environment and transfer into the food chain. Knowledge about uptake of these elements in plants from various ecosystems is crucial for our understanding of the cycling of these elements, and therefore deserves further investigation.

The element compositions of *J. balticus* and *S. exigua* showed different chemical fingerprints at contaminated and non-contaminated habitats. High variation in element concentrations was found in *J. balticus* in contaminated sites compared to *S. exigua*. Concentrations of S, K, Mg, Mn, Fe, Cu, Zn, B, Sr, Al, and As were found to be higher in *J. balticus* from contaminated compared to non-contaminated habitats, which suggests a potential role for *J. balticus* in soil element monitoring and assessment. In contrast, *S. exigua* showed very low variation in element concentrations between contaminated and non-contaminated

sites. Similar results were reported by Landberg and Greger, (1996), where *Salix* species showed low concentrations of Cu, Zn and Cd in the leaves at both contaminated and non-contaminated sites. However, among the *Salix* species (*S. exigua, S. boothii,* and *S. wolfii*) from uncontaminated sites, each showed selective preference for some elements in their leaf tissue. For example, Ca and S concentrations were higher in *S. exigua*, while concentrations of Al, Mn, and Fe were higher in *S. boothii*, and Si was higher in *S. wolfii*. The variation in element concentrations in *Salix* species indicates that the uptake of elements in Salix is element and species specific.

5.5. Future Studies

- The seasonal differences and the effects of salinity on multi-element uptake in *Triglochin maritima* from hot spring and other habitats would particularly add further knowledge to our understanding of multi-element concentrations in this species.

- To our knowledge, this is the first time the association of a mycorrhizal fungus and *T. maritima* was confirmed. Due to presence of hyphae and vesicle in both soil treatment at all temperature levels, effect of mycorrhize on element uptake could not be discerned, therefore further research is needed to identify and further investigate the role of mycorrhizae in multi-element uptake of elements and survival of *T. maritima* in 'extreme' environments, such as hot springs.

- The multi-element concentrations for *J. balticus, S. exigua, S. wolfii,* and *S. boothii* presented in this dissertation were from leaf tissues only, because

collection of entire roots associated with individual plant is very difficult in the field. It was therefore not possible to examine the translocation of elements from root to shoots in these species, This should be addressed in future studies with these species, which should also include greenhouse experiments.

5.6. Conclusions

- *T. maritima* is mycorrhizal, but with which species remains to be investigated further.

- Multi-element composition in plants varied by plant species, habitat differences, growth conditions (greenhouse and field conditions), and temperature. Therefore, in multi-element uptake studies on plants, it is necessary to consider many factors simultaneously in order to understand the uptake patterns in plants.

- *T. maritima, E. rostellata,* and *J. balticus* showed species specific variation in element concentrations at hot spring influenced wetlands. *T. maritima* showed higher concentrations of Ca, P, S, Na, Mg, Li, and Sr. *E. rostellata* showed higher Si and K, whereas *J. balticus* showed higher concentrations of Mo, B, Al, and As.

- Habitat specific multi-element concentrations were observed in *Triglochin maritima*. The concentrations of Mn, B, and Li were consistently higher in hot spring influenced plants compared to temporary wetlands, whereas Ca, Mg, Fe, and Sr were higher in plants from temporary wetlands.

- Irrespective of habitat and growing conditions, *T. maritima* consistently showed higher concentrations of Na and K in leaf tissues compared to other elements, but concentrations of Na was almost double to K at all the conditions. This indicates that *T. maritima* regulated these two elements irrespective of the habitats.

- The majority of element concentrations (Ca, P, S, K, Na, Mg, B, Mn, Mo, Cu, Zn, Ag, Ba, Rb, Ti), contents (Ca, P, S, Na, Mg, Mn, Mo, Cu, Zn, Se, Ag, As, Ba, Cs, Sr, Ti) and translocation patterns (P,K, Na, Cr, Mo, Mn, Ni, Cu, B, Se, Li, Rb, Cs, Sr, Ti, Zr, and Ag) were affected by temperature in *T. maritima*, therefore temperature appeared to be an important environmental factor in element compositions of plants.

- *J. balticus* from contaminated sites showed higher concentrations of S, Cu, Zn, Sr, Al, and As. These element concentrations were lower in *J. balticus* from un-contaminated sites indicating that *J. balticus* can be used in the monitoring and assessment of contaminated sites.

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APPENDIX

Table A.1. Instrument detection limit (IDL) of inductively coupled plasma spectrometry mg L^{-1} in 2007 and 2008.

Elements	IDL	Elements	IDL
Ag	0.001	Mn	0.001
Al	0.009	Mo	0.010
As	0.021	Na	0.015
B	0.010	Ni	0.010
Ba	0.002	P	0.039
Be	0.000	Pb	0.033
Ca	0.006	S	0.043
Cd	0.001	Sb	0.043
Ce	0.011	Se	0.037
Co	0.007	Si	0.015
Cr	0.002	Sn	0.023
Cu	0.003	Sr	0.000
Fe	0.018	Ti	0.001
Hg	0.002	Tl	0.030
K	0.173	V	0.003
Li	0.001	Zn	0.006
Mg	0.034		

Table A.2. Method detection limit (MDL) of inductively coupled plasma spectrometry in mg L^{-1} in 2009.

Elements	IDL	Elements	IDL
Ag	0.002	Na	10
Al	100	Nb	0.01
As	0.1	Ni	0.1
Au	0.0002	P	10
B	1	Pb	0.01
Ba	0.1	Pd	0.002
Be	0.1	Pt	0.001
Bi	0.02	Rb	0.1
Ca	100	Re	0.001
Cd	0.01	S	100
Ce	0.01	Sb	0.02
Co	0.01	Sc	0.1
Cr	0.1	Se	0.1
Cs	0.005	Sn	0.02
Cu	0.01	Sr	0.5
Fe	10	Ta	0.001
Ga	0.1	Te	0.02
Ge	0.01	Th	0.01
Hf	0.001	Ti	1
Hg	0.001	Tl	0.02
In	0.02	U	0.01
K	100	V	2
La	0.01	W	0.1
Li	0.01	Y	0.001
Mg	10	Zn	0.1
Mn	1	Zr	0.01
Mo	0.01		