ABSTRACT

Title of Document: INVESTIGATING CRUMB RUBBER AMENDMENTS FOR EXTENSIVE GREEN ROOF SUBSTRATES

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Extensive green roof systems can mitigate urban stormwater by capturing rainfall and reducing runoff volume. Green roof substrates, often made from expanded shales, slates and clays are fundamental for roof hydraulic dynamics, and for providing optimal plant growth conditions. However, these substrates occasionally impose load limitations for retrofitting existing infrastructure. This research studied recycled-tire crumb rubber, as a light-weight material for amending green roof substrates. Zinc release from crumb rubber was quantified, and the interactions with commercial rooflite® substrate and the effect of high Zn concentrations on the growth and uptake by *Sedum* were studied. Zn was found to leach from crumb rubber in quantities that could negatively affect plant growth; however, Zn was adsorbed onto cation exchange sites of the mineral and/or organic portion of rooflite®, preventing negative growth effects in *Sedum*. Crumb rubber could be utilized as an amendment with substrates having high cation exchange capacities.

INVESTIGATING CRUMB RUBBER AMENDMENTS FOR EXTENSIVE GREEN ROOF SUBSTRATES.

By

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Dedication

This thesis is dedicated to my parents, who have always trusted and supported my abilities to reach my goals.

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I would like to express my sincere appreciation to all my committee members for the opportunity of working and learning from them. I owe my deepest gratitude to Dr. Andrew Ristvey, who always supported my research and assisted my understanding of this topic.

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Chapter 1: Literature Review

A. ***The Urban Context: Stormwater Runoff***

Water, essential for most plant and animal life, is fundamental for social, economic and biological systems. Globally, about 67% of the water utilized by humans is used for agriculture, 19% for industrial processes, and 9% for domestic use (Sharma, 2009). Clean and abundant water is necessary to sustain food production, human health and maintain habitats for wildlife. Unfortunately, as a consequence of accelerated population growth and unsustainable urban development, we are currently facing critical issues with water quality impairment and quantity management (Berghage *et al.,* 2009).

The change in land use from forest or agriculture to suburban or urban areas, particularly the introduction of impervious surfaces and constructed drainage networks, has disrupted the natural hydrologic balance. When more than 75% of a non-disturbed area is replaced with impervious or hardened surfaces, infiltration and evapotranspiration are significantly reduced and the proportion of runoff water increases to approximately 55% (Federal Interagency Stream Restoration Working Group (FISRWG), 1998). This is a dramatic modification, considering that in non-disturbed conditions, run off averages are approximately 10%. Urbanization of any magnitude has been demonstrated to negatively affect in-stream water quality (National Water Council, 2008).

A direct consequence of the high volume of stormwater runoff is the change in peak discharge and velocity. Runoff water can convey a number of pollutants, for instance, physical debris (from microscopic to large particles), chemical constituents (both dissolved and immiscible), and changes other physical properties such as water temperature (National Water Council, 2008). After urban development and during dry periods, some contaminants (e.g. oils, sediments, pesticides and heavy metals) can accumulate on impervious surfaces. These pollutants can be dramatically released during the first stormwater flush (Prowell, 2006).

In addition to the surface pollution problems, many old cities, particularly in the Northeast and Great Lakes regions, contribute to water impairment by discharging untreated human, commercial, and industrial waste directly into waterways (Kloss and Stoner, 2006). These events occur when the flow of combined sewer systems, containing both stormwater and sewage, exceeds the capacity of the system. Pathogens from sanitary overflows can have a negative impact on drinking water supply, fish consumption, shellfish harvesting and recreation (USEPA, 2004). Sanitary overflows can be avoided by separating combined sewers, expanding treatment capacity or storage within the sewer system, or by replacing broken or decaying pipes. However, cost and disruption issues often prevent these solutions from being implemented (USEPA, 2008).

The urban stormwater problematic is currently acknowledged in North America. For example, Mike Shapiro, Acting Assistant Administrator of the U.S. Environmental Protection Agency, testified his concerns before Congress in 2009: "*In September 2007, the USEPA Inspector General concluded that stormwater discharges in the Chesapeake Bay, associated with increased impervious surface* *area, which was attributable to development, were far outstripping gains made from addressing other sources of degradation"* (Shapiro, 2009).

Certainly, the deterioration of the Chesapeake Bay watershed as a consequence of intensive changes in land use exemplifies the stormwater problem. According to Copper (1995), the Chesapeake Bay area experienced progressive changes in land use, from basic agriculture during the settlement of Native Americans prior the 17th Century, to extensive urban areas as a consequence of the tremendous population growth observed during the late 19th Century. Nowadays, the demand for residential development continues. By 2006, the population in the watershed had reached 16.6 million people, according to the U.S. Geological Survey and the Bay Program. Predictions indicate the population will exceed 18 million in 2020 (Chesapeake Bay Program, 2008). Currently, the impervious area in the watershed is estimated to be approximately 1.1 million acres (445,156 hectares) (Chesapeake Bay Program, 2008).

Stormwater regulations have been in effect since 1987, when the U.S. Environmental Protection Agency (USEPA), under the framework of the Clean Water Act, was requested to control certain stormwater discharges as part of the National Pollutant Discharge Elimination System. Two permitting programs were implemented in 1990 (Phase I) and 1999 (Phase II) in order to set the requirements for municipal separate storm sewer systems and industrial activities including construction (National Water Council, 2008).

These regulations focus on specific pollutants discharged from permitted points; however, a series of limitations prevent the Federal Stormwater Program from completely restoring the nation's waters. The National Research Council reported the following limitations in the Urban Stormwater Management Report (2004):

- The volume of discharges is ignored because flow or alternative measures have not yet been implemented.
- The Clean Water Act does not provide the authority to restrict land development.
- The Urban Stormwater Program lacks the resources to continuously and effectively monitoring discharge points.
- The state and local governments do not possess the adequate financial support to rigorously implement the stormwater program.
- USEPA does not exercise a vigilant regulatory oversight in the licensing of products that contribute to stormwater pollution in a significant way.

Because of these limitations, the Environmental Protection Agency Office of Water encourages the implementation of green infrastructure (Shapiro, 2009), especially in light of the new Chesapeake Bay Presidential Order and legally binding agreements (Chesapeake Bay Foundation, 2010). Green infrastructure refers to systems and practices that use or mimic natural processes to infiltrate, evapotranspirate or reuse stormwater or runoff on the site where it is generated (USEPA, 2008). Current approaches include green roofs, trees and tree boxes, rain gardens, vegetated swales, pocket wetlands, infiltration planters, porous and permeable pavements, vegetated median strips, reforestation/revegetation, and protection and enhancement of riparian buffers and floodplains (USEPA, 2008).

By using these techniques, several problems as stormwater, combined sewer overflows and non point source discharges can be better managed (Shapiro, 2009). The benefits of green infrastructure tend to be particularly important in urban and suburban areas. The United States Environmental Protection Agency (USEPA, 2010a) summarizes the following benefits of green infrastructure technologies:

- Reduction and delay stormwater runoff volumes.
- Potential improvement of aquifer recharge rate.
- Reduction of pollutant levels from stormwater when infiltration occurs.
- Potential cooling effects from vegetated systems.
- Creation of habitats for wildlife.
- Perceived improvement of human emotional wellbeing.

This thesis focuses on the role of extensive green roofs, constructed for mitigating storm water runoff through the installation of substrates and the establishment of vegetation on the rooftops of buildings. Consistent with the general benefits described for green infrastructure, green roofs also generate several environmental, economical and social benefits.

The green roof industry in North America is very young in comparison to Europe, particularly Germany, which is the leader of green roof technologies. The Guideline for the Planning, Execution and Upkeep of Green Roof Sites, published by the Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau (FLL, 2002), is a relevant compilation of the technical experience accumulated in Germany. Specific research for United States (U.S.) conditions is necessary to validate the developing industry by testing commercial components and green roof designs and performance. Climatic and environmental conditions are substantially more variable in the U.S. than in Germany and will greatly affect green roof performance. Snodgrass and Snodgrass (2006) acknowledge that some lessons can be extrapolated from the Europe; however, specific research about green roof media composition, depth, and plant performance needs to be conducted in order to ensure success under North American conditions. In general, more local technical knowledge needs to be generated to protect the customer's investment and to achieve the environmental services expected from green roofs. The research described in this thesis investigates the effect of a recycled tire material (hereafter referred to as "crumb rubber") as a sustainable amendment for extensive green roof substrates.

B. ***Green Roofs***

B.1. Historical Background and Definition

A green roof is defined as a contained green space on top of a man made structure above, at or below grade (Green Roofs for Healthy Cities, 2008). Historically, greening roofs dates from thousands of years ago. For example, the Hanging Gardens of Babylon constitute an example of gardens constructed on rooftops (Snodgrass and Snodgrass, 2006). Although no definitive proof of their existence have been found, they are probably considered the most famous gardens in history (Osmundson, 1999). Scandinavian roofs also were covered with vegetation during the Viking and Middle Ages (Berg, 1989). This technology used several layers of birch bark for waterproofing purposes (Stern *et al.,* 2006) and included an uppermost layer of sod or dry turf to hold the birch bark in place and to allow for the growth of grasses (Vreim, 1966). In very dry areas, the use of *Sedum*, *Allium* and *Sempervivum* species was recommended (Nordhagen, 1934 and Melheim, 1933). For landscaping purposes, sod houses were planted with wildflowers. A representation of a sod house exists in Epcot's Park Norway pavilion in Orlando, Florida. This roof displays *Evolvulus* species, (blue daze), *Vinca* (vinca or periwinkle) and *Impatiens* species (impatiens) (Markey, 2006).

The contemporary use of vegetation and supporting structures, intrinsically integrated to the buildings, represents the modern concept and technology generated in Germany and Central Europe (Dunnet and Kingsbury, 2008). In the 1880's, sand and gravel were used in the top of a highly flammable tar for reducing fire hazards and it was later observed that natural seed colonization occurred (Getter and Rowe, 2006). For more than one hundred years, 50 of these pioneer roofs have remained functional (Kohler and Keeley, 2005).

One of the earliest green roofs in the United States is located in the Rockefeller Center, New York (Osmundson, 1999). Established in 1936, this project is 76,400 square feet (approximately 0.7 Hectares) in area (Greenroofs.com, 2010a). The adoption of green roofs as a sustainable practice has been encouraged by the governments of United States and Canada since the

1990's. (Snodgrass and Snodgrass, 2006). A current estimate of the total green roof square footage in the United States is over 10.5 million square feet or over 97 ha (Greenroofs.com, 2010b).

B.2. Green Roof Systems Classification

Contemporary green roofs integrate the plants and its supportive structures in the construction or retrofit of buildings. The new approach has established two main categories, based principally on the amount of maintenance required: intensive and extensive green roofs. Intensive green roofs are designed to reproduce conventional gardens and expect to involve the individuals in recreation purposes (Dunnet and Kingsbury, 2008). They display a whole range of vegetation types, from herbaceous plants to trees and shrubs (Getter and Rowe, 2006); in order to sustain these species, they require a deep soil layer (at least 6 inches, equivalent 15 cm), typically rich in organic matter (Snodgrass and Snodgrass, 2006). High maintenance is required in the form of weeding, fertilizing and watering (Berndtsson, 2010).

In contrast, extensive green roofs, which are typically not accessible to the public, are meant to fulfill ecological functions (Dunnet and Kingsbury, 2008), for instance, stormwater mitigation and habitat creation. Extensive green roof systems are usually composed by the following layers (from bottom to top): deck, waterproofing, insulation, root barrier, drainage, root permeable filter, substrate and vegetation (Snodgrass and Snodgrass, 2006). Hardy succulents are the most extensively used plant species (Snodgrass and Snodgrass, 2006) but herbs, grasses and mosses have also been used in installations (Getter and Rowe, 2006). In general, green roofs require relatively minimal maintenance; however, basic maintenance such as replanting, irrigating, fertilizing, and weeding is fundamental until plant coverage reaches approximately 80% of the surface area (Getter and Rowe, 2006). Some authors refer to a third group of roof called “semi-intensive,” where the elements from intensive and extensive green roofs are combined. Dunnet and Kingsbury (2008) consider semi-intensive green roofs to provide an alternative to enhance aesthetics and biodiversity. The following table summarizes the general characteristics of intensive, semi-intensive and extensive green roofs.

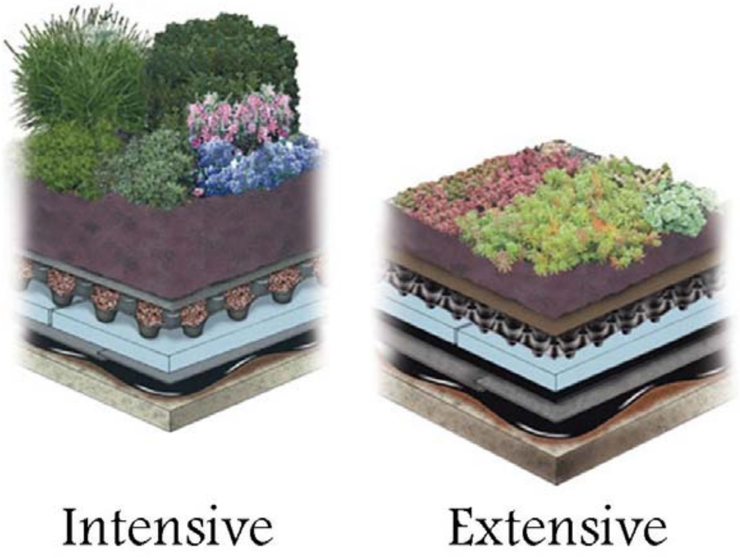


Fig. 1.1. An example of intensive and extensive green roof components. Source: American Hydrotech Inc., 2010.

Since this thesis focuses on extensive green roofs, the rest of this literature review will refer exclusively to this category.

Table 1.1. General characteristics of different green roof categories. Source: Green Roofs for Healthy Cities, 2008.

Characteristic	Extensive	Semi-Intensive	Intensive
Substrate depth	6 inches (15 cm) or less	25% above or below 6 inches (15 cm)	More than 6 inches (15 cm)
Accessibility	Often inaccessible	May be partially accessible	Usually accessible
Fully saturated weight	Low 10 - 35 lb/ft^2 (48.8 – 170.9 kg/m^2)	Varies 35 - 50 lb/ft^2 (170.9 – 244.1 kg/m^2)	Varies 35 - 300 lb/ft^2 (170.9 – 1,464.7 kg/m^2)
Plant diversity	Low	Greater	Greatest
Cost	Low	Varies	High
Maintenance	Minimal	Varies	Varies, but is is generally high

B.3. Intent and Benefits of Extensive Green Roof Systems

The motivation to construct an extensive green roof depends on the primary benefit expected from the project. As an example of green infrastructure, extensive green roofs contribute to stormwater management, increase biodiversity and, typically, reduce energy costs during the summer (Getter and Rowe, 2006). Other specific benefits derived from this technology are the extension of the life of the roof membrane (a major cost-consideration), and the improvement of building aesthetic characteristics. Several authors have categorized green roof benefits according to three main areas: environmental, economical and aesthetic

(Dunnet and Kingsbury, 2008); other authors divide the green roof benefits as public and private (Peck and Kuhn, 2000). The following descriptions are based on Dunnet and Kingsbury's (2008) classification.

B.3.1. Environmental Benefits

The following aspects constitute the major environmental benefits of extensive green roofs:

- Stormwater management and water quality improvement.
- Habitat creation.
- Potential reduction of the urban heat island.

B.3.1.1. Stormwater Management and Water Quality Improvement

The reduction of storm water runoff quantity is possibly the most important single benefit of extensive green roof (Getter and Rowe, 2006). Since impervious surfaces in urban areas often exceed 40%, green roofs have an important role in best management practices for stormwater mitigation (Dunnet and Kingsbury, 2008). The most researched green roof topic has been the reduction and management of stormwater runoff (Dunnet and Kingsbury, 2008). As a result, it is well understood that:

- Water is retained in the pore spaces of the substrate or taken up by absorbent materials in the mix (Dunnet and Kingsbury, 2008).
- Water is also used by plants, which require it for physiological processes, including transpiration (this is one of the ways water is rapidly removed

from the green roof substrate and returned to the atmosphere (Getter and Rowe, 2006).

- Water can be stored and retained by the substrate, becoming a buffer between the atmosphere and the roof top (Dunnet and Kingsbury, 2008).
- As a result of the green roof system, peak flow runoff volumes are reduced and there is a delay in stormwater draining from the roof (Getter and Rowe, 2006).

The storage capacity of extensive green roofs is dependent upon the season of the year, the depth of the substrate, the number and type of layers used for its construction, angle of slope of the roof, the physical properties of the growing media, the type of plants incorporated, and the rainfall intensity (Dunnet and Kingsbury, 2008). Most research has determined yearly reductions in runoff between 40 and 60%, but reduction values above 80% have been reported for specific localities and environments (Dunnet and Kingsbury, 2008). Typically, the average runoff volume from rainfall events can be reduced from 30 to 60% according to multiple authors (Getter and Rowe, 2006).

Water quality can be modified when green roofs replace conventional roofing materials. The characteristics of runoff water from green roofs are, by and large, dependent on the quality of the rainwater and the characteristics of the media (Dunnet and Kingsbury, 2008). The levels of some nutrients in the green roof leachate can increase while other nutrients can be reduced, for this reason, Berghage *et al*. (2007) points out that “the increased concentration of a chemical element should not be seen in isolation”. According to Dunnet and Kingsbury

(2008) the actual load of certain nutrient concentration could be comparable to same levels of nutrients leached from other urban vegetated areas or could be the simple result of much reduced flow from the roof.

Two factors can contribute to elevated levels of nutrients in green roofs: the composition of the media and fertilization practices (Dunnet and Kingsbury, 2008). The first factor tends to be only important during the early life of the roof, when first stormwater flushes occur, and it can be minimized by reducing the amount of organic matter (Hunton *et al.,* 2006). In regards to fertilization, this should be also a requirement almost restricted to the establishment of plants, since the ultimate goal for extensive green roofs is to be self-sustainable through steady-state nutrient cycling. In the case of green roof systems that are nutritionally poor, follow-up fertilization events in intervals of several years are suggested until reaching the desired plant coverage (FLL, 2002). Furthermore, high fertility has been shown to increase unwanted species competition (Getter and Rowe, 2006; Dunnett and Kingsbury, 2008).

Once the potential sources of pollution from green roofs are better understood, it is important to focus on the benefits derived from the reduced volume and speed of the stormwater runoff from the system. Less stormwater- in waterways translates into less erosion, sedimentation, and reduced overflow from combined sewer systems (Getter and Rowe, 2006). Additionally, green roofs also have the capability to buffer acid rain, until the point in which all the negative charges of the particle surface become saturated (Berghage *et al.,* 2009).

Therefore, by reducing the volume of stormwater runoff, water quality is improved.

B.3.1.2. Habitat Creation

Another important benefit realized from the implementation of extensive green roofs is the creation of habitat for wild species. Switzerland has led the way in conducting research and creating regulations to promote a strategy of biodiversity. Federal legislation on the conservation of nature and cultural heritage requires the protection of endangered species by using well-designed green roofs to provide habitat and to compensate by the land-use changes (Brenneisen, 2006).

In order to meet these legislated objectives, some modifications of design criteria are implemented, such as increasing the thickness of the substrate and incorporating natural soils from nearby areas (Brenneisen, 2006). The use of natural and structural soils have been demonstrated to favor the colonization of approximately 79 species of beetles and 40 of spiders in the most biodiverse roof installation investigated to date: the Rhypark Building, in Basel, Switzerland (App. Fig. A1). Variations of green roofs are "brown roofs", which mimic urban wastelands i.e. brown field sites, which host rare invertebrates and ground-nesting birds (Dunnet and Kingsbury, 2008). In this case, urban substrates such as brick rubble, crushed concrete, sands, gravels, and subsoils are utilized, and installed irregularly to recreate a mini-topography able to maximize the ecological variety (Dunnet and Kingsbury, 2008), (App. Fig. A2). To date, green roofs and brown roofs have not been compared under equal conditions; however, best design practices involve characteristics of both types to maximize biodiversity (Dunnet and Kingsbury, 2008).

B.3.1.3. Potential Reduction of the Urban Heat Island

Rooftops are reflective surfaces that can accumulate heat. For example, during NASA flyovers, the rooftops of the cities of Baton Rouge, Houston, Sacramento and Salt Lake City reached temperatures of 160 °F (71 °C), while vegetation and water recorded surface temperatures between 75 and 95 °F (24 and 35 °C) (Wong, 2005). The cooling effect of evapotranspiration is clear at the microclimatic scale (Dunnet and Kingsbury, 2008). By transforming solar energy into water vapor, the production of heat in the impervious surfaces is prevented or reduced (Bass, 2001). Little research has been conducted in this area, but it is suggested that, the larger the individual green areas are, the greater is the range of temperature moderation between them and impervious surfaces (Dunnet and Kingsbury, 2008). In a holistic way, green roofs are connectors that allow the continuum of environmental services across the different types of green infrastructure that can be present in a city or residential area.

B.3.2. Economic Benefits

The following are major economic benefits of extensive green roofs:

- Increased roof life;
- Cooling, insulation and energy efficiency, and
- Green Building assessment and public relations.

B.3.2.1 Increased Roof Life

The various components of the green roof protect the waterproofing membrane against solar exposure and ultraviolet radiation (Getter and Rowe, 2006). By avoiding drastic changes in temperature, the roof membrane does not expand and contract as it occurs in non-vegetated roofs (Getter and Rowe, 2006). A study conducted in Canada compared the maximum temperatures reached by membranes covered and not covered by vegetation and the related parts of the green roof. The membrane protected by the components of the green roof reached a temperature of 77 °F (25 °C), while the membrane not covered by vegetation registered 158 °F (70 °C) (Liu and Baskaran, 2003). Under these circumstances, it is quite possible that a membrane protected by the vegetation of a green roof could be useful for two to three times the life cycle of that of a non-vegetated membrane (Peck *et al*., 1999).

B.3.2.2. Cooling, Insulation and Energy Efficiency

The vegetation, substrate and additional components of a green roof can reduce solar energy gain by up to 90% compared with non-shaded buildings (Getter and Rowe, 2006). It is estimated that for every 0.5 C reduction in internal building air temperature, this may reduce electricity use for air-conditioning by up to 8% (Dunnett and Kingsbury, 2004). The greater energy savings occur during summer, when, more often, the spaces between substrate particles are filled with air, since water is a poor insulator (Getter and Rowe, 2006). Potential energy savings in cities is extremely relevant because buildings consume 36% of the total energy use and 65% of the total electricity consumption in cities (Kula, 2005). In

2003, it was estimated that if all the buildings of Chicago had green roofs, the potential savings could be $100M per year (Laberge, 2003). Since then, the cost of energy has risen considerably.

B.3.2.3. Green Building Assessment and Public Relations

Construction projects that implement green roofs can opt for various assessment and rating schemes for sustainable or green building. For example, the LEED (Leadership in Energy and Environmental Design) Program (U.S. Green Building Council, 2010) rewards green roof systems for their contribution to stormwater management and reduction of heat island effects (Oberlander *et al.*, 2002).

B.3.3. Aesthetic Benefits

Humans experience beneficial health effects when observing green plants and nature, for example, stress reduction, lowered blood pressure, reduced muscle tension, and increased feelings of well-being (Ulrich and Simmons, 1986). These are essential emotions desirable to sustain work productivity. Kaplan *et al.,* (1988) reported that employees who had a view of natural landscapes were less stressed, experienced greater job satisfaction, and reported fewer headaches and other illnesses than those who had no natural view. Ulrich (1984) also related the exposure to natural environments with the faster recovery of patients after surgery. Related to the economic benefits, the aesthetic component can add value to real estate and services (e.g. hotels, restaurants, condominiums etc.) (Dunnett and Kingsbury, 2004).

B.4. Extensive Green Roof System Components and Construction

The components of a green roof can be classified as either physical, including the deck, waterproofing membrane, insulation, root barrier, drainage layer and the root permeable filter layer, or dynamic, including substrate and vegetation (Weiler and Scholz-Barth, 2009). Designers have the flexibility to choose among different materials and technologies in order to achieve the overall design intent of the project. The order of the physical layers may vary among projects; however, in general, these layers are installed in the way that provides the maximum protection to the waterproof membrane so that the life of the project is maximized.

B.4.1. The Deck

This is the base of the green roof. It can be constructed from concrete, wood, metal, plastic, gypsum, or composite (Snodgrass 2009). In the United States, plywood is the most common material used in residential projects and concrete for buildings (Snodgrass and Snodgrass, 2006). Appendix Fig. A3 provides an example of the installation of a deck in a residential project.

B.4.2. Waterproofing Layer

There are three types of waterproofing methods: the built-up roof, the single-ply membrane and the fluid-applied membrane (Osmundson, 1999). Built-up roofs are composed of bitumen/asphalt felt or bituminized fabrics. Given the short life of these materials (15-20 years), they are not recommendable for green roofs purposes (Dunnet and Kingsbury, 2008).

Single-ply membranes are root resistant and efficient if correctly installed. They consist of rolled sheets of inorganic materials as plastic, polyvinyl chloride (PCV), thermoplastic polyolefin (TPO) or synthetic rubber, usually with heat-welded seams (Snodgrass and Snodgrass, 2006). Appendix Fig. A4 illustrates the installation of a single-ply membrane in a commercial project. Fluid-applied membranes are hot or cold liquids that can be sprayed or painted on the surface, and are very convenient for irregular surfaces (Dunnet and Kingsbury, 2008) and solid concrete decks (Snodgrass and Snodgrass, 2006). Once dry, they act like a seal, preventing leaks in the joints (Osmundson, 1999).

B.4.3. Insulation Layer

Insulation materials translate into in energy saving advantages for buildings (Snodgrass and Snodgrass, 2006). This layer can be located above or below the waterproofing membrane, however, by locating it above, mildew problems can be prevented and extra protection against ultraviolet (UV) degradation is provided to the membrane (Snodgrass and Snodgrass, 2006). Styrofoam is an example of the materials used for insulation purposes (Dunnet and Kingsbury, 2008). The installation of Styrofoam in a commercial project is illustrated in Appendix Fig. A5.

B.4.4. Root Barrier

This protection layer is absolutely necessary if the deck is made of biodegradable products such as: wood, asphalt and bitumen (Snodgrass and Snodgrass, 2006). Among the products used to prevent root penetration are PVC rolls and high-density polyethylene sheets (Snodgrass and Snodgrass, 2006). Alternatively, an innovative pre-constructed system uses aluminum surfaces in order to prevent root and moisture penetration and avoids the use of PVC because of environmental concerns, (Corus Building Systems, 2008). Appendix Fig. A6. illustrates the installation of a root barrier.

B.4.5. Drainage Layer

Drainage is essential to maintain the aeration in the root zone (Snodgrass and Snodgrass, 2006). Prolonged saturation of a roof can bring physiological disorders to the plants and could favor the colonization of pathogenic organisms. The thermal insulation properties are also lost when the green roof is permanently wet (Dunnet and Kingsbury, 2008). Green roofs installed on flat roofs or in high rainfall regime areas usually require additional means to remove the water retained in the substrate (Snodgrass and Snodgrass, 2006). According to Dunnet and Kingsbury (2008), three main types of materials can be used for drainage i.e., granular, porous mats and lightweight plastic or polystyrene modules.

Granular materials include: gravel, stone chips, broken clay tiles, clinker, scoria (lava rock), pumice, expanded shale and expanded clay granules (Dunnet and Kingsbury, 2008). A layer of granular materials can be incorporated underneath the substrate profile increasing the root space for plants (Dunnet and Kingsbury, 2008). Porous mats, made of a range of materials such as recycled clothing and car seats, act like sponges that absorbs the excessive water. Some materials can negatively affect plants since they tend to extract the available water necessary for plant growth (Dunnet and Kingsbury, 2008).

Lightweight plastic or polystyrene modules exhibit great flexibility in design and appearance. Usually thinner than 1 inch (2.5 cm), some sheets contain reservoirs to retain water, and others can be filled with granular media (Dunnet and Kingsbury, 2008). In some cases, the drainage layer enables irrigation water to be introduced from the base (Dunnet and Kingsbury, 2008). Drainage outlets must be kept free of substrate particles at all times in order to maintain their functionality (Dunnet and Kingsbury, 2008). The installation of a modular drainage layer in a residential project is illustrated in Appendix Fig. A7.

B.4.6. Root Permeable Filter Layer

This layer is responsible for keeping the substrate in place, and preventing blockage or damage of drainage outlets. It is highly recommended to use a filter cloth or mat, such as semi-permeable polypropylene fabric, to prevent the movement of fine particles from the substrate into the drainage layer (Snodgrass and Snodgrass, 2006). The root permeable layer should overlap 8 inches (20 cm) when laid out (Dunnet and Kingsbury, 2008). Appendix Fig. A8 illustrates the installation of this layer in a commercial project.

B.4.7. Substrate Layer

Green roof substrates (also known as growing media) are a specifically formulated mix of mineral materials, stabilized organic amendments and stabilized lightweight aggregates (Weiler and Scholz-Barth, 2009). The ideal substrate for extensive green roofs should retain the following characteristics:

- Lightweight;

- Well-drained;
- Adequate water and nutrient-holding capacity;
- Able to filter pollutants;
- Sustainable;
- Durable and stable.

The shallow layer of substrate, between 0.8 and 6 inches (between 2 and 15 cm) is predominantly inorganic (Dunnet and Kingsbury, 2008) and generally, the organic fraction should not exceed 8% by mass (FLL, 2002). In order to have a substrate that efficiently absorbs and retains water and nutrients, while at the same time exhibits free draining characteristics, it is necessary to use granular materials that achieve these objectives with the different pore sizes created between the particles (Miller, 2003). The granular products can be roughly classified as natural minerals, artificial minerals and recycled or waste materials (Dunnet and Kingsbury, 2008).

Currently, most of the projects in the east of the United States use commercial mixes based on expanded shales, slates and clays. Shale is a detrital sedimentary rock composed of very fine clay-size particles from the decomposition of feldspar, quarts, mica, pyrite and organic matter (Powell, 2010). A lamination process occurs when layers of other sediments lithify the silt and mud of the shale surface (Powell, 2010). Shales have a high cation exchange capacity and provide some nutrients to plants (Handreck and Black, 2005). Expanded shales are an excellent material to remove pollutants. This material has been proven to retain phosphorus, ammonia and metals from synthetic acid rain

(Long *et al.,* 2006). Slate is a foliated metamorphic rock derived from the metamorphism of shale (Powell, 2010).

Table 1.2. Examples of materials utilized as extensive green roof substrates. Sources: Dunnet and Kingsbury, 2008, and Green Roofs for Healthy Cities, 2008.

Natural Minerals	**Artificial Minerals**	**Recycled or Waste Materials**
Sand	Perlite	Crushed clay brick or tiles, brick rubble
Lava (scoria)	Vermiculite	Crushed concrete
Pumice	Expanded shales, slates and clays	Subsoil
Gravel	Rockwool	

A study conducted in the Center for Green Roof Research, Penn State University Park, demonstrated that expanded slates are less efficient than expanded shales and clays for pollutant retention. However, expanded slates are efficient in removing nutrients and metals on a weight basis (Long *et al.,* 2006). Clays are soil particles smaller than 2 µm (0.002 mm), composed of crystalline sheets of silica and alumina (Sylvia *et al.,* 2005). One sheet of silica and one sheet of alumina is classified as a 1:1 clay, like kaolinite; and a sheet of alumina between two sheets of silica is classified as a 2:1 clay, like smectite (Handreck and Black, 2005). These sheets give a layered effect to clays and increase their surface area (Sylvia *et al.,* 2005). The different arrangement of atoms in clays makes these particles very active from a chemical point of view (Handreck and Black, 2005).

The combination of activeness and large surface area of clays have important repercussions for the soils and substrates dynamics (Handreck and Black, 2005). The adsorption of water, nutrients and gases, and the attraction between particles are all surface phenomena (Sylvia *et al.,* 2005).

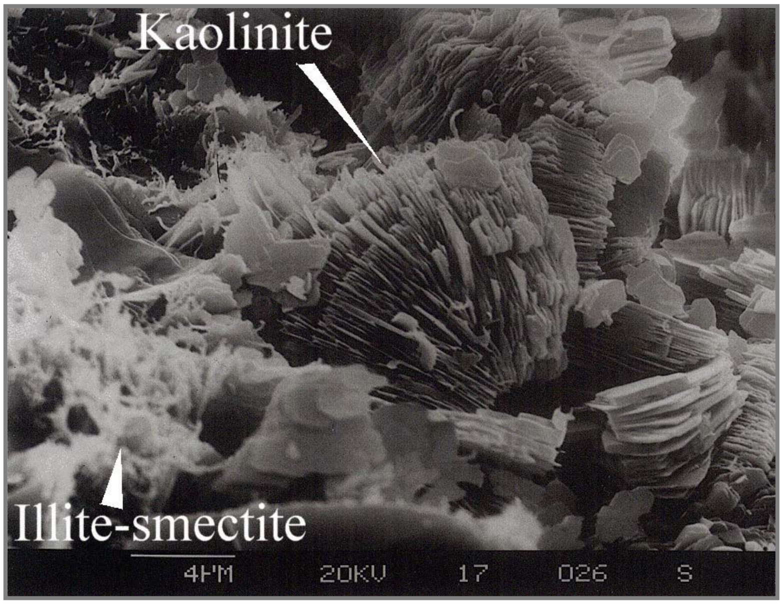


Fig. 1.2. A scanning electron micrograph showing the layered characteristics of clays. Source: US Geological Survey Online Publications Directory, 2009.

Heat expanded clays tend to increase the pH buffering capacity and cation exchange of substrate mixes. These particles also tend to hold water very tightly (Handreck and Black, 2005). The water holding characteristics of a substrate made of expanded clays could be problematic for horticultural crops but it could be adequate for drought tolerant species in extensive green roofs, especially if the substrate has a balanced particle size distribution.

Given the mineral characteristics of shales, slates and clays, the lightweight expanded mixes derived from these materials have a good cation exchange capacity, which is defined as the ability of a soil or substrate to hold positive ions (Sylvia *et al.,* 2005). For extensive green roof effects, the cation exchange capacity of a substrate is important for pollutant filtration and plant nutrition (Long *et al*., 2006; Berghage *et al*., 2009).

Another important chemical characteristic of substrates is pH, which has a direct influence in the availability of nutrients to plants. It affects the total amount of nutrients held by soils, influencing nutrient deficiencies, toxicities and microbiological activity (Handreck and Black, 2005). The maximum nutrient availability occurs between pH 6 to 7 for most soils (Handreck and Black, 2005). Most microorganisms prefer a pH range in which most nutrients are available (Sylvia *et al.,* 2005). Microorganisms also have the ability to modify the pH by producing organic acids under anaerobic conditions, and by producing H^+ when oxidizing ammonia and sulfur under aerobic conditions (Sylvia *et al.,* 2005).

The chemical and physical properties of green roof substrates are achieved by combining different particle sizes of different, chemically-dynamic materials. According to FLL (2002), the recommended content of clay and silt is 7% by mass in extensive single course constructions. If the depth of the substrate layer is less than 10 cm (4 inches), the largest particle size recommended is 1.2 cm (0.47 inches). If the media is deeper than 10 cm, the maximum particle size recommended is 1.6 cm (0.63 inches), (FLL, 2002). The FLL manual (2002) offers guidelines for the maximum and minimum particle distribution for green roof media with an appropriate particle size percentage, by mass. By following these guidelines the resulting substrate should contain a well-balanced amount of small and large pores between the inorganic particles, which in conjunction with the organic matter, determines the dynamics for water retention.

The substrate water-holding capacity, defined as the amount of water that a substrate can retain after saturation and drainage, plays an important role in extensive green roofs (Handreck and Black, 2005). This property is responsible for retaining stormwater, and for continuously providing the air and water required for plant development. FLL guidelines (FLL, 2002) suggest substrate water-holding capacities between 20% and 35%, depending on construction specifications.

Bulk density is the physical property that describes the mass of substrate that occupies a certain volume. In practice, the load (weight) of the substrate has major structural implications for green roof installations. Under saturated conditions, extensive green roofs can be relatively heavy for existing buildings that need to be retrofitted. For example, Cumberland Hall, a residential project of the University of Maryland that houses 489 students, only allowed the installation of a green roof on 65% of the total available roof area, because of structural load limitations of the building (Department of Environmental Safety, UMD, 2010).

In addition to the load limitations, extensive green roof substrates have been criticized for their high-embodied energy (Rustagi *et al.*, 2008). According to Elliot (2007), the embodied energy required for the manufacture of expanded shales, slates and clays (as a category) from the mining point to the manufacturer shipping point is approximately 1.34 million British Thermal Units (BTU's) (184.9 MJ/hl. In this process, mining and hauling demand 4.3%, the kiln utilizes 91.4%, and the sorting/screening step (possibly over estimated) requires 4.3% of the energy.

Because of the high-embodied energy required for the manufacture of expanded shales, slates and clays, and because of the expectancy of the industry and the communities for the development of lighter and more sustainable materials, some recycled products are being considered for amending green roof substrates. Recycled products are readily available and they have the potential of reducing the environmental impact of production and the manufacturing costs (Emilsson, 2008).

B.4.8. Vegetation Layer

When selecting plant material, certain aspects must be considered, for instance: the design intent, aesthetic appeal, local environmental conditions, plant characteristics, disease and pest resistance, and substrate composition and depth (Getter and Rowe, 2006). Some of the desirable characteristics for extensive green roof plants include: easy propagation, rapid establishment, and high ground cover density (White and Snodgrass, 2003). It is also important, for sustaining a full coverage, that the plants possess the mechanisms to perpetuate their propagation in the long term, as long as the environmental conditions are favorable (Getter and Rowe, 2006). In the United States, succulent, native and short grass prairie species are the major plant categories preferred for extensive green roofs (Snodgrass and Snodgrass, 2006; Sutton, 2009). A typical green roof plant installation is illustrated in Appendix Fig. A9.

B.4.8.1. Succulent Plants

The most adaptable green roof plant species have low growing habits, shallow and perennial root systems, and exhibit a high tolerance to extreme environmental and biological conditions (Snodgrass and Snodgrass, 2006). Succulent plants adapt well in these conditions (Getter and Rowe, 2006); for this reason, they are widely-used in extensive green roof projects.

According to Snodgrass *et al*. (2006), *Sedum*, *Sempervivum, Talinum, Jovibarba* and *Delosperma* are the best-adapted genera of succulents. *Sedum*, in particular, has shown the greatest survival in a wide range of conditions (Snodgrass and Snodgrass, 2006). Appendix Fig. A10 illustrates a mature extensive green roof planted with *Sedum* species. Hardy succulents have crassulacean acid metabolism (CAM), which allows the plants to increase the water-use efficiency by opening the stomata and storing CO_2 during the night, when evaporation rates are the lowest (Lüttge, 2004).

The genus *Sedum*, which belongs to the Crassulaceae family, seems to be facultative CAM species. Lee and Griffiths (1987) supported this theory by demonstrating that *Sedum telephium* has the potential to use the regular C_3 metabolism under well-watered conditions, and it expresses a transition to CAM when drought conditions are experienced. This includes a continuum of different stages of CAM expression that are repeatedly reversible under changing drought and watering regimes. The survival of *Sedum* species under extended drought conditions has been extensively documented. One of the outstanding species of this genus, *Sedum album* L (white stonecrop), has survived after more than 100 days without water (Lasalle, 1998). This is likely mostly attributable to this species ability to reduce water loss to a minimum under extreme conditions.

Occasionally, project owners and designers intend to increase the biological diversity of the green roof system by incorporating beneficial microorganisms. According to Brundrett (2009), the Crassulaceae family does not establish mycorrhizal associations; however, other succulent plants, such as members of the Agavaceae, Cactaceae and Euphorbiaceae families have arbuscular mycorrhizal roots (Brundret, 2009).

B.4.8.2. Native Plants and Short Grass Prairie Species

The selection of native and natural prairie species has being promoted for green roof installations in the Midwest regions of the US (Getter and Rowe, 2006). Native plants are already adapted to the existing climate but the potential fire hazard (particularly for grasses) represents a major concern for green roof installations on rooftops (Monterusso *et al.,* 2005). A second disadvantage is the high dependence of native plants for irrigation, since the majority of native plants rely on deep tap roots under natural conditions (Getter and Rowe, 2006). Through some plant selection studies, four native species were found to be well adapted to green roof conditions: *Allium cernuum* L. (nodding wild onion), *Coreopsis lanceolata* L. (lanceleaf coreopsis), *Opuntia humifusa* Raf. (prickly pear), and *Tradescantia ohiensis* L. (spiderwort), (Monterusso *et al.,* 2005). In addition to fire hazards, a disadvantage of green roof grasses is the access requirement for hard pruning before the onset of new growth and other basic maintenance activities (Snodgrass and Snodgrass, 2006).

The use of grasses is attractive for adding motion and texture to the design, and for providing habitat to birds and insects; however, because of the high biomass production requirements, a deeper layer of growing media is generally necessary to sustain this type of vegetation (Snodgrass and Snodgrass, 2006). As an exception, blue grama (*Bouteloua gracilis*) is better adapted to shallow rooting and high evapotranspiration environments, low rainfall, mycorrhizal symbiosis, and pulsed nutritional requirements (Sutton, 2009).

C. ***Investigating Crumb Rubber as a Potential Amendment for Extensive Green Roof Substrates***

C.1. Crumb Rubber and Zinc

Used automotive tire disposal is a major environmental concern. Each year, approximately 270 million automobile and truck tires are removed from service and scrapped in the United States (Geosyntec Consultants, 2008). Several programs, laws or regulations throughout 48 states in the US have encouraged the management of this waste (USEPA, 2010b). For example, in Maryland, the HB 1202 Scrap Tire Recycling Act, enacted in 1991, regulates the proper disposal of scrap tires (USEPA, 1999). Nowadays, this recycled product has several industrial applications. Geosyntec Consultants (2008) summarized the following uses:

- Alternative fuel source for electric generation;
- Fuel source for cement kiln operations;
- Raw material for the production of industrial and consumer goods;
- Raw material for landfills and septic systems;
- Lightweight fill material for embankment or retaining wall construction;
- Rubber mats in horse stalls;
- Sound barrier at highways and
- Rubberized asphalt.

Crumb rubber (CR), defined as rubber granules derived from a waste tire that are less than or equal to one-quarter inch or six millimeters in size, is a product that, among other applications, has been investigated to amend substrates in horticultural production (Newman *et al.,* 1997) turf grass and playground installations (Groenevelt and Grunthal, 1998).

Significant reductions in soil hardness and soil shear strength, as well as improvements in soil aeration and drainage have been reported with CR incorporation (Groenevelt and Grunthal, 1998). CR, as a potential lightweight amendment for extensive green roof substrates could reduce substrate loads, decreasing engineering costs for buildings (Anderson *et al.*, 2006) and may also improve the porosity (Ristvey *et al*., 2010) and longevity of many green roof substrates. However, it has also been noticed that plants growing in CR amended soils and substrates exhibit a high Zinc (Zn) foliar content (Groenevelt and Grunthal, 1998) and in some cases, the exposure to CR has resulted in yield reduction (Newman *et al.,* 1997) and phytotoxicity (Handreck and Black, 2005).

Zn is a bluish-white lustrous metal with atomic number of 30 and atomic mass of 65.38, constitutes between 0.0005% and 0.02% of the Earth's crust (Irwin *et al.*, 2007). Zn is widely used by industry is an essential element for all organisms (Landner and Lindeström, 1998). Mean Zn concentrations in soils range from 50 to 66 μg total Zn/g of soil, and plants require Zn as an essential component of proteins (Broadley *et al.*, 2007). High soil pH influences the availability of Zn because of increased adsorption to cation exchange sites (Broadley *et al.*, 2007). Zn toxicity in soils is uncommon; however, it is usually observed when the concentration in the leaves exceeds 300 μg Zn/g of dry weight (Broadley *et al.*, 2007). Plants capable of accumulating more than 3,000 μg Zn/g of dry weight in the shoots are considered highly tolerant to Zn, and a plant is classified as a hyperaccumulator if more than 10,000 μg Zn/g of dry weight in the aerial parts of plant species occurs under the natural growing environment (Broadley *et al.*, 2007).

Some polymers, metals and additives are incorporated to the natural and synthetic rubber elastomers during the tire manufacture in order to enhance performance (Geosyntec Consultants, 2008). Zn oxide is used as a vulcanizing initiator for rubber fabrication (Gordon *et al.,* 2003). Therefore, rubber tires contain between 2.5% (FLL, 2002) and 5% Zn (Handreck, 1996) as Zn oxide.

According to a generic six-reservoir cycle (Fig. 1.3) for mineral resources, the Zn cycle includes the following stages: source (extraction from the environment), processing, fabrication, use, waste management, and a sink reservoir (Gordon *et al.,* 2003). The information from this cycle provides a framework for resource management, environmental science, and policy analysis (Gordon *et al.,* 2003).

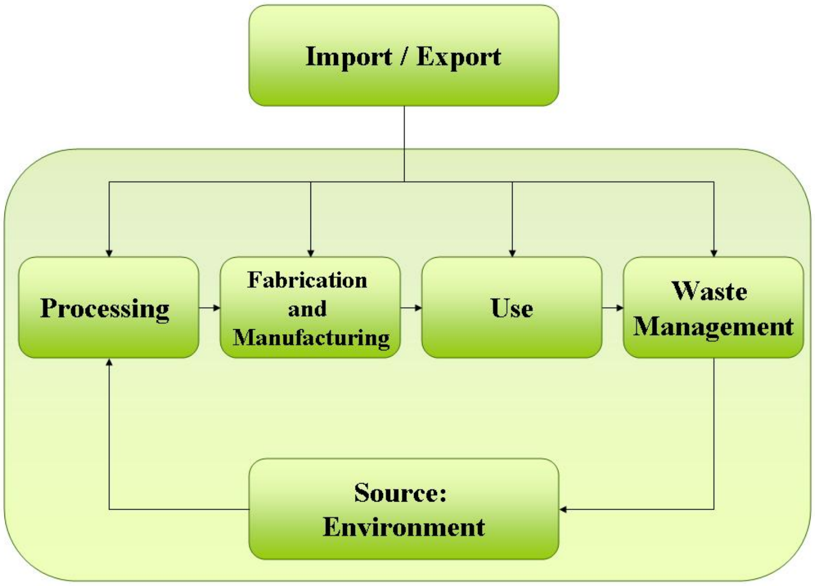


Fig. 1.3. The generic six-reservoir cycle for a mineral resource. Source: (Gordon *et al.,* 2003).

Presently, around 70% of the Zn produced worldwide originates from mined ores, and 30% is estimated to be from recycled or secondary Zn sources (International Zinc Association, 2010a). After the processing stage, Zn is used to fabricate coated steels, brass, Zn-based alloys and chemical compounds, among other products (International Zinc Association, 2010b). According to estimations in 2003, the final use of Zn occurs in the following sectors: construction (45%), transportation (25%), consumer and electrical goods (23%), and general engineering (7%) (International Zinc Association, 2003b). Moderate amounts of Zn leaches into the environment during the use phase (Gordon *et al.,* 2003). Fig.

1.4. exemplifies different sources of Zn dissipation that affect the quality of urban runoff.

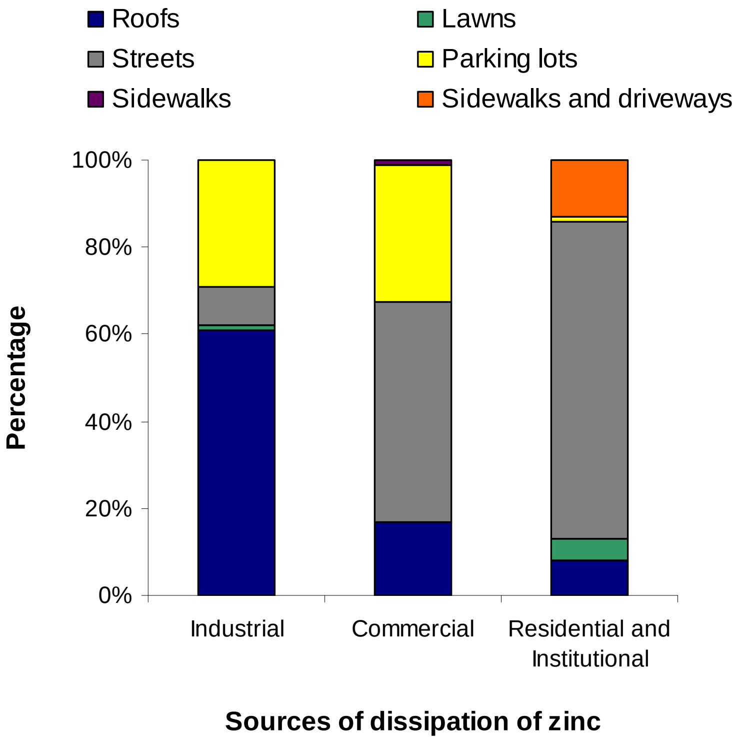


Fig. 1.4. Sources of total Zn in urban runoff. Adapted from University of Wisconsin-Extension, 1997.

The addition of Zn to aquatic environments imposes a hazard for plants and animals. This pollutant can cause mortality, growth retardation, and reproductive impairments to aquatic species (Eisler, 1993). The following levels are the national recommended water criteria for Zn. The Criteria Maximum Concentration (CMC) represents the highest concentration of a material in surface water to which an aquatic community can be briefly exposed without resulting in an unacceptable effect. The Criterion Continuous Concentration (CCC) refers to the highest concentration of a material in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect (USEPA, 2009).

Table 1.3. National Recommended Water Criteria for Zn. (USEPA, 2009).

	Freshwater		**Saltwater**		**Human Health for the Consumption of**	
Pollutant Category	**CMC (acute) (µg/L)**	**CCC (chronic) (µg/L)**	**CMC (acute) (µg/L)**	**CCC (chronic) (µg/L)**	**Water organism (µg/L)**	**Organism Only (µg/L)**
Prioritary	120	120	90	81	7400	26000

Several factors influence the bioavailability of Zn, for example: temperature, pH, water hardness, and the presence of other contaminants (Environmental Protection Division, British Columbia, 1999). For example, Zn is more toxic to fish in soft, acidic waters with low total alkalinity (Wurts and Durburow, 1992). The oral ingestion of 45 mg of elemental Zn per day has proven to be non toxic for human adults (Prasad, 1993). However, excessive Zn can promote copper deficiency, anemia, decreased levels of high-density lipoprotein (HDL) cholesterol (Mrini, 2003), pancreas damage, headache, and abdominal pain (Irwin *et al*., 1997).

Post-industrial and post-consumer Zn materials are reused, recycled, deposited in landfills, or lost to the environment. During waste management stages, few uses of Zn are suitable for easy recycling (Gordon *et al.,* 2003). With regard to rubber tires, an industrial technology for recovering the metal has not been developed, probably because the Zn concentration after the use stage is not significant compared to other materials such as brass and galvanizing residues (Gordon *et al.,* 2003).

The import and export of Zn divides products by category (Gordon *et al.,* 2003):

- Production: ore concentrate and smelted, and refined Zn.
- Semi-production: Zn ingot, sheet, plated steel, among others; to be used for final products.
- Final products: commercial and industrial products containing Zn or Zn alloy.

Import and export records are important to reflect the accumulation or depletion of regional Zn stocks (Gordon *et al.,* 2003). For example, during 1985 and 2005, Central and East Europe reduced its refined Zn demand by 809 kilotons, while China increased its' demand by 2599 kilotons (International Zinc Association, 2010c). From a sustainability aspect, the usage trend of Zn can not be sustained indefinitely because of the observed rates of depletion (Kesler, 1994). The reuse of Zn (processed, in-use or discarded) is highly recommended, as well as a critical reflection about the anthropogenic inputs of Zn into the environment (Gordon *et al.,* 2003).

D. *Objectives of this Research*

Several research objectives were developed within the framework of this study, to determine whether CR could be used as an environmentally safe amendment for extensive green roof substrates. These were:

- To quantify the amount of Zn released from CR.
- To determine if what conditions influence the release of Zn from CR.
- To determine the interactions between CR Zn and an expanded shale, slate and clay substrate mix (rooflite®).
- To compare any leachate from CR or amended substrates, with water quality parameters.
- To determine if *Sedum album* (L), *S. reflexum* (L) and *S. kamtschaticum* (Fisch) are tolerant to the amount of Zn released from CR amended substrates.
- To describe the response of *Sedum kamtschaticum* to elevated concentrations of Zn commonly used during hypertolerance studies.

Chapter 2: Substrate Based Studies

A. Introduction

Extensive green roof systems are designed primarily to mitigate storm water runoff from impervious surfaces in dense urban areas. Additionally, extensive green roof systems have other proven ecological and economic benefits, for example energy conservation, mitigation of the urban heat island effect, and improvements in urban aesthetics (Dunnet and Kingsbury, 2008).

A key design component of extensive green roof systems are lightweight substrates, usually made from heat-expanded shales, slates and clays. These substrates are able to buffer a large proportion of a typical rainfall event, thereby mitigating runoff from urban areas (Getter and Rowe, 2006). The physical properties of extensive green roof substrates, primarily particle size, are an important determinant of water-holding capacity and air-filled porosity. These characteristics, when adequately balanced, allow for the development of healthy plant root systems and optimal stormwater holding capacity.

Crumb rubber (CR), a recycled material from scrap tires, has been suggested as a lightweight amendment for green roof substrates. To some extent, the use of CR in the green roof industry could alleviate a major environmental concern. Each year, over 270 million automobile and truck tires are disposed of in the United States (Geosyntec Consultants, 2008). CR amendments in extensive green roof substrates have been demonstrated to improve air-filled porosity while reducing substrate weight. This might increase the potential for retrofit of green roofs on older buildings (Ristvey *et al.*, 2010).

However, a disadvantage that needs to be overcome is the presence of zinc (Zn) in CR. Tires containing between 2.5% (FLL, 2002) and 5% Zn (Handreck, 1996) as Zn oxide could represent a major input of Zn in urban runoff, which could exceed water quality standards at the point of discharge (University of Wisconsin-Extension, 1997). To date, 489 cases of Zn water impairment have been reported in the List of Specific State Causes of Impairment Other Than Mercury (USEPA, 2010c). Geosyntec Consultants (2008) reported that the release of Zn from tire-derived materials over 20 months was below the allowable effluent concentration (no specific concentration noted) from a 2 ft (0.6 m) tire chip layer in a contained landfill. Soluble Zn from CR used in green roof systems could therefore be toxic for the growth of *Sedum* species, and it could also represent a pollutant for aquatic environments at relatively low concentrations (USEPA, 2009).

High levels of Zn have been reported when growing plants in ground tire amended products (Handreck and Black, 2005; Newman *et al.* 1997; Zhao, 1995; Bowman *et al.*, 1994). In these cases, the negative results of CR possibly occurred as a combined effect of several factors, which determined the availability of Zn in the soil or substrate solution. For example, the original tire Zn content, the volume of the amendment, the pH of the soil and solution, and the cation exchange capacity (CEC) of the substrate materials could contribute to Zn availability.

CEC refers to the ability of the substrate to exchange and retain positively charged ions.

Zn toxicity risk increases when the substrate is kept below a 6.5 pH (Newman and Meneley, 2005) and when combined with substrates of a low cation exchange capacity (CEC). The source materials in many green roof substrates (expanded shales, slates, clays and organics) have chemical characteristics that determine CEC (Austin, 2006). A commercial substrate, (rooflite®, Skyland USA, Avondale, PA) containing 80% expanded shale, slate and clay, with less than 65 g/L of organic material was utilized during this research. Based on an analysis conducted by A&L Eastern Laboratories, Inc., the average CEC of rooflite® is 7.45 meq/100 g at a pH of 7 (Appendix B1). Compared to many soil colloids, this CEC is low, but it represents a moderate CEC for many green roof substrates according to Soil Control Lab and Turf Diagnosis and Design, cited by Green Roofs for Healthy Cities (2008).

Under natural conditions, Zn is adsorbed by soil sediments and organic components in aquatic ecosystems (He *et al*., 2005), as a consequence of cation exchange capacity. Three important questions therefore arise when considering the use of CR as a potential amendment for extensive green roof substrates:

1) how much Zn is actually released from CR, over time?
2) how does Zn interact with the green roof substrates?, and
3) how much Zn could be expected to leach from a commercial green roof amended with CR?

To understand and quantify the dynamics between CR and substrates made of expanded shales, slates and clays, three experiments were conducted to investigate the following objectives:

- Quantify the rate and total release of Zn that could potentially leach out of CR (8-12 sieve mesh) in two different pH treatments over a 16 day (384-hour) period.
- Determine if substrates made of expanded shales, slates and clays have the ability to adsorb Zn released from CR amendments, over time.
- Quantify the amount and concentration of Zn in leachates from several substrate mixes, including CR, rooflite® and 3 mm glass beads (Walter Stern, Inc., Port Washington, NY).

B. Methodology

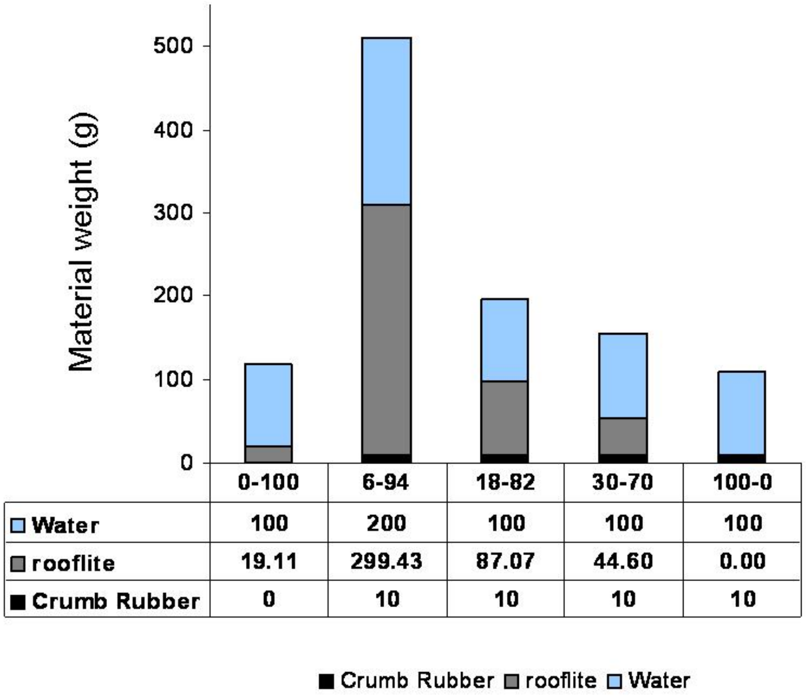
B.1. Experiment 1: **Quantification of Zn release over time from crumb rubber exposed to acidified and non-acidified reverse-osmosis water solutions**

Exactly 10 grams of CR (8-12 sieve mesh) was weighed and placed in 60 replicate 125 ml Erlenmeyer flasks, 10 for each of six sample times (12, 24, 48, 96, 192 and 384 hours). Two water treatments, namely untreated reverse osmosis (RO) water (pH 5.5), and RO water adjusted to a pH of approximately 4.1 with sulfuric acid, were prepared. Half of the 60 flasks were filled with 50 ml of RO water and the other half were filled with 50 ml of acidified RO water. Acidified water was used as a treatment based on Lynch *et al.,* (2004), who consistently recorded pH values of 4.4 from rainwater in central Pennsylvania. All flasks were sealed with parafilm. At each sample time, five replicate flasks per water treatment (10 total) were filtered, and the resulting supernatant decanted into 20-ml scintillation vials, and frozen until analyzed for available Zn by Inductively Coupled Plasma (ICP) spectrometry at the University of Delaware Soils Testing Laboratory (Newark, DE). Leachate results from each sample period were analyzed with a Mixed Procedure, ANOVA to determine statistical significance between treatments (SAS v. 9.1; SAS Corporation, NC).

The null hypotheses established for this experiment were: (1) the cumulative amount of Zn released from CR would not be significantly different after 16 days of immersion in acidified and non acidified RO water, and (2) the rate of Zn release would be equivalent at all sample times.

B.2. Experiment 2: Adsorption of Zn in crumb rubber amended green roof substrates

Five proportions of CR (0%, 6%, 18%, 30% and 100% by volume) were combined with a green roof substrate (rooflite®) in 300 ml flasks, with 10 replicates per treatment, sampled at four times during the study. The weight of CR was constant (10 g) and the mass of rooflite® was adjusted according to the treatment ratio. One hundred ml of RO water was added to each flask (200 ml for the 6% treatment). The pH of the water was adjusted to 5.5 before addition to treatments. Fig. 2.1 indicates the exact weight of each component combined to achieve the proportions of interest.



	0-100	6-94	18-82	30-70	100-0
☐ Water	100	200	100	100	100
▨ rooflite	19.11	299.43	87.07	44.60	0.00
■ Crumb Rubber	0	10	10	10	10

Fig. 2.1. Mass of water, rooflite® and CR combined to formulate and saturate five different proportions of CR amended substrates.

Note treatment nomenclature: the first number corresponds to the volumetric percentage of CR and the second to the volumetric percentage of rooflite®.

Water samples were decanted from the replicate flasks at 0, 2, 8 and 16 days after the start of the experiment, each sampling day having a separate set (n=10) of treatment replicates. Flasks were agitated 2 hours before sampling to prevent any stratification of the substrate/supernatant. Supernatants were sampled as described in the previous experiment and Zn sample analyses were performed at the University of Delaware Soil Testing Laboratory, as previously noted. The resultant Zn concentration values were normalized (by multiplying by the expressed solution volume) to obtain Zn content per gram of CR. A mixed PROC

ANOVA was used to determine statistical significance between the Zn content of the various treatments (SAS v. 9.1).

The null hypotheses established for this experiment were: (1) there would not be a significant difference in available Zn, between treatments and the 100% CR control, and (2) the availability of free Zn would not be significantly decreased with increasing proportions of rooflite®.

B.3. Experiment 3: Quantifying available Zn leachates from green roof substrates, with and without crumb rubber

Three different substrate mixes were formulated: (1) 30% glass beads and 70% rooflite®; (2) 30% CR and 70% rooflite®, and (3) 30% CR and 70% glass beads. Glass beads are made of borosilicate glass; this material possesses a very low intrinsic cation exchange capacity, however, it has a limited ability to adsorb some heavy metals in soils (Kim and Hill, 1993). Compared to rooflite®, glass beads are considered to have almost no cation exchange capacity (see Appendix B1 and B3 for laboratory results). Each treatment was replicated six times. Each replicate consisted of one 4” Oyama pot (AV Planters, San Lorenzo, CA). Oyama pots are bottom-watering planters, having an inner pot, which serves as a container for the substrate, and an outer pot, which is the water reservoir (Fig. 2.2). In this experiment, the outer pot allowed for the repeated collection of leachate samples, over time.

The pots were filled to 83% of their volume capacity. At the start of the study, 250 ml of RO water was added to each pot from the top, to imitate a saturating rainfall. This volume of water simulated optimal growing conditions for the development of *Sedum* species, based on additional experimentation (see Chapter 3).

The outer pot's function is comparable to a modular drainage layer in a green roof installation, as it allows the stored water to be taken up into the substrate by capillary action. The properties of adhesion and cohesion of water and solid particles are influenced by the size of the pores and the height of the column. It is therefore likely that the water content in the pots exhibited a gradient from the bottom (more saturated) to the top (more dry). The conditions of this experiment, although artificial, were intended to simulate water dynamics in a green roof with regular rainfall. The experimental units were randomly arranged in a growth chamber with an average temperature of 23 ˚C, 60% relative humidity.

In contrast to outdoor and greenhouse environments, the use of a growth chamber allows for the maintenance of constant environmental conditions. These variables influence photosynthesis, evapotranspiration and growth rate which could alter the concentration of Zn in leachates and in plant tissue. By reducing environmental variability, we presume the accuracy of the experimental results is improved.

The surfaces of the pots were covered to prevent evaporation. One week after the start of the study, the pots were taken out of the growth chamber and substrate leachates were sampled. The treatments were sorted by anticipated Zn concentration, from low to high, to prevent potential cross-contamination when collecting the leachates.

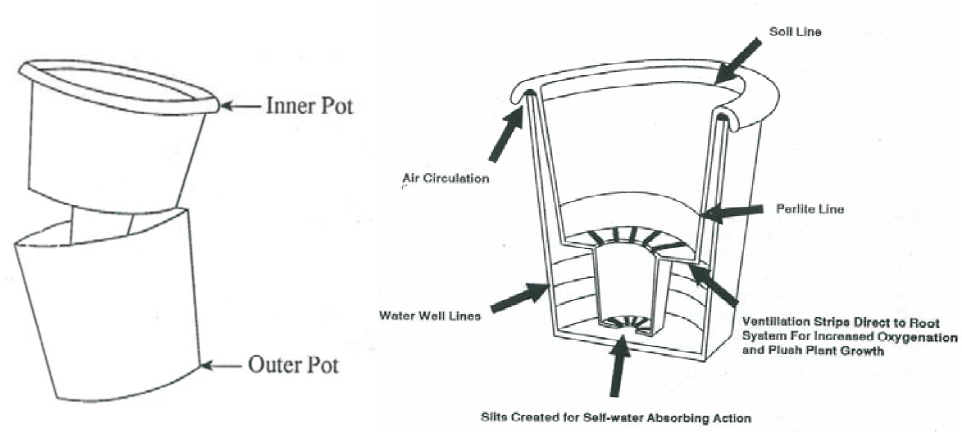


Fig. 2.2. Oyama pot description. Source: oyamaplanters.com (2006).

Fig. 2.3. Addition of 250 ml of RO water to Oyama pot containing the treatment of 30% CR and 70% glass beads.

The individual volume of the leachates was measured each week by using a 250 mL graduated cylinder. Initially, a measured volume of 40 ml was collected, and used to rinse the walls of the graduated cylinder to prevent cross-contamination of samples; this first aliquot was then discarded. The remaining leachate was then measured and a 20 ml subsample was taken for Zn analysis. After measurement and sampling all replicate sets from each treatment, the graduated cylinder was washed with soap, repeatedly rinsed with RO water and dried. The next six replicates of the following treatment were then sampled, as previously described. When all leachates from all treatment replicates had been collected, measured and sampled, the pots were refilled with 250 mL of fresh RO water and re-randomized back in the growth chamber under the same aforementioned conditions. This procedure was repeated every week for twelve weeks, resulting in 72 samples per treatment.

The data from this experiment were not normally distributed and exhibited heterogeneous variance, which could not be corrected by transformation. For this reason, a non-parametric General Linear Models Mixed ANOVA with repeated measures (SAS v. 9.1) was used to determine the statistical significance between treatments over time.

The null hypothesis established for this experiment was that were would be no significant difference in the availability of Zn between the treatments.

C. Results

C.1. Experiment 1: Quantification of Zn release over time from crumb rubber exposed to acidified and non-acidified RO water solutions

The analysis of the water samples indicated that Zn leaches from CR in a relatively linear fashion over time after the first 12 hours, up to 16 days (Fig. 2.4). The final cumulative release of Zn per gram of CR averaged 64.7 µg (± 6.51 SE) and 53.9 µg (± 4.62 SE) with the acidified and non-acidified RO water, respectively. During the first 12 hours, significantly more Zn was released from the acid water treatment ($P < 0.05$), but thereafter no significant differences were found in the cumulative Zn leached between either pH treatments. Interestingly, the results exhibited a relatively large sample to sample variation.

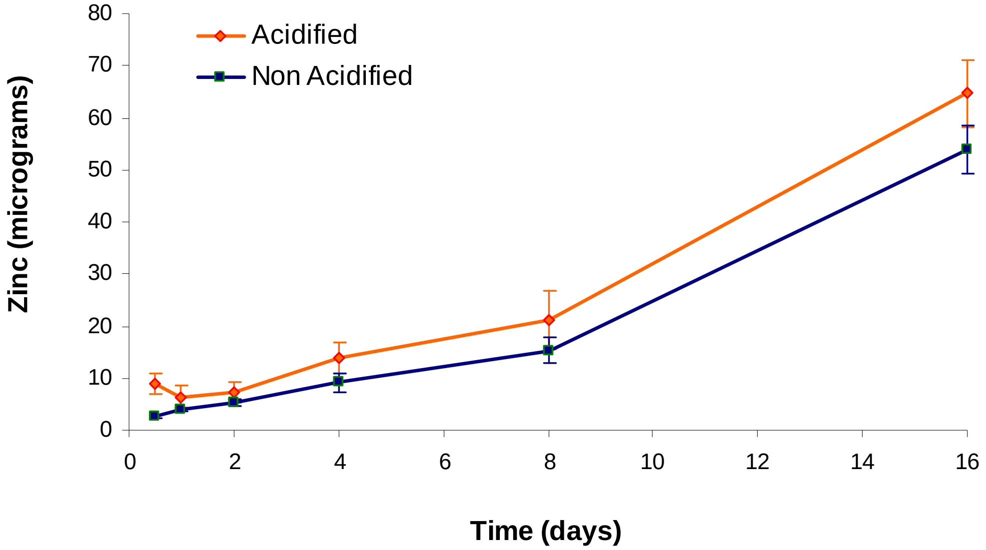


Fig. 2.4. Micrograms of Zn released from CR for different exposure times in acidified and non-acidified solutions.

Fig. 2.5 illustrates that Zn was initially released at an average rate of 0.74 (± 0.167 SE) μg Zn per gram of CR per hour during the first 12 hours in the acidified RO water treatment. This initial rate of release was significantly different from CR soaked in non-acidified RO water ($P<0.05$), which leached at a rate of 0.22 (± 0.002 SE) μg Zn/g CR/hr. Similar to the cumulative Zn release results after 12 hours, the Zn release rate of both treatments was not significantly different. Both rates of Zn released per gram of CR decreased and stabilized after the first 12 hours (Fig. 2.5).

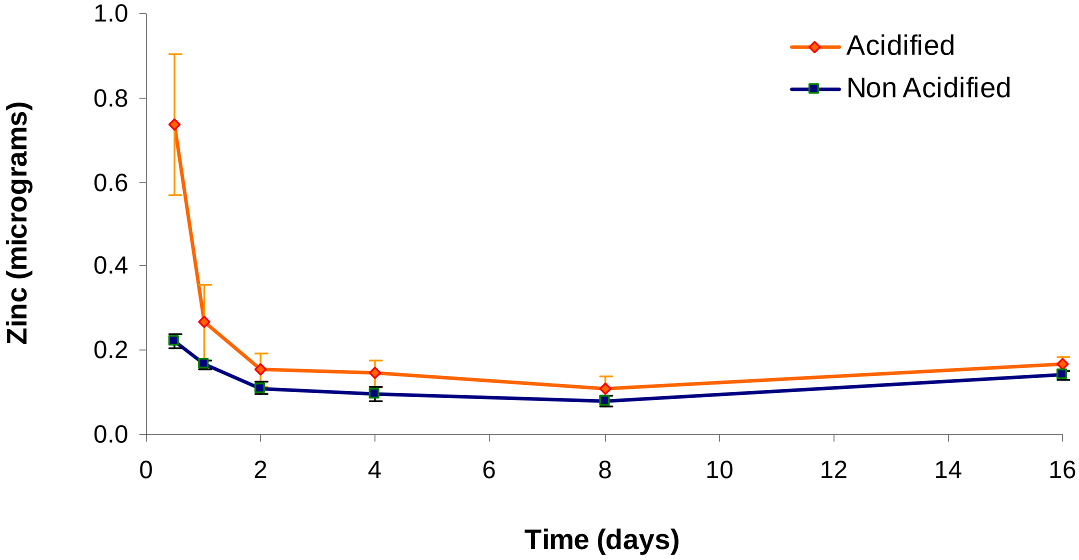


Fig. 2.5. Micrograms of Zn released per hour from rubber crumb exposed to different times in acidified and non-acidified solutions.

C.2. Experiment 2: **Adsorption of Zn in crumb rubber amended green roof substrates**

The average amount of available Zn released in µg per gram of CR over time is shown in Fig. 2.6. After 16 days, the 100% CR control leachate contained 99.5 (± 2.63 SE) µg/g CR, while the 30% CR treatment exhibited only 1.1 (± 0.16 SE) µg Zn/g CR (Fig. 2.6). This demonstrates that all treatments containing rooflite® were able to reduce the amount of available Zn by nearly 100 times.

Although the 100% CR treatment response was evidently different from the rest of the treatments, it imposed limitations for statistical analysis. In general, the data from this experiment were highly variable because of the treatment intervals chosen, preventing us from meeting the assumptions for analysis of variance (ANOVA). Successful data transformation was only possible when the 100% CR treatment was completely excluded from the data set. In order to therefore identify significant differences in the total average release of Zn and the average Zn release rate between the lower levels of CR, the 100% CR was excluded from analysis and a $\log_{10}$ data transformation was performed.

Significant differences for the total average release of Zn (µg Zn/g CR) at study initiation (time zero) were found between all treatments except 6% and 30% CR ($P< 0.05$). Two and eight days after study initiation, all differences in Zn release were significant among treatments. At the end of the experiment (day 16), all the treatments except 18% and 30% were significantly different. The rate of Zn released for each treatment over the 16-day period, expressed as µg of Zn per gram of CR per hour, is depicted in Fig. 2.7. Zn concentrations were measured at each sampling period, and normalized for Zn content by multiplying with sample volume collected. Rates were extrapolated by dividing the Zn content by the intervals of time between samples.

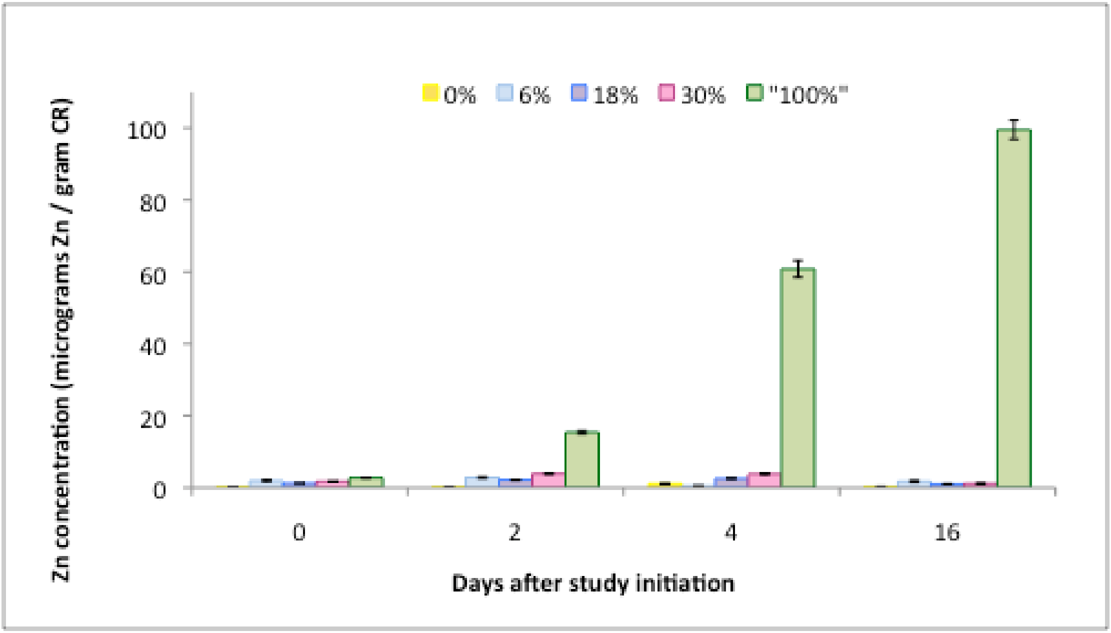


Fig. 2.6. Zn availability in the leachate from five volumetric proportions of Zn (CR) and rooflite® during sixteen day study period.

As can be seen from Fig. 2.7, Zn release rates at the initiation of the experiment were higher in comparison to the subsequent sampling periods; the 100% CR control treatment release rate was substantially higher than all other treatments levels. The rates for all treatment levels decreased by more than ten-fold their initial value within 48 hours compared to the 100% CR control, which continued to have a substantially higher release rate throughout the study. During the final 8-day period (day 9 through day 16), the 30% CR proportion exhibited an average release rate of 29 ng Zn/g CR/hr.

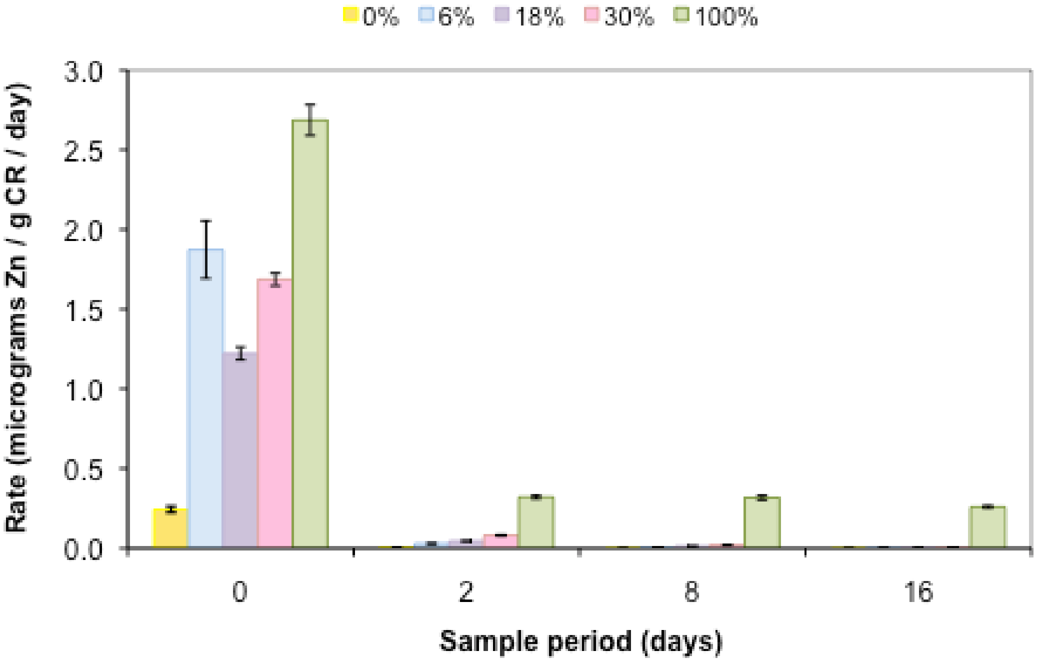


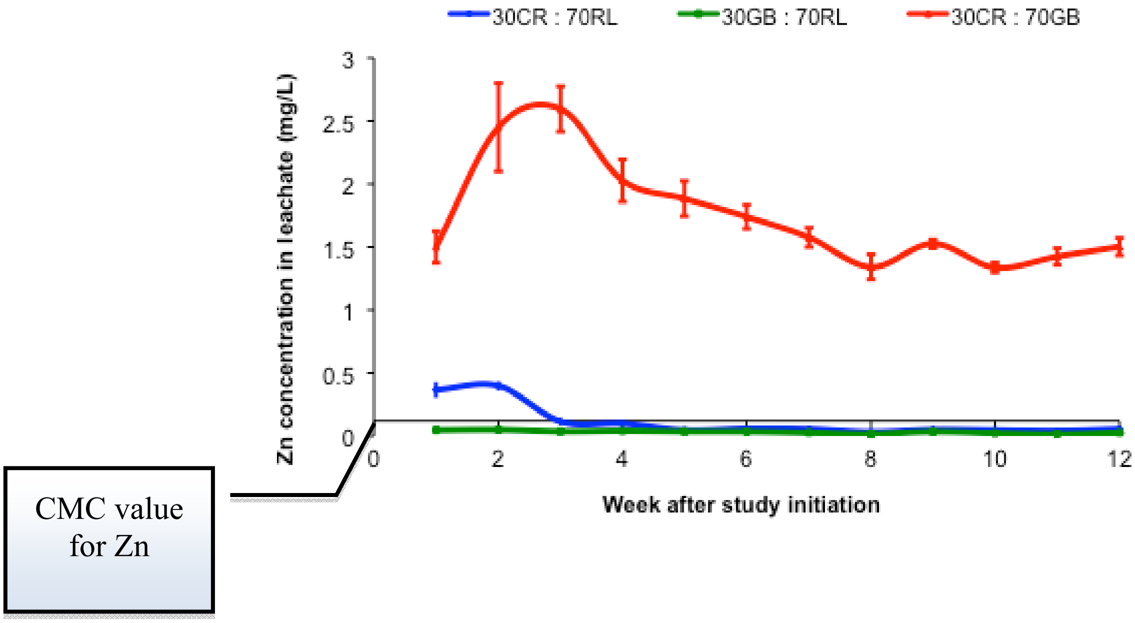
Fig. 2.7. Rate (µg/hr) of Zn released per gram of CR extrapolated between each sampling period, from five volumetric proportions (%) of CR and rooflite® during a sixteen day (384-hour) study period.

C.3. Experiment 3: Quantifying available Zn from leachates of substrates, with and without crumb rubber

Leachate Zn concentrations (mg/L) of the three formulated green roof amended substrates are shown in Fig. 2.8. The following treatment nomenclature are used from now on: 30CR : 70RL corresponds to 30% CR & 70% rooflite®; 30GB : 70RL to 30% glass beads and 70% rooflite®; 30CR : 70GB to 30% CR and 70% glass beads (volumetric proportions). The CMC (Criteria Maximum Concentration) value for Zn in freshwater is 0.12 mg/L (USEPA, 2009) and is indicated as a straight line in Fig. 2.8. A CMC value represents the highest concentration of a material in surface water to which an aquatic community can be briefly exposed without resulting in an unacceptable effect (USEPA, 2009). Our results showed that the 30% CR : 70% GB (control) treatment exceeded this CMC value at all sampling times, while the 30% CR : 70% RL treatment exceeded this threshold only during the first two weeks. The 30% GB : 70% RL treatment remained below this threshold at all sample times.

The highest leachate concentration in the 30% CR : 70% GB treatment (i.e. 2.60 mg Zn/L ± 0.180 SE), was observed during the third week. After this time, Zn concentrations decreased substantially. The minimum concentration of Zn from this treatment occurred during the 10th week with 1.34 mg Zn/L (± 0.038 SE). The highest concentration in the 30CR : 70RL treatment was 0.40 mg Zn/L (± 0.037 SE) and occurred during the second week. After this time, all concentrations were below the CMC value. The minimum Zn release occurred in the 8th week, and corresponded to 0.03 mg Zn/L (± 0.004 SE). The highest concentration in the 30GB : 70RL treatment occurred during the second week and the minimum during the 11th week, of 0.05 mg Zn/L (± 0.003 SE) and 0.02 mg Zn/L (± 0.002 SE), respectively.

The non-parametric repeated measures ANOVA indicated that the mean leachate Zn concentration from the 30CR : 70GB treatment was significantly different than the 30CR : 70RL and the 30GB : 70RL ($P < 0.05$).



CMC value for Zn

Fig. 2.8. Leachate Zn concentration (mg/L) from CR, rooflite® and glass beads formulations.

Fig. 2.9 illustrates the amount of Zn released from CR (from the 30CR : 70GB and the 30CR : 70RL treatments), and the amount of Zn released from rooflite® in the 30GB : 70RL treatment. Note that although CR was the main source of Zn in this study, rooflite® did contain a small amount of natural Zn. An analysis of rooflite® conducted by A&L Eastern Laboratories Inc (City, State) determined the average Zn concentration in rooflite® was 15.7 mg/Kg (Appendix B2).

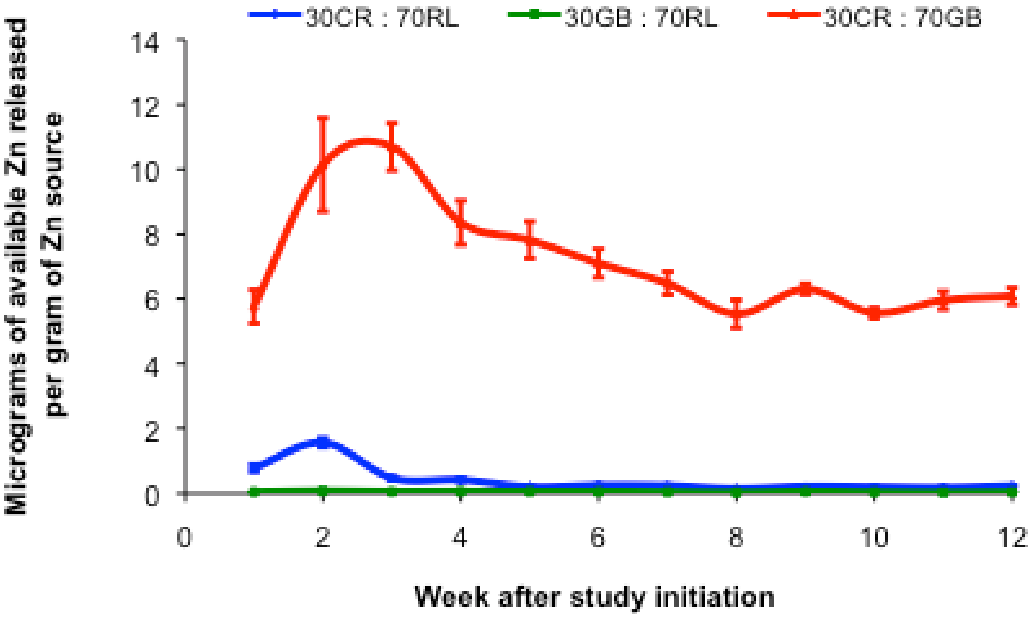


Fig. 2.9. Net Zn released (μg) from either crumb ruber (30CR : 70RL and 30CR : 70GB treatments) or rooflite® (30 GB: 70 RL treatment).

A general comparison of the maximum values observed during the experiment indicates there was 10.70 μg Zn/g CR (± 0.729 SE) in the leachate of the 30CR : 70GB treatment, compared to 1.57 μg Zn/g CR (± 0.145 SE) in the 30CR : 70RL treatment. In the GB : RL treatment, the maximum amount of available Zn was 0.06 μg Zn/g RL (± 0.004 SE). The non-parametric analysis indicated the Zn availability (μg) per gram of metal source was significantly different between 30CR : 70GB treatment and the 30CR : 70RL and 30GB : 70RL treatments ($P< 0.05$), during the entire experiment.

As a general example, App. Table B.4 presents the analysis of variance conducted for the analysis of this experiment.

D. Discussion

D.1. Experiment 1: **Quantification of Zn release over time from crumb rubber exposed to acidified and non-acidified RO water solutions**

During the first study, we found that Zn was leached from CR and that the acidity of the water can increase the rate of release during the first few hours of saturation in RO water. As seen in soils, the solubility of Zn increases at low pH (Broadly *et al.*, 2007). However, no significant differences in the final cumulative Zn release were noted after 16 days of saturation between the acidified and non-acidified treatments. Neither the cumulative Zn release nor the rate of Zn released per hour showed significant differences after the first 12 hours in the two water treatments.

Pollutants are generally released during two stages of a product's life cycle: early and late life (Clark et al., 2008). From this experiment, it is presumed that the likelihood of phytotoxic effects or the occurrence of anthropogenic contamination in a CR amended green roof would be higher after the first flush of water (rain or irrigation), especially in locations where acid rain occurs. Over time, it appears that the effect of the acidified water (rainfall) would not be different compared to non-acidified rainfall. It also seems plausible that rubber crumb incorporated into green roof substrates with the same proportion of CR would release the same amount of Zn over time, if the pH of rainfall were similar to our experimental conditions. We did observe a large sample to sample variation in this study, which may possibly reflect the variability of analysis, variability of sampling, or most likely, the variability of Zn in the rubber crumb samples (e.g. different brands or origins of tires).

D.2. Experiment 2: **Adsorption of Zn in crumb rubber amended green roof substrates**

In the second study, an interaction was noted between the Zn released from CR and the mineral and organic particles of rooflite®. This specific green roof substrate had the capability to almost totally adsorb the Zn released from 30% CR, incorporated on a volume basis. From these results, it appears likely that rooflite® could mitigate the potential negative effects of Zn released from CR, at least during the first three months of the life of a green roof.

Extrapolating from these results, a green roof with a 30% CR could potentially release an average of 15.4 mg of Zn per square meter in 16 days if consistently saturated. Under real green roof conditions, the Zn release would be affected by several external factors, including plant uptake, weathering and exposure, storm water pH, volume, frequency and duration of the rainfall events, and total cation adsorption ability of rooflite®. With an average CEC of 7.45 meq/100 g, rooflite® demonstrated the capability to absorb the majority of the relatively low amounts of Zn released from CR under these experimental conditions. This adsorption response is very important for preventing Zn phytotoxicity effects from CR in plants, and will be further discussed below, and in Chapter 3.

However, we do not know the time it would take for Zn to be completely depleted from CR. Consequently, the exchange sites in rooflite® could quickly become saturated. In the worse-case scenario, excessive Zn could become available in solution and high enough concentrations to affect the growth of the green roof plants. Furthermore, if substantial Zn would be leached out from the green roof system, it would be considered as a source of anthropogenic pollution. It should be noted that the 18% CR and the 30% CR treatments were not significantly different from each other. This response suggests that by amending a substrate with 30% CR instead 18%, the bulk density of the substrate could be decreased without adding more significant Zn to the leachate solution. Ristvey *et al*., (2010) noted that rooflite® amended with 18% CR reduces the bulk density of rooflite® by 6%, while maintaining water holding capacity and air filled porosity characteristics within FLL limits (FLL, 2002).

D.3. Experiment 3: Quantifying available Zn from leachates of substrates, with and without crumb rubber

The last study's results were very consistent with the previous findings. The 30CR : 70GB (control) treatment released a significantly higher concentration of Zn, compared to the 30CR : 70RL and 30GB : 70 RL treatments.

These last two treatments were not significantly different from each other according to the non-parametric analysis. These results are important since the average concentration of the 30CR : 70RL treatment was very close to the criteria maximum concentration (USEPA, 2009) during the first two weeks, and after that, it was always below the CMC level (Fig 2.8). From these results, the Zn leachate from an urban green roof system amended with 30% would be insignificant particularly considering this concentration would be quickly diluted with additional runoff water at the point of discharge.

A significantly higher amount of Zn was released from the 30CR : 70GB treatment, since the glass beads had little or no cation adsorption capability. Interestingly, the amount of Zn released from the 30CR : 70RL and the 30GB : 70RL was not significantly different between treatments. The low level of Zn leached from the 30GB : 70RL treatment was released by the shale component, which is derived from fine sediments of inorganic and organic origin, and are known to contain larger amounts of trace elements including Cu, Zn, Mn, Pb, and Cd. (He *et al*., 2005).

In summary, rooflite® was shown to adsorb substantial quantities of Zn leached from CR. As demonstrated by the third study, rooflite® maintained the ability to adsorb Zn released from CR for up to three months, even though laboratory analysis reported a relatively low CEC for this product. Other green roof substrates have claimed relatively higher CEC's; those substrates may therefore be ideal for further research in the use of CR and Zn sequestration.

Chapter 3: Plant – Substrate Interactions

A. Introduction

Low impact development techniques are now the preferred method of stormwater management in new and urban redevelopment projects (Andrus *et al.*, 2009). The green roof industry grew by 35% in North America in 2008 (Green Roofs for Healthy Cities, 2009). Increased interest in green roof systems for urban stormwater management has prompted the need for research to determine green roof plant/substrate interactions and the efficacy of these systems for urban stormwater remediation (Snodgrass, 2006, Berghage *et al.,* 2009; Clark *et al.*, 2008; Getter and Rowe, 2006).

Substrates are fundamental to green roof system performance. They provide the matrix to sustain plant growth and to reduce peak flows during rainfall events (Getter and Rowe, 2006). Most commercial green roof media combine a lightweight aggregate like expanded clay, slate or shale (80-90% by volume) with organic components (10-20% by volume or 2-5% by weight). Crumb rubber (CR), a product made from recycled tires is a potential light-weight amendment that could reduce substrate weight, decreasing live loads of green roofs and engineering costs for buildings (Anderson *et al.*, 2006); they may also improve the porosity and longevity of many green roof substrates. However, tire formulations are known to contain between 2.5% (FLL, 2002) and 5% Zinc (Zn) (Handreck, 1996), primarily as Zn oxide, which is used as an activator in the vulcanization process (Li *et al.*, 2007). The Zn content of CR may therefore preclude large additions to green roof substrates unless the Zn is adsorbed by the substrate and/or *Sedum* species can tolerate or hyperaccumulate the metal in large quantities.

Previous studies have reported negative effects in ornamental plants when CR was incorporated into substrates (Newman et al., 1997; Handreck and Black, 2005). According to the results from our previous studies (Chapter 2), Zn was effectively leached from CR, but adsorbed in the most part by the substrate, presumably as a result of substrate cation-exchange capacity. However, some available Zn remained in solution and could be a source of toxicity to green roof *Sedum* plants and aquatic life.

In order to determine if *Sedum* plants can tolerate CR amendments up to 30% (maximum volumetric amendment evaluated during this thesis), an experiment was conducted with the following objectives:

- To determine if the plant growth quality of three *Sedum* species was affected by increasing proportions of CR amendments to a typical green roof substrate.
- To ascertain if plant growth and dry mass are reduced when plants were exposed to CR amended substrates.
- To quantify the shoot Zn content of the three species exposed to CR amended substrates, as well as the root Zn content of one of the species.

Two additional hydroponic experiments were conducted to determine if *Sedum kamtschaticum* (Fisch.) is a hyper-tolerant species. *S. kamtschaticum* is a vibrant green species with rapid growth rate, commonly use to create a dense plant carpet (Stephenson, 2002). It belongs to the subgenus *Sedum*, Aizoon group, which presents the following characteristics: herbaceous flat leaves, thin stems with a maximum height of approximately 6 inches (15 cm), and a thick woody rootstock (Stephenson, 2002).

Fig. 3.1. Shoot and roots of *Sedum kamtschaticum.*

In addition to the desirable morphologic characteristics of this plant, which makes feasible to collect samples from both shoot and root tissues, *S. kamtschaticum* was the species that showed the smallest natural variation in the first experiment. Furthermore, this is one of the plants most commonly chosen for green roof installations in the United States (Snodgrass, 2009).

The objectives of this experiment were:

- To determine if *S. kamtschaticum* would express phytotoxicity symptoms as a product of elevated levels of Zn in either of the two substrates.
- To compare the effects of the substrate composition on plant growth.

- To compare the effect of the substrate composition and Zn levels on shoot and root tissue Zn content.

B. Methods and Materials

B.1. Experiment 1: **Tolerance of *Sedum* spp. to various ratios of crumb rubber amendments in green roof substrate**

Three *Sedum* species, *S. album* (.L), *S. reflexum* (L.), and *S. kamtschaticum*, were grown in a green roof substrate (rooflite®, Skyland USA, Avondale, PA) containing primarily expanded shale, slate and clay, with less than 65 g/L of organic material. The substrate was amended with 0%, 6%, 12%, 18%, 24%, or 30% CR by volume. Each substrate was mixed in a large container and was then subsequently distributed among replicate containers. Ten replicate *Sedum* plugs per treatment combination (180 experimental units) were placed in 10 cm (4 inch) containers in an incompletely randomized block design; plants were then established for one month (Fig. 3.2).

Fig. 3.2 *Sedum album, S. reflexum* and *S. kamtschaticum* plants arranged in an incompletely randomized block design in the greenhouse.

During the first 6 weeks of the study, plants were fertilized weekly with 200 mg N/L using a soluble 20-4.4-16.6 (N : P_2O_5 : K_2O) fertilizer, with microelements. This fertilization regime applied less than 50 μg chelated Zn to each plant over the whole study.

Five months after the start of the study, the Foliar Volume Index (FVI) was estimated by using the following formula:

FVI (cm^3) = (H x W x D) x QF

where height (H), width (W) and depth (D) were measured in cm, and "QF" represented a subjective quality factor to express the degree of leaf coverage inside the three-dimensional space. The quality factor ranged from zero (no foliar area) to 0.99. At the termination of the study (immediately after FVI determination), plants were harvested, with shoots dried in an oven at 60 C degrees for 96 hours and weighed for dry mass. Leaves were not separated from stems due to insufficient dry mass for laboratory analysis. The shoot Zn concentration of *S. kamtschaticum* was determined by ICP analysis at the University of Delaware Soil Testing Laboratory (Newark, DE).

Our null hypotheses were: (1) the FVI of each species would not be significantly different between the 0% control and the four treatment levels; (2) no significant difference would be found between any *Sedum* species dry mass of the 0% control and the four treatment levels; and (3) no significant difference in (a) shoot Zn concentration or (b) shoot Zn content would occur between the 0% control and the four treatment levels of *S. kamtschaticum*.

The final leaf volume index measurement was subjected to regression analysis, and dry mass and Zn results were analyzed by the Mixed Procedure, ANOVA (SAS v. 9.1; SAS Corporation, NC).

B.2. Experiment 2: **Response of *Sedum kamtschaticum* to elevated doses of Zn in two different substrates under hydroponic conditions**

This experiment was conducted to determine the Zn tolerance of *S. kamtschaticum* during a 90-day hydroponic study between April and June 2009, comparing two substrates and three Zn levels applied in an otherwise balanced Hoagland's solution. This experiment was conducted simultaneously under greenhouse and growth chamber conditions, with the purpose of ascertaining any growth differences due to differing environmental conditions.

The growth chamber (Conviron Model DDR36; Conviron, Winnepeg, Canada) was programmed to maintain, on average, the following conditions:

- Day / night length: 12 hours.
- Temperature: 23 ˚C
- Relative humidity: 60%
- Light intensity: #4 (500 ± 20 μmol/m^2/s).

While the greenhouse showed greater environmental variation, the growth chamber environment conditions were held constant during the course of the experiment.

This experiment was restricted to only once species because of its intensive nature. *Sedum kamtschaticum* was selected because it showed the smallest variability during previous experiments, with the most sensitivity to Zn uptake. Plants in a dormant state were transplanted from plugs and allowed to establish in 4” Oyama pots (AV Planters, San Lorenzo, CA) containing either rooflite® green roof media (rooflite®, Skyland USA, Avondale, PA) or 3 mm glass beads (Walter Stern, Inc., Port Washington, NY). During the first month (plant establishment), all plants received only water. At the end of the fourth week all the plants were pruned to a height of 10 cm. In general, the coloration of the plants suggested some degree of nutritional deficiency, since growing for a month without nutrients diluted the nutrient content of the plugs.

After 4 weeks, the, plants were exposed to three Zn treatment regimens including 0.3 ppm (to match an ambient substrate Zn concentration), 80 ppm, and 160 ppm Zn in solution. These rates will be referred to hereafter as low, medium and high Zn, respectively. Additionally, the following concentration of nutrients (μmol/L) were applied to all the treatments: 2000 $Ca(NO_3)_2{\bullet}4H_2O$, 1000 KH_2PO_4, 500 $MgSO_4{\bullet}7H_2O$, 100 KCl, 700 K_2SO_4, 10 H_3BO_3, 0.50 $MnSO_4{\bullet}H_2O$, 0.20 $CuSO_4{\bullet}5H_2O$, 0.01 $(NH_4)_6Mo_7O_{24}$, 100 Fe-EDTA(ethylenediaminetetracetate). Treatments were developed based on similar Zn phytotoxicity research done by Qui *et al*., (2006).

A weekly volume of 300 ml of solution was necessary to maintain container capacity conditions for the plants growing in glass beads. However, the same volume didn’t seem adequate for the rooflite® substrate, as the single application of 300 ml of water rapidly saturated the rooflite® and Zn would have leached from the container. For this reason, the plants growing in rooflite® received 150 ml of the solution twice a week.

Fig. 3.3. *S. kamtschaticum* growing in rooflite® and glass beads.

This irrigation regime did not represent a treatment in and of itself, but a small management adjustment to maintain adequate aeration and moisture in both substrates throughout the experiment. Even though the irrigation frequency was different, both substrate treatments received the same volume of solution and concentration of Zn in the Hoagland's solution every week.

Plants were harvested at 30, 60 and 90 days after treatment. During harvests, shoots and roots were separated. Roots were removed from media, washed with tap water and set in 20 mM Na2-EDTA (disodium ethylenediaminetetraacetate) for 20 min to remove any Zn adhering to the root surfaces (Yang *et al*., 2004). Plant tissues were dried at 60 °C (140 °F) and weighed for dry mass determination. Due to very small dry mass quantities, shoots and roots were individually pulverized with a mortar and pestle, for tissue analysis to determine the average Zn uptake. Extreme care was taken to avoid cross-contamination of samples, by cleaning with ethanol between every sample. Tissue Zn concentrations were determined by Inductively Coupled Plasma Mass Spectrometry at the University of Delaware Soil Testing Laboratory.

The data from this study exhibited heterogeneous variances and, in most cases, non normal distribution. Both square root and log10 transformation data failed to correct the inequality of variances, therefore, the assumptions for a MIXED procedure were not met. For this reason, a non parametric generalized linear mixed model (GLIMMIX procedure) was chosen, since it fits statistical models to data with correlations or no constant variability and where the response is not necessarily normally distributed (SAS v. 9.1; SAS Corporation, NC).

The null hypotheses for this experiment were: (1) the different Zn treatments would not significantly affect the dry mass of *S. kamtschaticum*; (2) plant dry mass would not be significantly different when growing in rooflite® compared to glass beads; (3) different treatment levels of Zn would not significantly affect Zn uptake of *S. kamtschaticum,* and (4) Zn uptake by *S. kamtschaticum* would not be significantly different when growing in either substrate (rooflite® vs. glass beads).

C. Results

***C.1. Experiment 1:* Tolerance of *Sedum* spp. to various ratios of crumb rubber amendments in rooflite®**

C.1.1. Shoot Volume Index and Dry Mass

An apparent downward trend in FVI was noticed when the volumetric proportion of CR increased in the substrate; however, the low R-squared values for these regression analyses (Figs. 3.4 A, B and C) indicated an insignificant Zn treatment effect on growth and quality at the end of the study within all *Sedum* species. Before harvest, *S. album* shoot volume ranged from 20.3 cm^3 to 36.5 cm^3 (Fig. 3.4 A); *S. reflexum* ranged from 6.8 cm^3 to 20.1 cm^3 (Fig. 3.4 B) and *S. kamtschaticum* ranged from 67.1 cm^3 to 112.7 cm^3 (Fig. 3.4 C). In general, the growth quality response was highly variable and FVI could not discriminate CR treatment differences between *Sedum* species. In contrast the dry mass results of the final shoot dry mass indicated some significant effects of percent CR for each of the species (Fig. 3.5).

The maximum average shoot dry mass in *S. album* (Fig. 3.5) was observed in the 0% CR treatment and corresponded to 2.78 g (± 0.39 SE). This was significantly higher than the other treatments (See App. Table C.1 A for P-values). The smallest average dry mass of *S. album* was 1.21 g (± 0.12 SE) and occurred in the 24% CR treatment, although this was not statistically different from any of the CR-amended substrates.

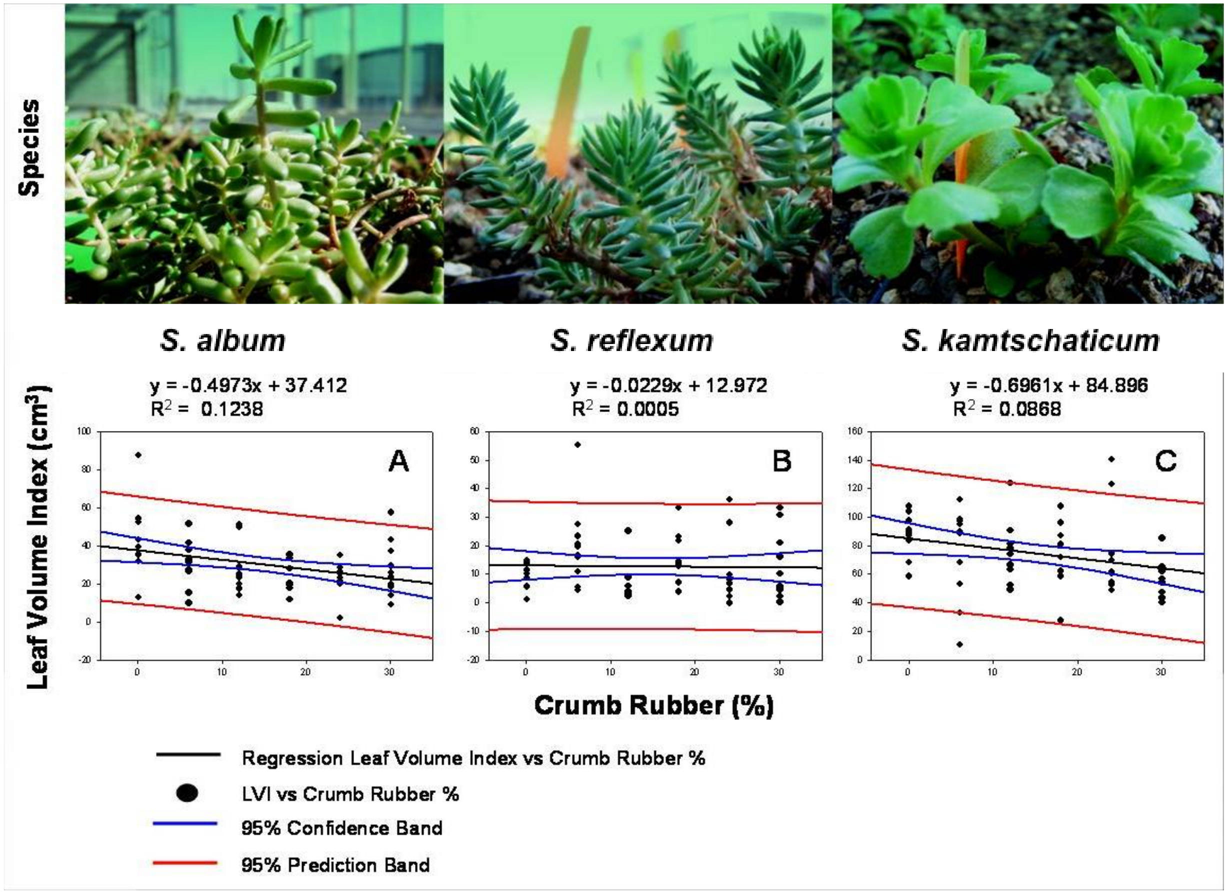


Fig. 3.4. Foliar volume index of three *Sedum* species: A) *S. album*, B) *S. reflexum*, C) *S. kamtschaticum* grown in several proportions of crumb rubber amended rooflite®.

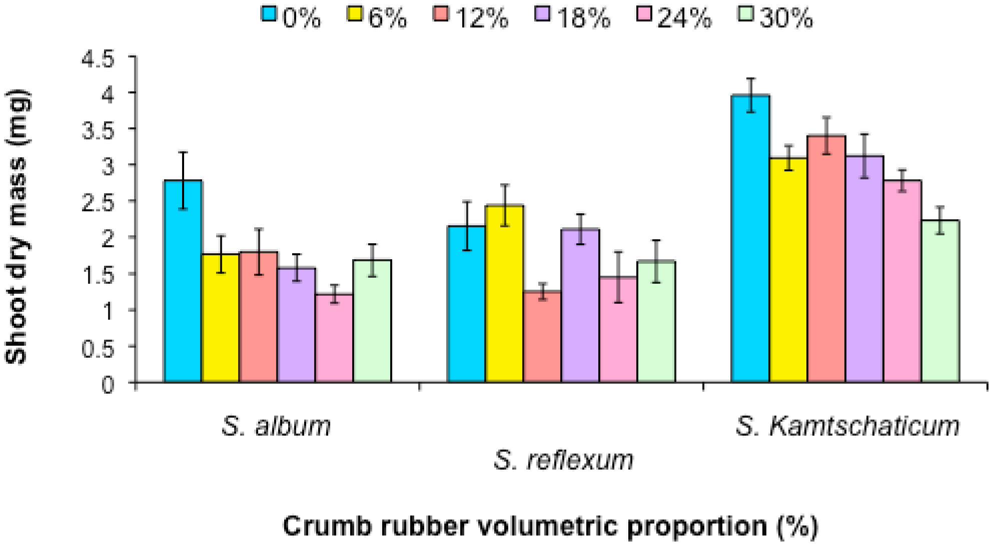


Fig. 3.5 Shoot dry mass of *S. album*, *S. reflexum*, and *S. kamtschaticum* grown in several proportions of a CR amended green roof substrate.

Sedum reflexum average shoot dry mass results ranged from 1.25 g (± 0.11 SE) to 2.43 g (± 0.28 SE); however, the response to the different CR levels was highly variable and didn't exhibit any logical trend (Fig 3.5). Significant differences between treatments are shown in App. Table C.1 B. *Sedum kamtschaticum* exhibited a significant reduction in average shoot dry mass with increasing proportions of CR (Fig. 3.5; App. Table C.1 C for P-values). The minimum averaged dry mass was obtained with the 30% CR (2.23g ± 0.18 SE) and the maximum in the 0% CR (3.96 g ± 0.23 SE).

C.1.2. Average shoot Zn Concentration and Zn Content of Sedum kamtschaticum

The average shoot Zn concentration in *S. kamtschaticum* increased linearly (with low variability) as the proportion of CR increased in the substrate (Fig. 3.6 A). The average concentrations ranged from 41.2 ppm (± 2.25 SE) to 86.4 ppm (± 1.98 SE). Significant differences were found for all the treatments except 6% and 12% CR, and 24 and 30% CR (See App. Table C.2 for P-values).

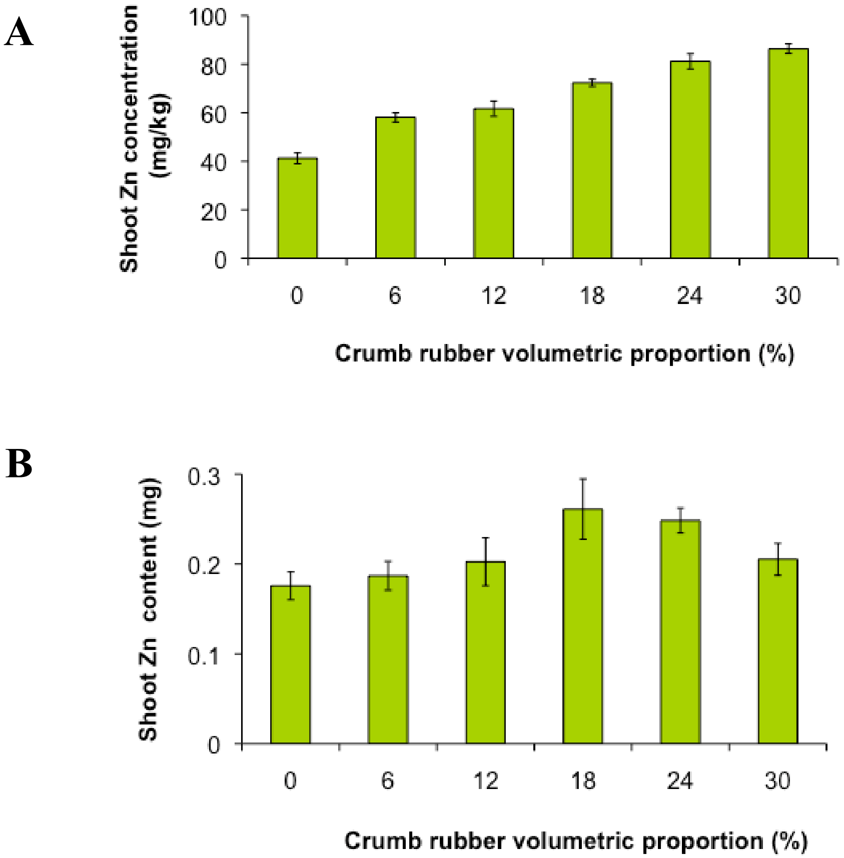


Fig. 3.6 (A) Shoot Zn concentration and (B) Zn content of *S. kamtschaticum* grown in several proportions of a CR amended rooflite®.

The average shoot Zn content (Fig 3.6 B) was obtained by multiplying the average leaf dry mass by leaf concentration, to normalize any differences in tissue concentration that were confounded by growth differences. No significant differences were found in shoot Zn content between any level of CR (Fig. 3.6 B)., *in S. kamtschaticum.* The minimum and maximum calculated contents were 0.17 mg (± 0.02 SE) and 0.26 mg (± 0.03 SE).

C.2. Experiment 2: Response of Sedum kamtschaticum to elevated doses of Zn in two different substrates under hydroponic conditions

C.2.1. Greenhouse Experiment

C.2.1.1. Root Comparisons

The average root dry mass of plants grown in rooflite® was significantly larger than plants grown in glass beads by the end of the study (Fig. 3.7 A). The increasing concentrations of Zn did not cause significant differences between plants grown in rooflite®. In contrast, the average root dry mass of plants was negatively affected by the medium and high Zn levels in glass beads. Similarly, the low Zn solution treatment in glass beads allowed for normal shoot growth and resulted in a significantly higher average shoot dry mass (Fig. 3.8 A) than in the medium ($P < 0.05$) and high ($P < 0.01$) Zn treatments (which were not significantly different from each other). All Zn treatment levels in rooflite® were significantly different from all Zn levels in glass beads (App. Table C.3 A).

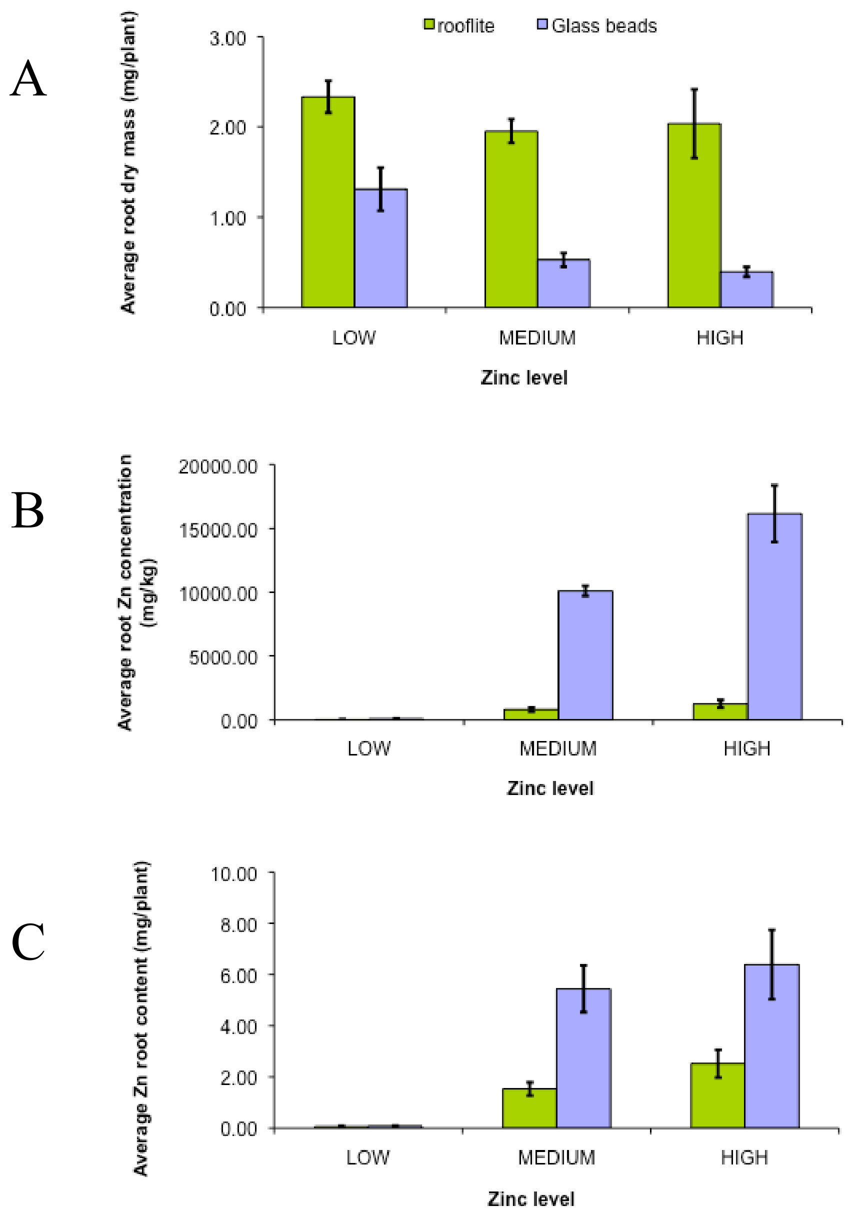


Fig. 3.7. (A) Average root dry mass, (B) Zn concentration and (C) Zn content of *S. kamtschaticum*, grown in glass beads or rooflite®, fertigated with three Zn concentration levels (0.03, 80 and 160 ppm) and grown under greenhouse conditions for three months.

In general, the average Zn concentration in roots of plants grown in glass beads was higher than the Zn concentration of roots grown in rooflite® (Fig. 3.7 B). While non-significant differences occurred at any Zn level in the rooflite® treatment, the average root Zn concentration between every Zn treatment level in glass beads was significantly different ($P < 0.0001$). Multiple means comparisons showed the low Zn treatment in glass beads was significantly different from all Zn levels in rooflite® (App. Table C.3 B).

Fig. 3.7 C indicates that the only significant difference in the average root Zn content was found between the low Zn level and the high level ($P < 0.05$) in the rooflite® treatment, due to high variability within treatments. In the glass bead treatment, the Zn content with the low Zn treatment was significantly different from the medium and high Zn treatments ($P < 0.0001$), which were not significantly different from each other. The low Zn treatment in glass beads was significantly different from the low and medium Zn levels in rooflite® (App. Table C.3 C).

C.2.1.2. Shoot Comparisons

The average shoot dry mass of plants grown in rooflite® was higher compared to plants grown in glass beads (Fig. 3.8 A). All Zn treatment levels in rooflite® were significantly different from all Zn levels in glass beads (App. Table C.4 A). No significant differences in shoot dry mass occurred between the three levels of Zn solution in rooflite® substrate. In contrast, the low Zn solution treatment in glass beads resulted in a significantly higher dry mass than with the medium and high Zn levels ($P < 0.001$), which were not significantly different from each other. App. Table B.5 is provided as an example of the statistical analyses conducted during this experiment.

In general, the average shoot Zn concentration of plants grown in glass beads was significantly higher than in plants grown in rooflite® (Fig. 3.8 B).

A comparison between rooflite® treatments showed no significant differences in Zn shoot concentration at any Zn level (Fig 3.7b). In contrast, the low and medium Zn levels in glass beads were not significantly different from each other, but the low Zn level average shoot concentration was significantly lower than the high Zn level ($P < 0.0001$) and the medium Zn level was significantly lower than in the high Zn level ($P < 0.05$). A multiple means comparison showed the medium Zn treatment in glass beads was significantly different from the low Zn level in rooflite®, and the high Zn level in glass beads was significantly different from all Zn levels in rooflite® (App. Table C.4 B).

Fig. 3.8 (C) shows the average shoot Zn content at the end of the 3-month study was not different in glass beads, independent of the Zn treatment, due to the reduction in shoot dry mass. For rooflite®, only the low Zn level had significantly less Zn, in comparison to the medium ($P < 0.001$) and the high Zn additions ($P < 0.0001$), which were not significantly different from each other. Significant differences between treatment combinations occurred in all the cases except the low Zn treatment in rooflite® compared to all Zn levels in glass beads (App. Table C.4 C).

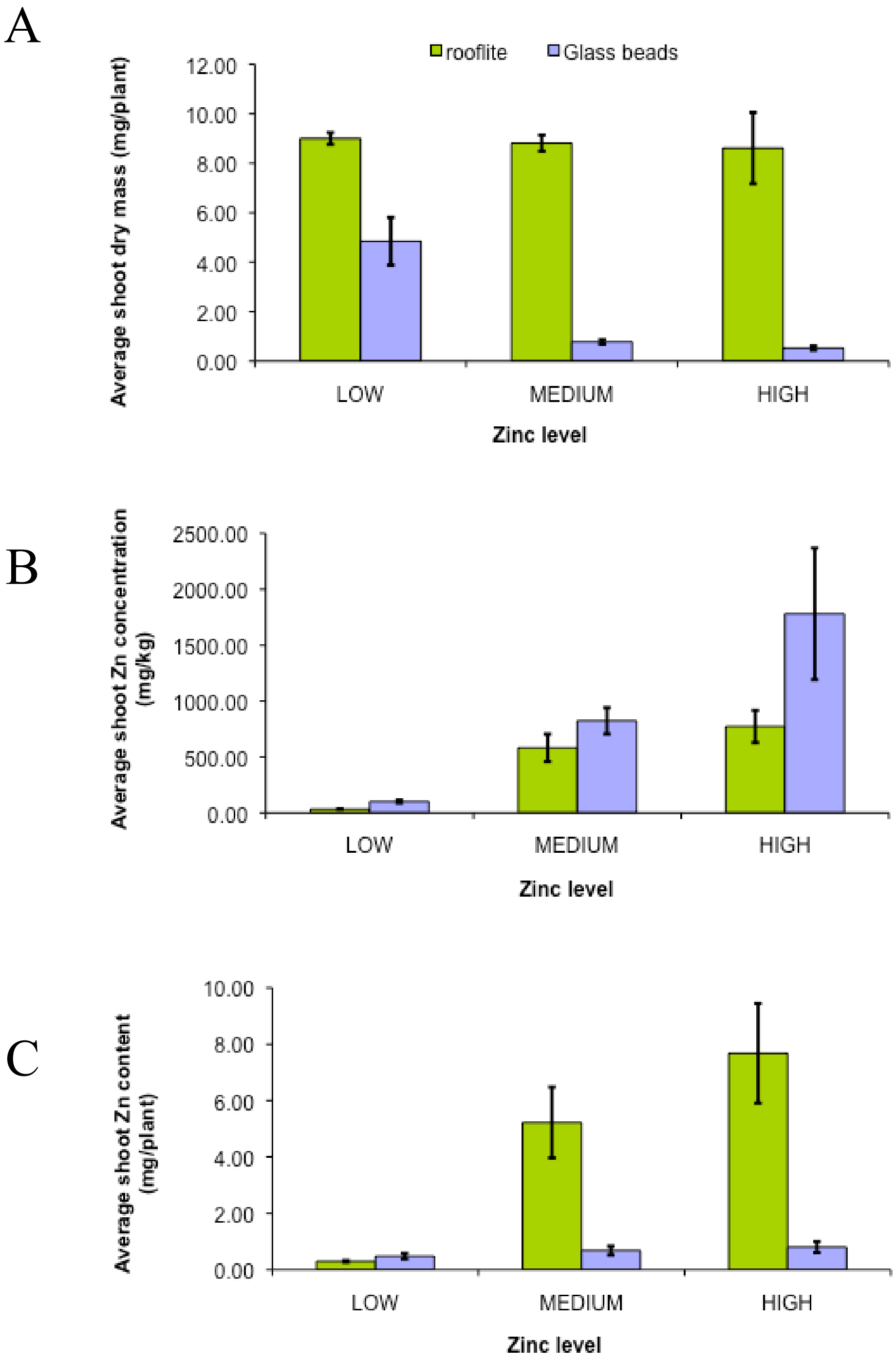


Fig. 3.8. (A) Average shoot dry mass, (B) Zn concentration and (C) Zn content of *S. kamtschaticum*, grown in glass beads or rooflite®, fertigated with three Zn concentration levels (0.03, 80 and 160 ppm) and grown under greenhouse conditions, three months after study initiation.

C.2.2. Growth Chamber Experiment

C.2.2.1. Root Comparisons

No significant differences were found in root dry mass between treatments in rooflite® (Fig. 3.9 A). In contrast, the low Zn solution treatment in glass beads resulted in a significantly higher dry mass compared to the medium and high Zn additions ($P < 0.05$), which were not significantly different from each other. A multiple means comparisons between treatment combinations showed the low Zn levels in glass beads was not significantly different from all the Zn levels in rooflite® (App. Table C.5 A).

Fig. 3.9 B illustrates the average root Zn concentrations in each treatment at the end of the study. No significant differences between any Zn level in the rooflite® occurred. In contrast, all Zn levels in glass beads were significantly different to each other as indicated in Fig. 3.9 B and App. Table C.5.B ($P < 0.0001$). A multiple means comparisons between treatment combinations showed the low Zn levels in glass beads was not significantly different from all the Zn levels in rooflite® (App. Table C.5 A).

No significant differences were found in the average root Zn content between treatments in rooflite® (Fig. 3.9 C). In contrast, all Zn levels were significantly different from each other in the glass beads treatment ($P < 0.05$ for the low-medium Zn level comparison; $P < 0.01$ for the medium-high Zn level comparison and $P < 0.0001$ for the low-high Zn level comparison).

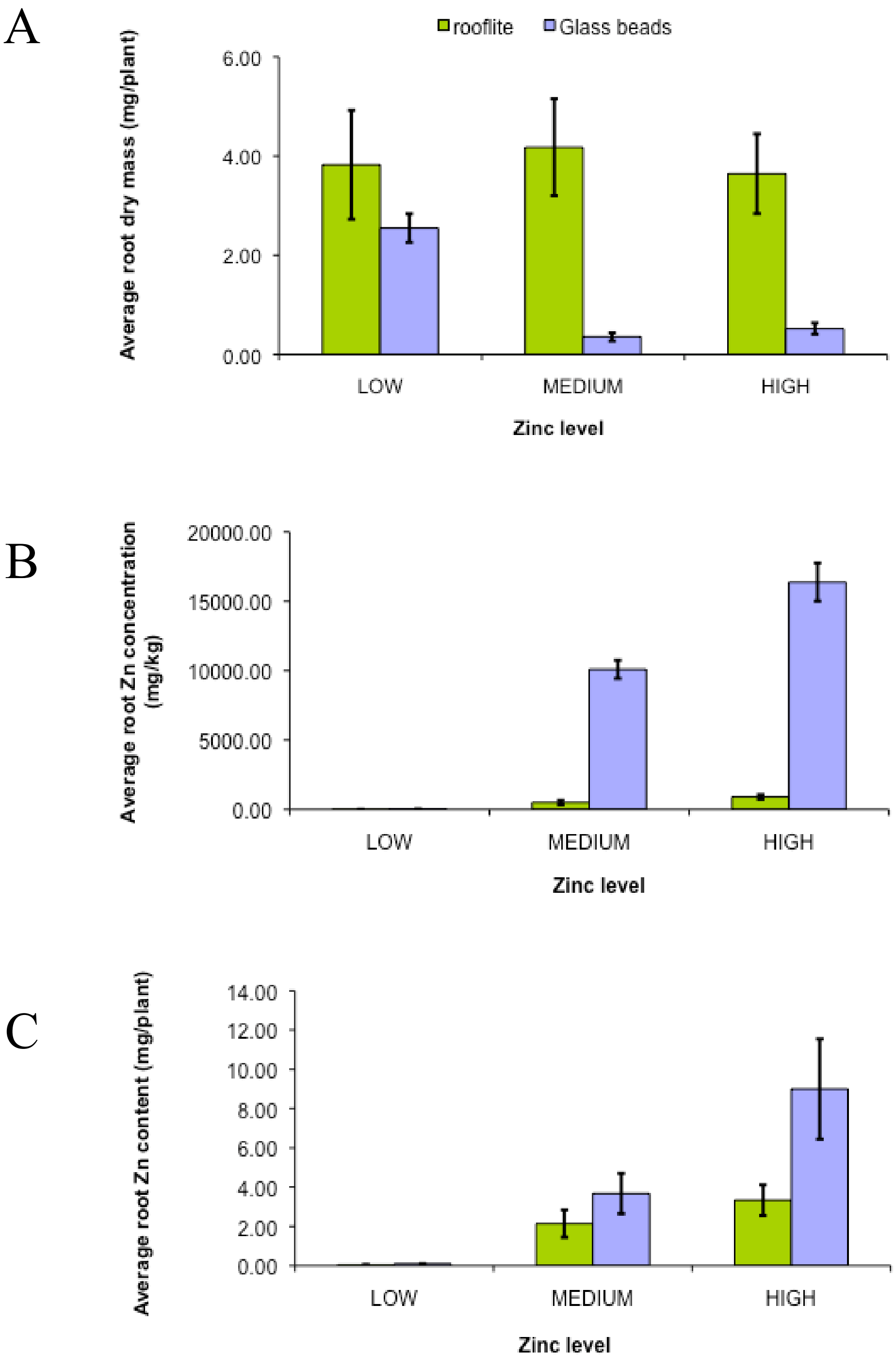


Fig. 3.9. (A) Average root dry mass, (B) Zn concentration and (C) Zn content of S. *kamtschaticum*, grown in glass beads or rooflite®, fertigated with three Zn concentration levels (0.03, 80 and 160 ppm) and grown under growth chamber conditions, three months after study initiation.

The treatment comparison showed significant differences between the medium Zn level in glass beads compared to the low Zn level in rooflite®, as well as in the high Zn level in glass beads compared to all Zn levels in rooflite® (App. Table C.5 C).

C.2.2.2. Shoot Comparisons

No significant differences in shoot dry mass were found between treatments in rooflite® (Fig. 3.10 A). In contrast, the lowest Zn treatment resulted in a significantly higher dry mass in glass beads than with the medium and high Zn treatments ($P < 0.05$), which were not significantly different from each other. All Zn treatment levels in rooflite® were significantly different from all Zn levels in glass beads except the pair of treatments constituted by both low Zn levels (App. Table C.6 A).

No significant differences in the average shoot concentration were found between Zn additions in the rooflite® treatment (Fig. 3.10 B). In contrast, in the glass bead treatment the low Zn vs. high Zn levels resulted in significantly different leaf Zn contents ($P < 0.0001$), as well as between medium and high levels of Zn ($P < 0.0001$). Multiple means comparisons between treatments showed significant differences between the high Zn level in glass beads compared to all Zn levels in rooflite® (App. Table C6 B).

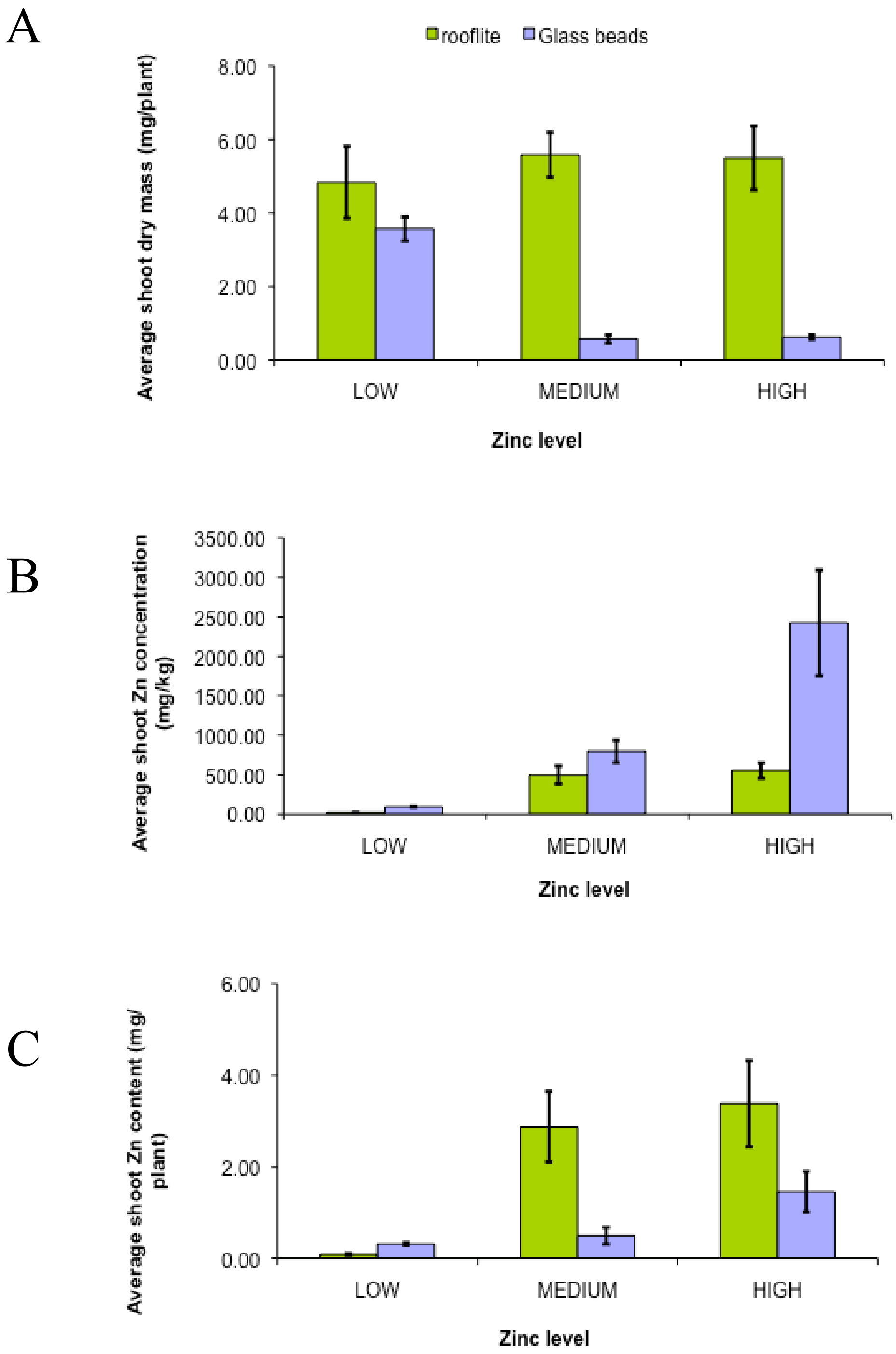


Fig. 3.10. (A) Average shoot dry mass, (B) Zn concentration and (C) Zn content of *S. kamtschaticum*, grown in glass beads or rooflite®, fertigated with three Zn concentration levels (0.03, 80 and 160 ppm) and grown under growth chamber conditions, three months after study initiation.

No significant differences in the average shoot Zn content were found between the three Zn treatments in glass beads, primarily because of the very low dry mass (growth) of plants at the highest Zn additions (Fig. 3.10 C). In contrast, the low Zn solution treatment had a significantly lower Zn content to the other treatments ($P < 0.001$), due to a larger dry mass. Multiple means comparisons between treatment combinations showed non-significant differences between the low Zn level in rooflite® compared to all Zn levels in glass beads, and non-significant differences either between the medium Zn level in rooflite® compared to the high Zn level in glass beads (App. Table C.6 C).

C.3. Additional Chronologic Observations

Throughout the time course of this study, the dry mass, Zn concentration and Zn content responses of shoot and root tissues exhibited comparatively similar results among the Zn and substrate treatments, in both greenhouse and growth chamber environments. Appendix Figures C.1 through C.12 show the responses of all variables over time in both greenhouse and growth chamber experiments. For brevity, mostly greenhouse results are described here.

Although some differences in dry mass, tissue Zn concentrations and contents between environments were noted, presumably due to the very different environmental conditions during the studies, growth patterns by treatment were surprisingly similar. In other words, similar statistical significances for each treatment response were found between the greenhouse and the growth chamber studies.

In the greenhouse study, average shoot and root dry mass of plants growing in glass beads were not significantly different than the plants growing in rooflite® at low Zn concentrations during the first two months of the study. However, in the growth chamber environment, no significant differences in dry mass between substrates were noted in plants with the low Zn treatment throughout the entire study. We presume that the influence of low light conditions on growth may have influenced *Sedum* growth and Zn uptake.

However, there were initial significant differences seen in each response variable within each tissue, between substrates and Zn levels, starting at the first harvest. Simply put, the availability of Zn in the glass beads began to have an effect on plant growth and uptake of Zn by the first harvest, which continued to end of the experiment, which is why final evaluation results form the major focus of our discussion.

D. Discussion

D.1. Experiment 1: **Tolerance of *Sedum* spp. to various ratios of crumb rubber amendments in rooflite®**

The growth of the three *Sedum* species in this study showed no visible symptoms of Zn toxicity after 5 months in CR amended rooflite®. *Sedum album* and *S. kamtschaticum* fully explored the total capacity of the container and bloomed without showing any stress. *Sedum reflexum* in contrast, exhibited some physiological disorders, such as a slight chlorosis and the partial abscission of leaves. These symptoms were noted across all treatments, as the plants exhibited different degrees of stress that didn't correlate with the different levels of CR. Since this high variability in growth was equal across the CR treatments, it appears that something other than the CR affected the growth of *S. reflexum*, possibly natural variability or the effect of other unfavorable abiotic factors. It is possible that *S. reflexum* had different nutritional and / or irrigation requirements compared to *S. album* and *S. kamtschaticum.*

The absence of significant differences in the FVI of the three species suggested that the increasing proportions of CR do not cause any obvious effect on the growth of these three species of *Sedum*, which are extensively used in green roof plantings. However, it should be noted that this index did not have the resolution for detecting negative effects in plant growth, under the described experimental conditions.

In contrast, the average dry mass showed a statistically significant negative effect on growth due to CR addition. In *S. album*, the control (0%) treatment was had a higher biomass compared to the rest of the CR treatments. A variation in *S. album* response to CR addition was noticeable because of an inconsistent downward trend in plant dry mass as the proportion of CR increased. From a biological perspective, we expected a downward linear response with increasing levels of significance as the proportion of CR increased, due to the potential amount of Zn released from the CR.

As stated previously with *S. reflexum,* something other than CR affected the growth quality of *S. album*, given the variability across the treatments. No biological inference could be made from the results, but plants exhibiting different levels of stress could have an important impact in the variability of the results.

S. kamtschaticum provided the clearest response in the reduction of dry mass with increasing quantities of CR in the substrate. By comparing the probabilities of the significant differences in the simple effects (App. Table 3.1C), it is clear that some degree of variability also occurred in the response by this species. From the three species evaluated, S. *kamtschaticum* was considered the best for additional research with hydroponic response studies to increasing Zn. In addition, the morphological characteristics of *S. kamtschaticum* make this species a more practical model for intensive plant studies.

By simple observation, it was noticed that the CR particle distribution in this study was not uniform throughout the experimental replicates of each treatment level. During the mixing process, where CR was proportionately added to the rooflite® the homogeneity of particles may have been different between treatments. It appeared that the 500 ml pots used in this study did not have adequate volume to ensure that a representative sample of that CR proportion was contained by each replicate. If this was the case, more CR could have been added to some pots and less in others. In order to reduce this potential source of variability in subsequent studies, the substrate combinations were formulated individually for each experimental unit. The average densities of the substrates were used to assure the exact amounts of the materials were added.

Based on the significance of the results, some categorization of treatments can be inferred. For example, an amendment of 12% would not be different from 6%, and an amendment of 30% would not be different 24%. These considerations could be important to determine the maximum load reduction or the economic cost considerations of the amended substrate, while preventing toxic effects in the plants.

The average shoot Zn concentration and Zn content were determined for *S. kamtschaticum*. In general, the average shoot concentration response was consistent with the dry mass effect, i.e. a significant difference was found between the control and the CR amended treatments. Typically, as the dry mass decreased, the average Zn concentration increased. In terms of Zn uptake, by combining the effect of growth (dry mass) and the uptake potential of the plants under the specific treatments (Zn concentration), no significant differences were found between treatments (Fig 3.6B). The most vigorous plants (control treatment, i.e. 0% Zn addition) extracted the same amount of the metal from the substrate than the rest of the treatments. Because of the growth dilution effect, the average shoot concentration was lower than in treatments with higher CR proportions. In contrast, the 30% CR treatment, although having a significantly lower average dry mass to the other treatments, accumulated a higher concentration of Zn in the shoot tissue; this response however, was not significant when the shoot Zn contents were analyzed.

Even though a dry mass reduction occurred as a consequence of the CR amendments and the Zn concentration increased as a consequence of the CR amendments, the absence of phytotoxicity symptoms (supported by the insignificant results in the FVI evaluations) suggested the possibility that *Sedum* species were tolerant to Zn. Past research had shown one species of the same genus, *Sedum alfredii* Hance, could accumulate 5000 mg Zn/Kg dry weight (Yang et al., 2002). Furthermore, from the results of this study, it was not clear if sufficient Zn had been provided to create stressful conditions for these green roof species. For these reasons, the focus of our subsequent research was to determine if *Sedum kamtschaticum* could in fact hypertolerate or hyperaccumulate Zn.

D.2. Experiment 2: Response of *Sedum kamtschaticum* to elevated concentrations of Zn in two different substrates.

D.2.1. Common Response of Plants to the Medium (80 mg Zn/L) and High (160 mg Zn/L) Zn Concentration Levels in Both Experiments

This study was conducted under two different environmental conditions (greenhouse and growth chamber), to ensure the results were not affected by unknown environmental differences. The commonalities and differences from the results will be discussed; however, it should be noted that statistical comparisons between the two environments was not an objective of this study.

The agreement of significant responses in the substrate treatments and Zn levels between locations reinforced the general conclusions of this study. While the rooflite® substrate prevented negative effects on plant growth at all Zn levels, the medium and high Zn concentrations caused the senescence and death of plants in the glass bead treatments. In these cases, the Zn toxicity was initially manifested in the root system, which prevented subsequent root growth and caused the death of existing root tissues. Consequently, shoot growth was compromised, with visible symptoms showing early in the study. While still functional, the phloem transported a high amount of Zn. For this reason, the small shoot dry masses were associated with high Zn concentrations, and the Zn content varied as a function of dry mass and Zn concentration.

D.2.2. Morphological Differences Between Greenhouse and Growth Chamber Plants

Although some general growth and morphologic differences were observed between locations, the common denominator between both studies was the minimal growth and eventual collapse of the plants at the medium and high Zn levels in the glass bead treatment. While the plants in the greenhouse showed fully expanded internodes and leaves, all plants in the growth chamber developed as a rosette (very compact growth) with comparatively small leaves. Since the growth reduction was manifested in both substrates and all Zn levels, it is very likely this was caused by the light characteristics of the growth chamber environment, which were not fully investigated. Additionally, by simple observation, the temperature and probably the evapotranspiration rates were lower in the growth chamber compared to the greenhouse.

The reduced growth and differentiation of the growth chamber plants could have been a short-term adaptation to prevent the elevated cost of biomass production. Simply put, plant growth occurs only if the plants can meet the energetic expense for the metabolic processes involved, such as photosynthesis, transpiration, water and nutrient uptake (Larchner, 2003).

D.2.3. Root Growth, Zn Concentration and Zn Accumulation

In both locations, no significant differences in the average root dry mass were found between the different Zn levels in the rooflite® substrate; we therefore assume that all the Zn was adsorbed by the rooflite® during the course of the experiments, although we did not analyze the substrates at the end of the experiment. By mitigating the negative effects of excessive Zn, the rooflite® ensured that roots grew equally well with all Zn additions. In both locations it was also confirmed that the low Zn level was adequate for growing plants in glass beads; however, the inability of glass beads to adsorb the excessive Zn from the medium and high Zn treatments caused a significant reduction in average root dry mass in these treatments.

The average root Zn concentration response further explains the overall results. We observed in both locations that average root Zn concentrations were not significantly different in plants growing in rooflite®, regardless of Zn treatment. However, this was not the case for plants growing in glass beads. Extremely high Zn concentrations were found in the roots of plants grown in the medium and high Zn treatments in glass beads. This high accumulation has been described as a Zn^{2+} complex that occurs in the organic ligands of roots before translocation to shoots via xylem (Broadley *et al.*, 2007).

D.2.4. Shoot Growth, Zn Concentration and Zn Accumulation

The shoot growth of S. *kamtschaticum* was unrestricted by the addition of 0.3 ppm Zn to both substrates, similar to the root growth results noted from both greenhouse and growth chamber environments. Zn additions to rooflite® had no effect on shoot growth. We presume that the cation exchange capacity of the rooflite® particles measured at 7.5 meq/100g (App. A1), provided a mechanism for Zn to adsorb to the substrate exchange complex.

Vigorous and functional root systems were the precondition for the occurrence of equivalently large shoot systems. Therefore, when root development was negatively affected by elevated concentrations of Zn in the soil solution, the shoot dry mass subsequently exhibited a mass reduction.

The tendency of average shoot concentration of plants grown in rooflite® in both locations was similar to the root responses described previously: no significant differences were found in the average shoot concentration at any Zn level. In both locations, it was also found that the low and medium Zn concentration levels in glass beads were not significantly different. We think that the absence of a significant difference between the low and medium Zn treatment levels in glass beads could be due to large variability within these treatments and the inability of the non-parametric statistical analysis to discriminate between treatments. This is strengthened by the observation that the plants receiving 80 mg/L Zn had manifested severe symptoms of Zn toxicity and significant reductions in dry mass by the end of the experiment. Non-parametric statistics is a good resource for analyzing data that cannot be transformed, however, it is well known that the level of power is lower compared to parametric analysis.

The simple effects for the average Zn shoot content were consistent in both environments for both glass bead and rooflite® substrates. As a consequence of the function between dry mass and Zn concentration, the response in shoot Zn accumulation of plants growing in glass beads was not different at any Zn solution concentration Note however, that while plants from the low Zn level treatment remained functional, the medium and high Zn level basically reflected the extreme accumulation of the metal in necrotic tissue with minimal dry mass.

In the rooflite® treatment, it was also observed that functional healthy plants can accumulate more Zn when the metal is available in excess in the substrate solution. The chemical characteristics of the substrate buffered the substrate solution by sequestering

Zn. For this reason, Zn could be accumulated and tolerated by vigorously growing plants. Due the accumulation of larger dry mass, the Zn concentration was diluted in the shoots.

Since *S. kamtschaticum* could not tolerate at least 80 mg/L Zn (medium Zn level), it is concluded that this species is not a hyper-accumulator of Zn. For comparative purposes, this concentration was effective for accumulating 10,000 mg/Kg Zn in the shoots of *Potentilla griffithii* Hook, without exhibiting any toxicity symptoms (Qiu *et al*., 2006).

D.2.5. Additional Chronologic Observations

One of the major contributions from this study was the description of the plant responses between harvests during the study. Severe toxic effects in the medium Zn level became visually evident relatively late in the time course of this study, however, the early evaluation of average dry mass, Zn concentration and Zn content, particularly in roots, allowed us to understand that the Zn toxicity began to occur since the first evaluation time. We could not have determined these effects if the duration of this study had been reduced.

The interaction between Zn and the mineral and organic particles of rooflite® described in the second chapter reduced Zn availability for plant uptake. Therefore, *S. kamtschaticum* fertigated with potentially toxic concentrations of Zn could survive and develop as vigorously as plants grown under safe Zn concentrations. During the three months of treatments application, no visual insights suggested that there would be a reversion in plant growth. Hence, there is a possibility that, after three months, the cation exchange sites of the substrate had not been totally saturated. From a strict chemical point of view, the use of CR seems feasible as long as the substrates possess a high cation exchange capacity.

Chapter 4: Summary and Final Remarks

Extensive green roofs can improve the hydrologic balance in urban scenarios by significantly reducing the volume of storm water runoff from impervious surfaces. Since green roofs incorporate dynamic components (both substrate and plants), these systems provide several ecological services. From a hydrologic perspective, the substrate layer is the most important component for capturing stormwater by retention and with the plant component, slowly releasing the moisture through evapotranspiration. From a biological point of view, the substrate is essential for sustaining the plants and associated life forms, by providing the physical characteristics such as optimal air and water availability, which are necessary for promoting the development of vigorous plant growth. Additionally, diverse green roof systems can provide rich habitats for wildlife in urban landscapes.

The incorporation of recycled products into the substrates is a potential way to reduce substrate density, overall weight and remove waste materials from the environment. However, comprehensive testing of recycled materials is recommended because some could potentially release toxic substances into the environment.

Our primary objective in this study was to determine if recycled crumb rubber (CR) could be used as a sustainable green roof amendment by investigating substrate interactions and plant responses to various proportions of CR amendments and Zn concentrations. The model green roof substrate, rooflite®, utilized in these experiments was composed of expanded shales, slates and clays with less than 65 g/L of organic material (rooflite®; Skyland, 2010).

We reached three major conclusions from this research:

1. We confirmed that Zn leaches from CR and the release rate is initially influenced by the pH of the solution.
2. Available Zn can negatively affect *Sedum* plant growth.
3. Zn adheres to the cation exchange sites of the mineral and organic portion of rooflite®.

In the substrate – CR – plant complex, these interactions are multifarious, and thus we investigated the issue with five different experiments to focus on specific objectives. It was the individual conclusions from these experiments that allowed us to understand the dynamics of Zn release from CR, Zn sequestration in rooflite®, and Zn phytotoxicity.

We quantified Zn release from CR and concluded that the rate and amount released was only significantly greater during the first 12 hours when CR was exposed to acidified water compared to non-acidified water. We also found that the availability of Zn in the soil solution was significantly reduced when CR was combined with rooflite®, a substrate with an average cation exchange capacity (CEC) of 7.45 meq/100 g at a pH of 7 (App. B1). For this reason, the leachate Zn concentration from rooflite® amended with 30% CR was significantly lower than the leachate of a combination of 30% CR and 70% glass beads, a material with an average of 0.3 meq/100 g (App. B2), a negligible CEC. The acute criteria maximum concentration (CMC) of Zn in freshwater has been defined as 0.12 mg Zn/L by the US Environmental Protection Agency. The concentration of Zn leached from a CR-amended green roof substrate was slightly above this value during the first two weeks after incorporation and at substrate container capacity. During the following weeks, the Zn concentration decreased to a level below the CMC for Zn. Interestingly, after twelve weeks, the average Zn leaching from the CR amended substrate treatment was not statistically significant from the control treatment without CR, indicating that rooflite® had similar amounts of native Zn.

Several volumetric proportions of rooflite® and CR (0% to 30%) were tested to evaluate the plant response to the substrate mixtures. Although no visual symptoms of toxicity were observed and no significant differences in the foliar volume were noted, a small but statistically significant reduction in dry mass occurred with proportions of CR greater than 0%. For example, *Sedum kamtschaticum*, shoot dry mass was significantly less as proportions of CR increased in rooflite® (see Fig. 3.5). A recent study suggests that increasing proportions of CR could modify the physical characteristics of the substrate, which should also be considered. Ristvey *et al.*, (2010) investigated three different commercial green roof substrates amended with similar volumetric proportions of CR used in this study. In the particular case of rooflite®, the limit for retaining the air-filled porosity within the FLL recommendations was 18% CR. Other commercial green roof substrates sustained adequate air-filled porosities with higher proportions of CR.

During the hydroponic experiment, Zn concentrations of 80 mg/L and above caused severe toxicity and mortality in *Sedum kamtschaticum* grown in a glass-bead substrate. However, no negative plant growth effects occurred at the highest concentration of 160 mg/L Zn, as a consequence of growing in rooflite®. Since Zn was sequestered on cation exchange sites in the substrate, plants grown in rooflite® were able to develop normally during the three-month study, without the stress of highly available Zn in the substrate solution.

We presume that we tested the worse-case Zn scenario during the hydroponic studies, since the Zn released by a 30% CR amendment is negligible compared to the elevated doses we applied during these experiments. We must however recognize the limitations in extrapolating our results to long-term commercial installations. We do not know the long-term limit of Zn release from CR, nor do we know the long-term capacity of rooflite® to sequester Zn. Additionally, only a limited number of *Sedum* species were evaluated and more studies should be done to validate our results under specific circumstances. In the future, several approaches could be adopted with regard to CR research for the green roof industry. From an industrial, environmental and marketing point of view, there is an opportunity for improving the commercial CR material by extracting the initial amount of Zn released from the product prior to use. The results from our leachate studies suggest that acid washing might potentially be a viable procedure to reduce the initial amount of Zn leached and not adsorbed by the substrate, as long as the leached metal could be remediated in a conscious and environmentally responsible manner.

Research should also be oriented towards the selection of other green roof substrates with high cation exchange capacity that could adsorb the majority of the Zn released from CR. We also recommend the evaluation of how CR responds to freeze-thaw cycles, UV and heat degradation, and to assess the fire hazard of these materials. This is fundamental to ascertain if CR could be use as a long term, stable, and environmentally responsible green roof substrate amendment. Nevertheless, this research shows that proportions between 18% and 30% CR could be used in green roof installations with few plant growth and environmental concerns. However, specific proportions are dependent upon the substrate's capability to adsorb Zn and to maintain the physical attributes to retain water yet provide adequate air-filled porosity for healthy root growth.

Appendix A

App. Fig. A1. Green roof plant diversity creating habitat conditions. Rhypark extensive green roof. City of Basel, Switzerland. Source: greenroofs.com (2010c).

App. Fig. A2. After construction: green roof designed to attract ground-nesting and feeding birds. City of Basel, Switzerland. Source: Breinnsein, 2006.

App. Fig. A3. Construction of a deck in the Flowers-Muller Residence. Project located in Adams Morgan, Washington, DC. Source: Capitol Greenroofs, 2010a.

App. Fig. A4. Installation of a single-ply waterproofing membrane in Harvard University Institute. Project located in Cambridge, Massachussets. Source: Capitol Greenroofs, 2010b.

App. Fig. A5. Installation of insulating materials (Dow Styrofoam 40 lb density) in Harvard University Institute. Project located in Cambridge, Massachussets. Source: Capitol Greenroofs, 2010b.

App. Fig. A6. Installation of a polyethylene root barrier in Harverford College, Pennsylvania. Source: Harverford College, 2010.

App. Fig. A7. Installation of a modular drainage layer. Source: Landmark Living Roofs Ltd, 2010.

App. Fig. A8. Installation of a filter fabric in Harvard University Institute. Project located in Cambridge, Massachussets. Source: Capitol Greenroofs, 2010b.

App. Fig. A9. Installation of *Sedum* species in Harvard University Institute. Project located in Cambridge, Massachussets. Source: Capitol Greenroofs, 2010b.

App. Fig. A10. Mature extensive green roof with *Sedum* species. Project located in Ford Motor Company's River Rouge Plant. Dearborn, MI. Source: greenroofs.com

Appendix B

Appendix B.1

Cation Exchange Capacity analysis of rooflite®.

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10-104-0675 **Page:** 1 of 1

Account Number
00278

A&L Eastern Laboratories, Inc.
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Deborah Dant
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Queenstown , MD 21658

Client : GREEN ROOF

Submitted By : DEBORAH DANT
Purchase Order :
Report Date : 4/19/2010
Date Received : 4/14/2010

REPORT OF ANALYSIS

		Cation Exg Capacity
		SW-9081
Lab No	Sample ID Sample Date and Time	meq/100g
06501	#1 GR	7.40
06502	#2 GR	7.50

Method Reference:
USEPA, SW-846, Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, 3rd Ed. Current Revision

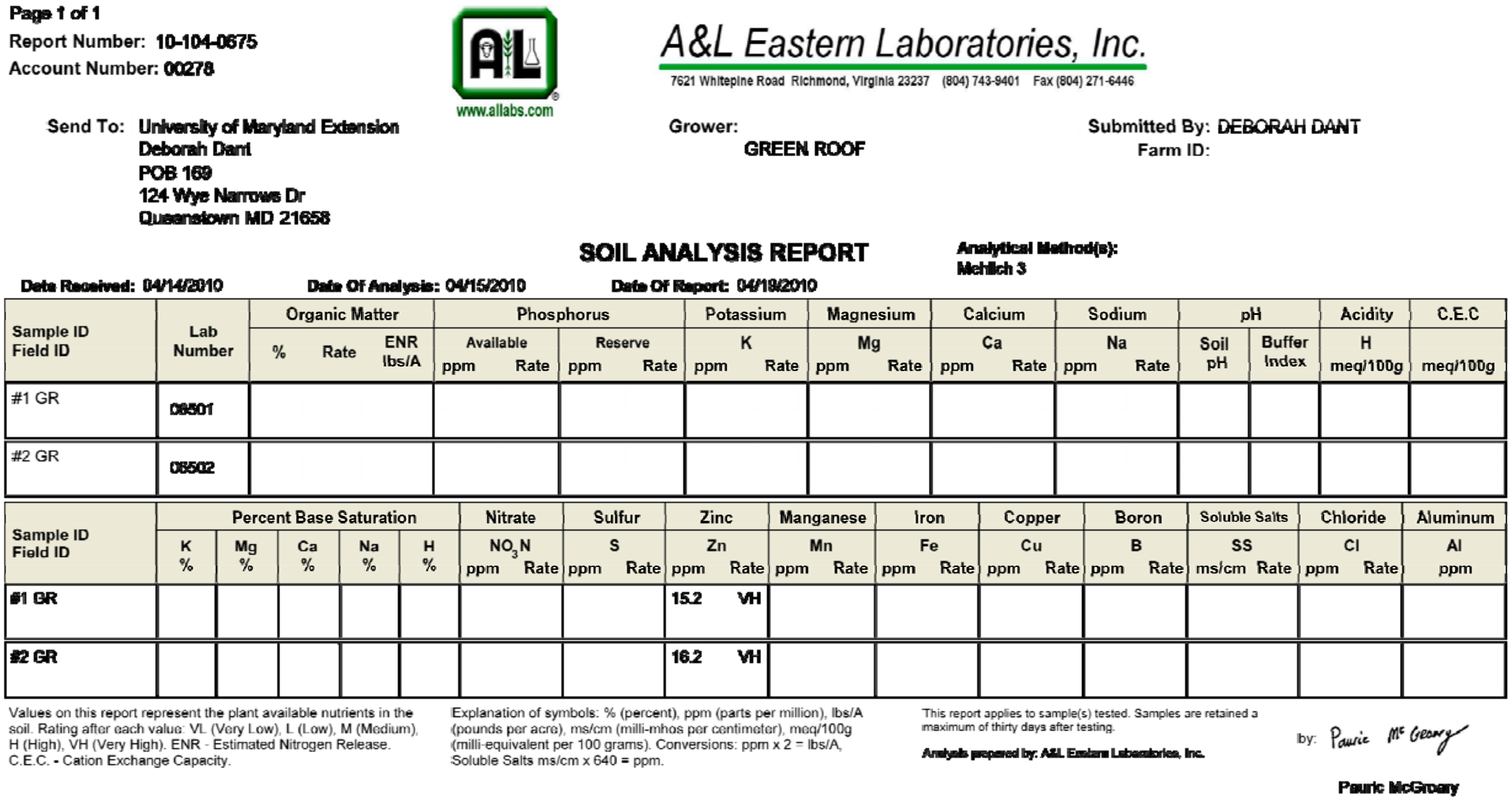
Pauric Mc Groary

Pauric McGroary

Sample results are reported 'as received' and are not moisture corrected unless noted

Appendix B.2

Zn concentration analysis of rooflite®.



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Grower:
GREEN ROOF

Submitted By: **DEBORAH DANT**
Farm ID:

SOIL ANALYSIS REPORT

Analytical Method(s):
Mehlich 3

Date Received: 04/14/2010 **Date Of Analysis: 04/15/2010** **Date Of Report: 04/19/2010**

Sample ID Field ID	Lab Number	Organic Matter			Phosphorus				Potassium		Magnesium		Calcium		Sodium		pH		Acidity	C.E.C
		%	Rate	ENR lbs/A	Available ppm	Rate	Reserve ppm	Rate	K ppm	Rate	Mg ppm	Rate	Ca ppm	Rate	Na ppm	Rate	Soil pH	Buffer Index	H meq/100g	meq/100g
#1 GR	08501																			
#2 GR	08502																			

Sample ID Field ID	Percent Base Saturation					Nitrate		Sulfur		Zinc		Manganese		Iron		Copper		Boron		Soluble Salts		Chloride		Aluminum
	K %	Mg %	Ca %	Na %	H %	NO_3N ppm	Rate	S ppm	Rate	Zn ppm	Rate	Mn ppm	Rate	Fe ppm	Rate	Cu ppm	Rate	B ppm	Rate	SS ms/cm	Rate	Cl ppm	Rate	Al ppm
#1 GR										15.2	VH													
#2 GR										16.2	VH													

Values on this report represent the plant available nutrients in the soil. Rating after each value: VL (Very Low), L (Low), M (Medium), H (High), VH (Very High). ENR - Estimated Nitrogen Release. C.E.C. - Cation Exchange Capacity.

Explanation of symbols: % (percent), ppm (parts per million), lbs/A (pounds per acre), ms/cm (milli-mhos per centimeter), meq/100g (milli-equivalent per 100 grams). Conversions: ppm x 2 = lbs/A, Soluble Salts ms/cm x 640 = ppm.

This report applies to sample(s) tested. Samples are retained a maximum of thirty days after testing.

Analysis prepared by: A&L Eastern Laboratories, Inc.

by: Pauric Mc Groary

Pauric McGroary

Appendix B.3

Cation exchange capacity analysis of glass beads.

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Account Number
00278

A&L Eastern Laboratories, Inc.

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Send To : University of Maryland Extension
Deborah Dant
POB 169
124 Wye Narrows Dr
Queenstown , MD 21658

Client : GREEN ROOF MEDIA

Submitted By : ANDREW RISTVEY
Purchase Order :
Report Date : 5/13/2010
Date Received : 5/10/2010

REPORT OF ANALYSIS

Cation Exg Capacity

SW-9081

Lab No	**Sample ID** **Sample Date and Time**	**meq/100g**
15327	GLASS BEAD-A	0.300
15328	GLASS BEAD-B	0.300

Method Reference:
Recommended Chemical Soil Test Procedures for the North Central Region. NCR Research Pub. No. 221 Revised.
USEPA, SW-846, Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, 3rd Ed. Current Revision

Pauric McGroary

Pauric McGroary

Sample results are reported 'as received' and are not moisture corrected unless noted

Appendix B.4

App. Table B.4. General Linear Model Mixed (GLIMMIX) Analysis of Variance for the quantification of the available Zn in leachates from green roof substrates with and without CR.

Leachate Zn concentration (mg/L) from CR, rooflite® and glass beads formulations(all times)

```
      Type III Tests of Fixed Effects
              Num  Den
Effect        DF   DF     F Value   Pr > F
Treatment     2    4      30.01     0.0039
tpoint        1    122    11.21     0.0011
```

```
Differences of Treatment Least Squares Means
                          Standard

Treatment  _  Treatment      Estimate   Error     DF  t Value   Pr > |t|
30GB : 70 RL  30CR : 70 RL   -0.08039   0.03053   4   -2.63     0.0580
30GB : 70 RL  30CR : 70 GB   -1.5065    0.2018    4   -7.46     0.0017
30CR : 70 RL  30CR : 70 GB   -1.4261    0.2018    4   -7.07     0.0021
```

Net Zn released (µg) from either crumb rubber (30CR : 70RL and 30CR : 70GB treatments) or rooflite® (30 GB: 70 RL treatment).

```
          Differences of Treatment Least Squares Means
                          Standard

Treatment  _  Treatment      Estimate   Error     DF  t Value   Pr > |t|
30GB : 70 RL  30CR : 70 RL   -0.3615    0.2008    4   -1.80     0.1462
30GB : 70 RL  30CR : 70      -7.1146    0.2008    4   -35.43    <.0001
30CR : 70 RL  30CR : 70      -6.7531    0.2008    4   -33.63    <.0001
```

Appendix B.5

App. Table B.5. General Linear Model Mixed (GLIMMIX) Analysis of Variance for the average dry mass of S. *kamtschaticum* grown in glass beads or rooflite®, fertigated with three Zn concentration levels (0.03, 80 and 160 ppm) and grown under greenhouse conditions, three months after study initiation.

```
             The GLIMMIX Procedure
          Type III Tests of Fixed Effects
                 Num    Den
Effect            DF     DF   F Value   Pr > F
Treatment          2     30      6.35   0.0050
SUBSTRATE          1     30    129.22   <.0001
Treatment*SUBSTRATE 2    30      4.80   0.0156

HIG    GLASS   HIG    ROOFL   -7.85   <.0001
HIG    GLASS   LOW    GLASS   -4.19   0.0002
HIG    GLASS   LOW    ROOFL   -8.23   <.0001
HIG    GLASS   MED    GLASS   -0.24   0.8083
HIG    GLASS   MED    ROOFL   -8.05   <.0001
HIG    ROOFL   LOW    GLASS    3.66   0.0010
HIG    ROOFL   LOW    ROOFL   -0.38   0.7094
HIG    ROOFL   MED    GLASS    7.61   <.0001
HIG    ROOFL   MED    ROOFL   -0.20   0.8456
LOW    GLASS   LOW    ROOFL   -4.03   0.0003
LOW    GLASS   MED    GLASS    3.95   0.0004
LOW    GLASS   MED    ROOFL   -3.85   0.0006
LOW    ROOFL   MED    GLASS    7.98   <.0001
LOW    ROOFL   MED    ROOFL    0.18   0.8586
MED    GLASS   MED    ROOFL   -7.80   <.0001
```

Appendix C

App. Table C.1. Summary of simple effects in average dry mass between CR treatments (A) *S. album*, (B) *S. reflexum*, (C) *S. kamtschaticum* (P-values for the significant differences are indicated).

A. *Sedum. album*

	0%	**6%**	**12%**	**18%**	**24%**	**30%**
0%		< 0.01	< 0.05	< 0.01	< 0.0001	< 0.01
6%	< 0.01					
12%	< 0.05					
18%	< 0.01					
24%	< 0.0001					
30%	< 0.01					

B. *Sedum. reflexum*

	0%	**6%**	**12%**	**18%**	**24%**	**30%**
0%			< 0.01			
6%			< 0.01		< 0.05	< 0.05
12%	< 0.01	< 0.01		< 0.05		
18%			< 0.05			
24%		< 0.05				
30%		< 0.05				

C. *Sedum. kamtschaticum*

	0%	**6%**	**12%**	**18%**	**24%**	**30%**
0%		< 0.01		< 0.01	< 0.001	< 0.0001
6%	< 0.01					< 0.05
12%						<0.01
18%	< 0.01					< 0.05
24%	< 0.001					
30%	< 0.0001	< 0.05	<0.01	< 0.05		

App. Table C.2. Summary of simple effects in average shoot Zn concentration in *S. kamtschaticum* as a result of increasing proportions of CR. (P-values for the significant differences are indicated).

Shoot average concentration of *Sedum. kamtschaticum*						
	0%	**6%**	**12%**	**18%**	**24%**	**30%**
0%		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
6%	< 0.0001			< 0.001	< 0.0001	< 0.0001
12%	< 0.0001			< 0.01	< 0.0001	< 0.0001
18%	< 0.0001	< 0.001	< 0.01		< 0.05	< 0.001
24%	< 0.0001	< 0.0001	< 0.0001	< 0.05		
30%	< 0.0001	< 0.0001	< 0.0001	< 0.001		

App. Table C.3. Multiple means comparison of average root dry mass of *S. kamtschaticum* root Zn concentration and root Zn content between the three Zn treatments and two substrates, rooflite® and glass beads grown in the greenhouse (P-values for significant differences are indicated).

A. AVERAGE ROOT DRY MASS

		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn	< 0.01	< 0.05	< 0.05
	Medium Zn	< 0.0001	< 0.0001	< 0.0001
	High Zn	< 0.0001	< 0.0001	< 0.0001

A. AVERAGE ROOT Zn CONCENTRATION

		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn			
	Medium Zn	< 0.0001	< 0.0001	< 0.0001
	High Zn	< 0.0001	< 0.0001	< 0.0001

C. AVERAGE ROOT Zn CONTENT

		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn			< 0.05
	Medium Zn	< 0.0001	< 0.001	< 0.01
	High Zn	< 0.0001	< 0.0001	< 0.001

App. Table C.4. Multiple means comparison of average shoot dry mass of *S. kamtschaticum* shoot Zn concentration and shoot Zn content between the three Zn treatments and two substrates, rooflite® and glass beads grown in the greenhouse (P-values for significant differences are indicated).

A. AVERAGE SHOOT DRY MASS

		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn	< 0.001	< 0.001	< 0.001
	Medium Zn	< 0.0001	< 0.0001	< 0.0001
	High Zn	< 0.0001	< 0.0001	< 0.0001

B. AVERAGED SHOOT Zn CONCENTRATION

		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn			
	Medium Zn	< 0.05		
	High Zn	< 0.0001	< 0.01	< 0.01

C. AVERAGED SHOOT Zn CONTENT

		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn		<0.001	<0.0001
	Medium Zn		<0.01	<0.0001
	High Zn		<0.1	<0.0001

App. Table C.5. Multiple means comparison of average root dry mass of *S. kamtschaticum* root Zn concentration and root Zn content between the three Zn treatments and two substrates, rooflite® and glass beads grown in the growth chamber (P-values for significant differences are indicated).

A. AVERAGE ROOT DRY MASS

		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn			
	Medium Zn	< 0.01	< 0.001	< 0.01
	High Zn	< 0.01	< 0.001	< 0.01

B. AVERAGE ROOT Zn CONCENTRATION

		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn			
	Medium Zn	< 0.0001	< 0.0001	< 0.0001
	High Zn	< 0.0001	< 0.0001	< 0.0001

C. AVERAGE ROOT Zn CONTENT

		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn			
	Medium Zn	< 0.05		
	High Zn	< 0.0001	< 0.001	< 0.01

App. Table C.6. Multiple means comparison of average shoot dry mass of *S. kamtschaticum* shoot Zn concentration and shoot Zn content between the three Zn treatments and two substrates, rooflite® and glass beads grown in the growth chamber (P-values for significant differences are indicated).

A. AVERAGE SHOOT DRY MASS

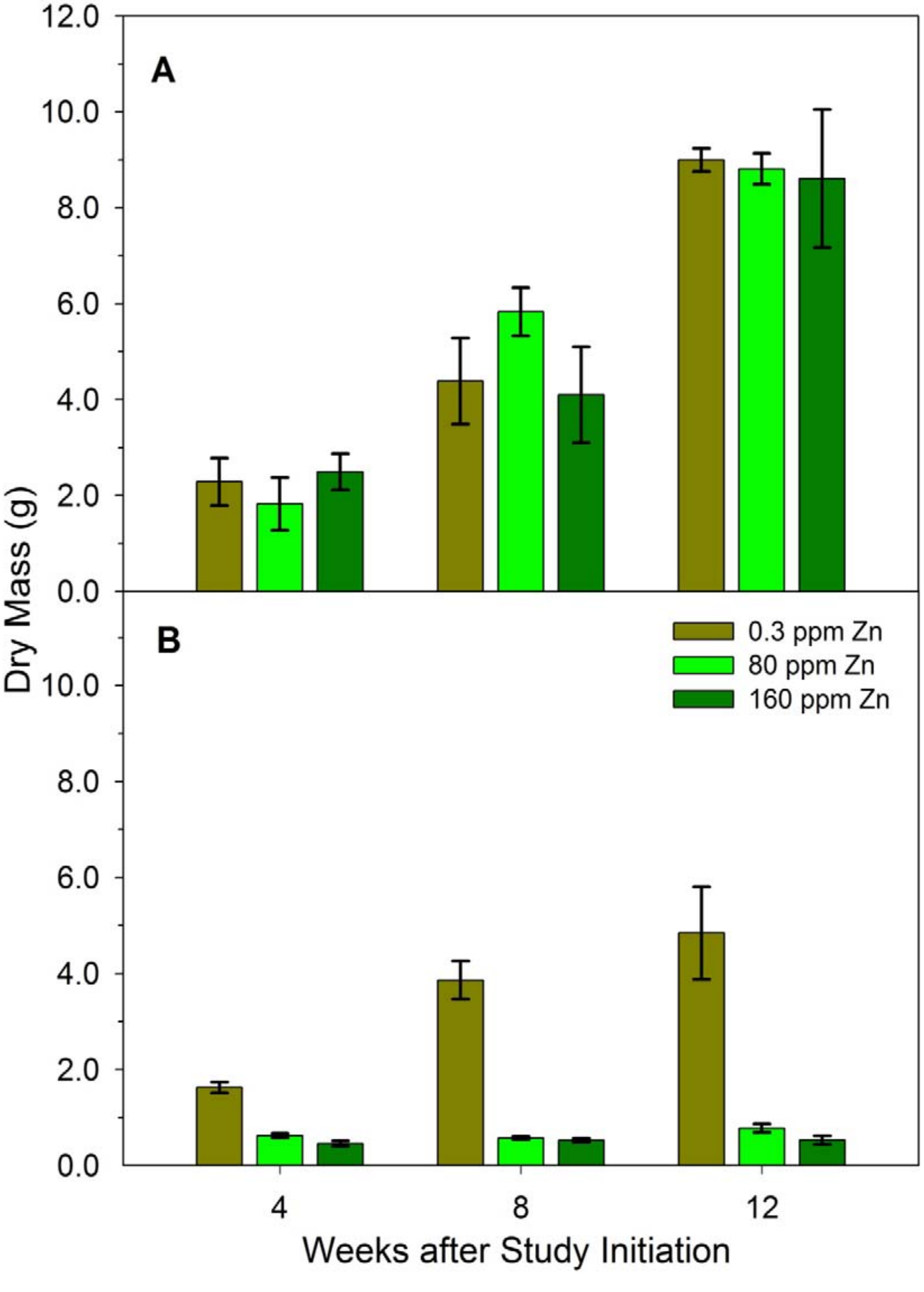
		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn		< 0.05	< 0.05
	Medium Zn	< 0.001	< 0.001	< 0.001
	High Zn	< 0.001	< 0.001	< 0.001

B. AVERAGE SHOOT Zn CONCENTRATION

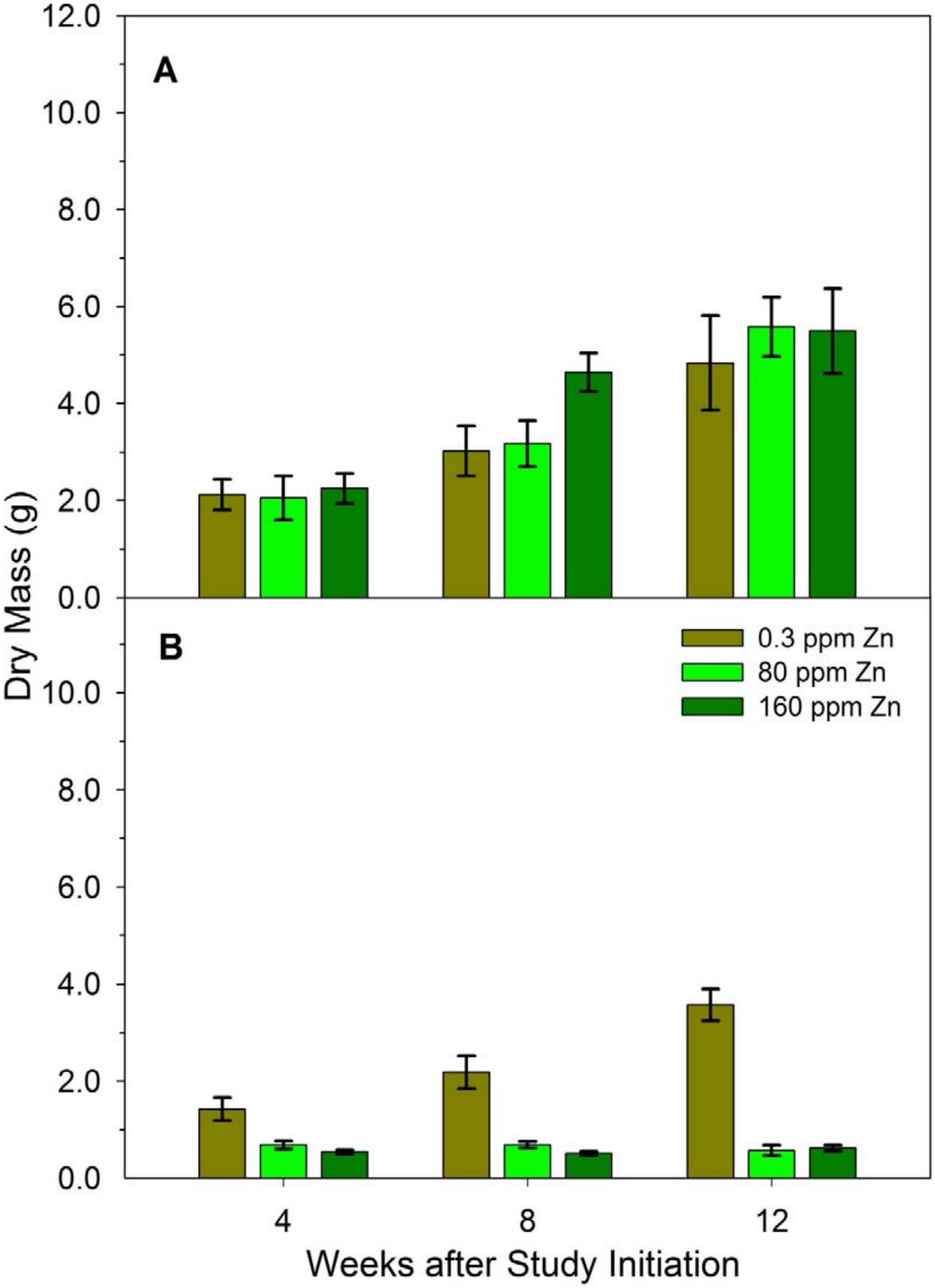
		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn			
	Medium Zn			
	High Zn	< 0.0001	< 0.0001	< 0.0001

C. AVERAGE SHOOT Zn CONTENT

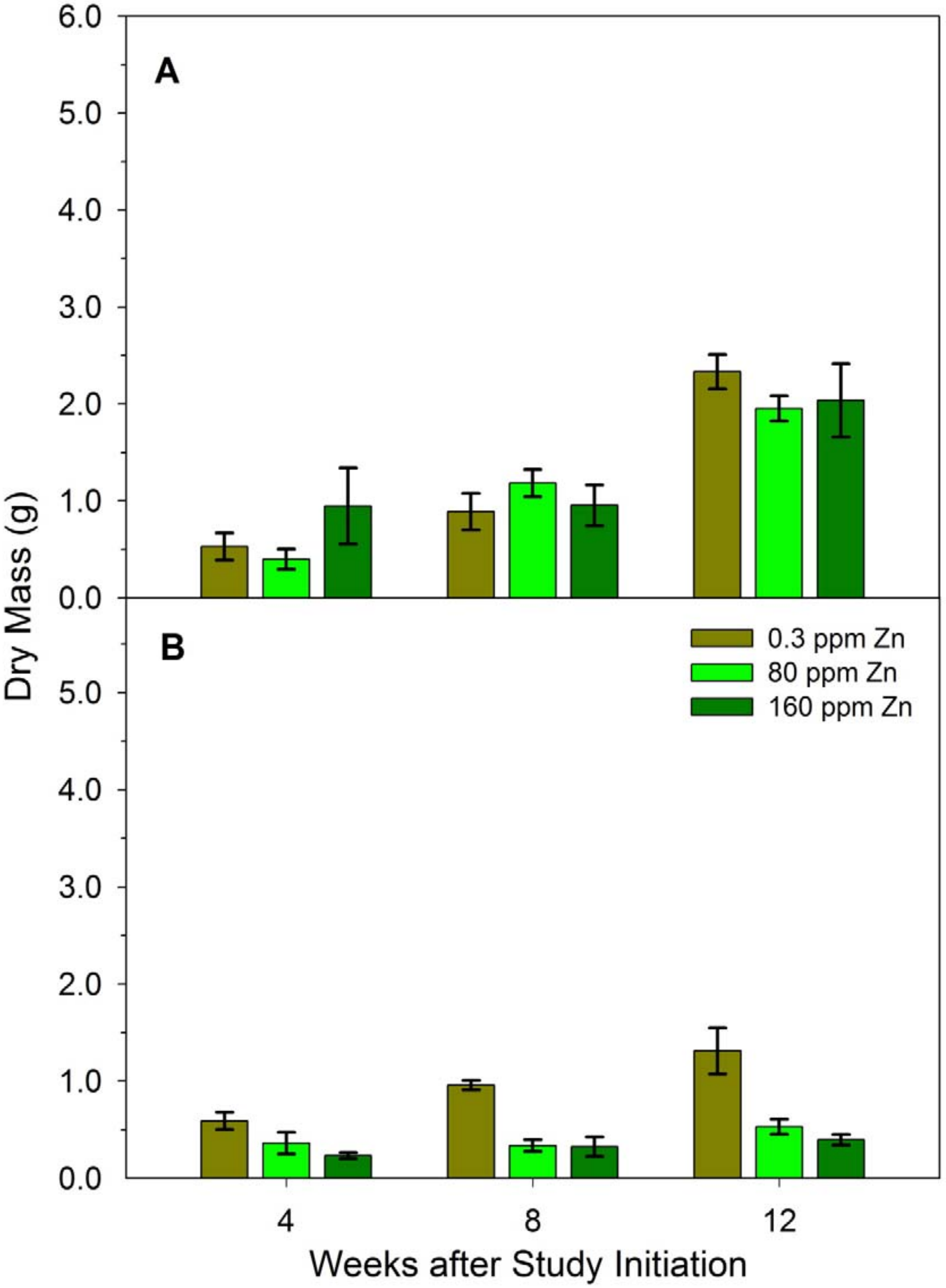
		rooflite®		
		Low Zn	Medium Zn	High Zn
Glass beads	Low Zn		< 0.01	< 0.001
	Medium Zn		< 0.01	< 0.001
	High Zn			< 0.05



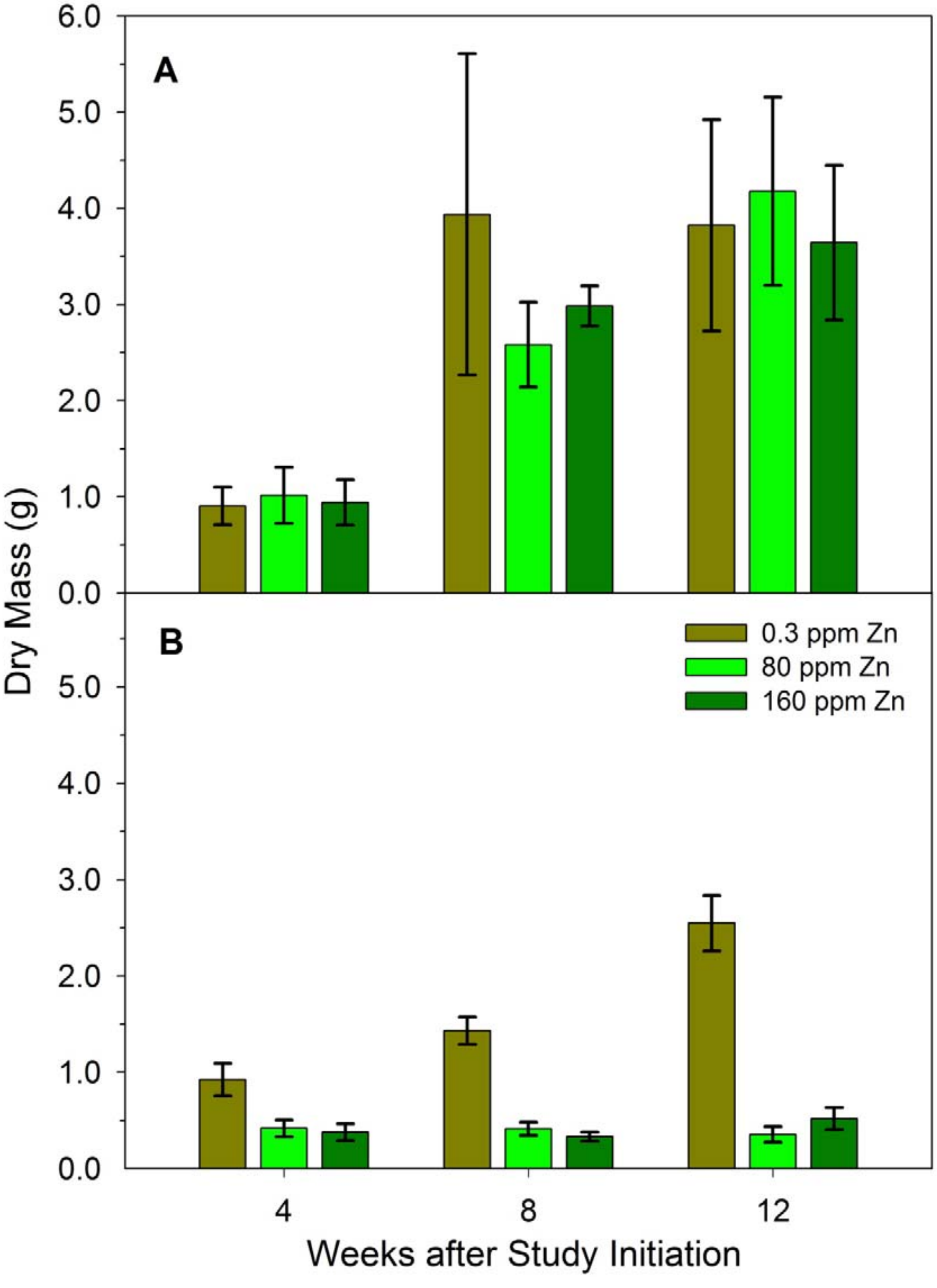
App. Fig. C1. Comparison of average shoot dry mass of *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a greenhouse environment.



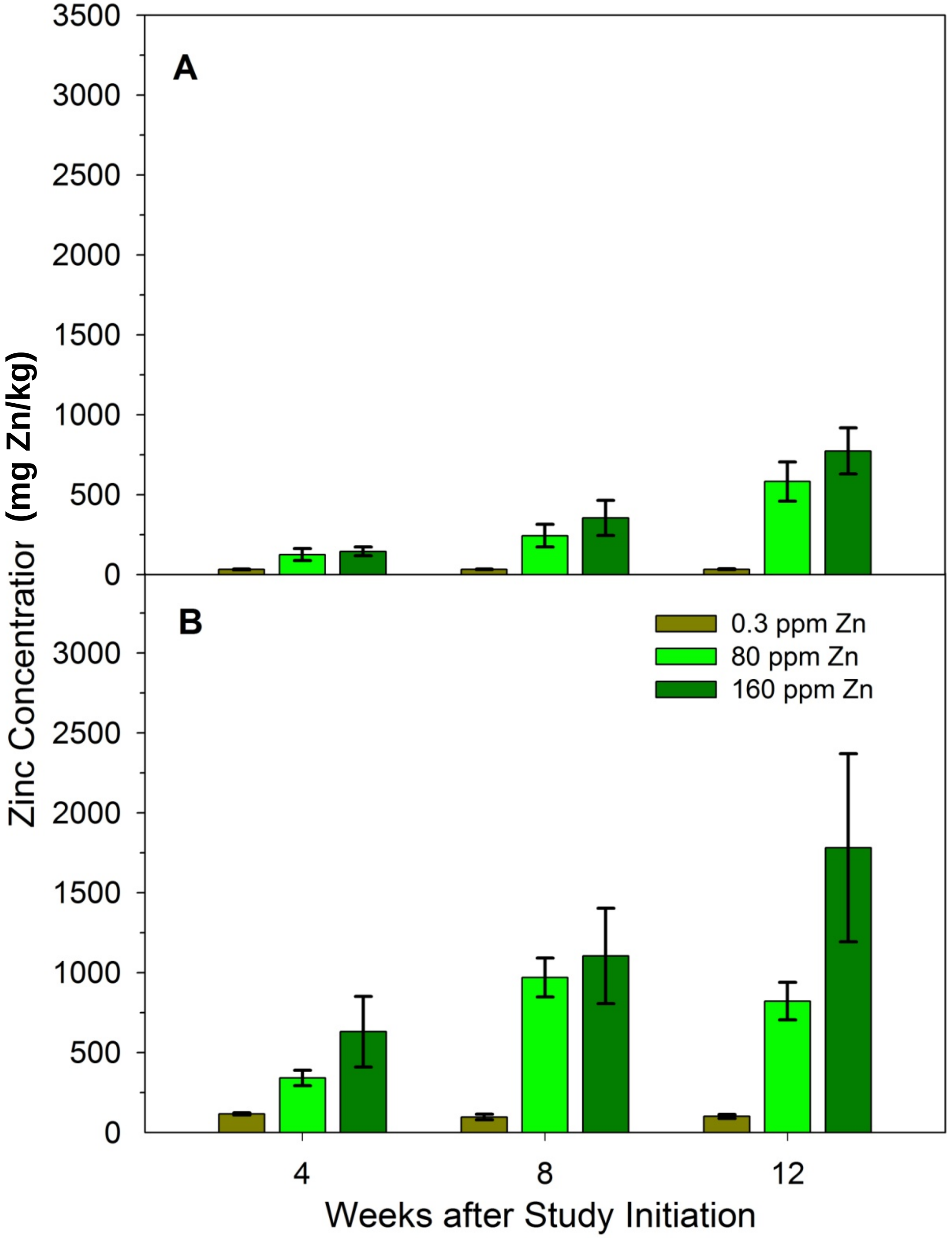
App. Fig. C2. Comparison of average shoot dry mass of S. *kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a growth chamber environment.



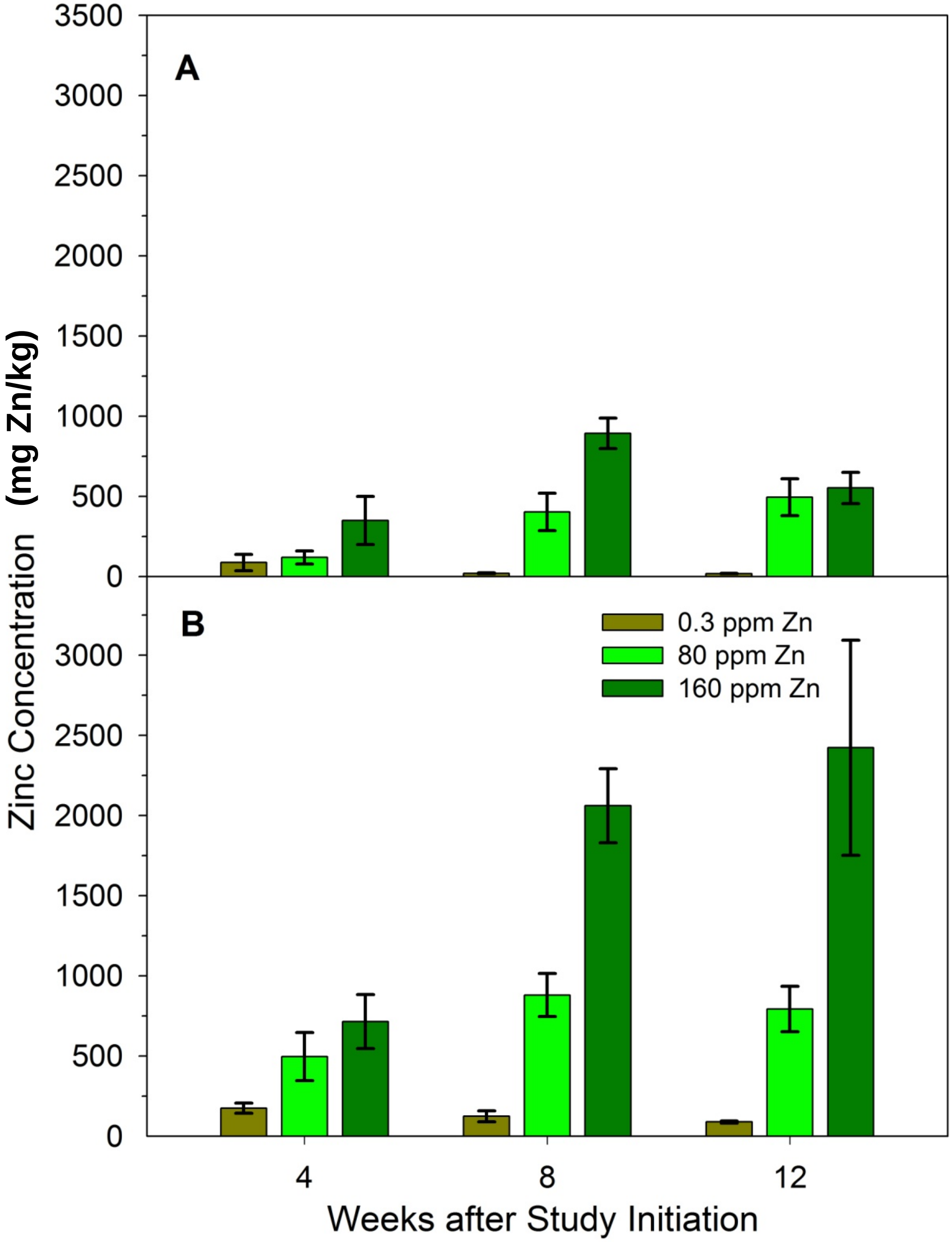
App. Fig. C3. Comparison of average root dry mass of *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a greenhouse environment.



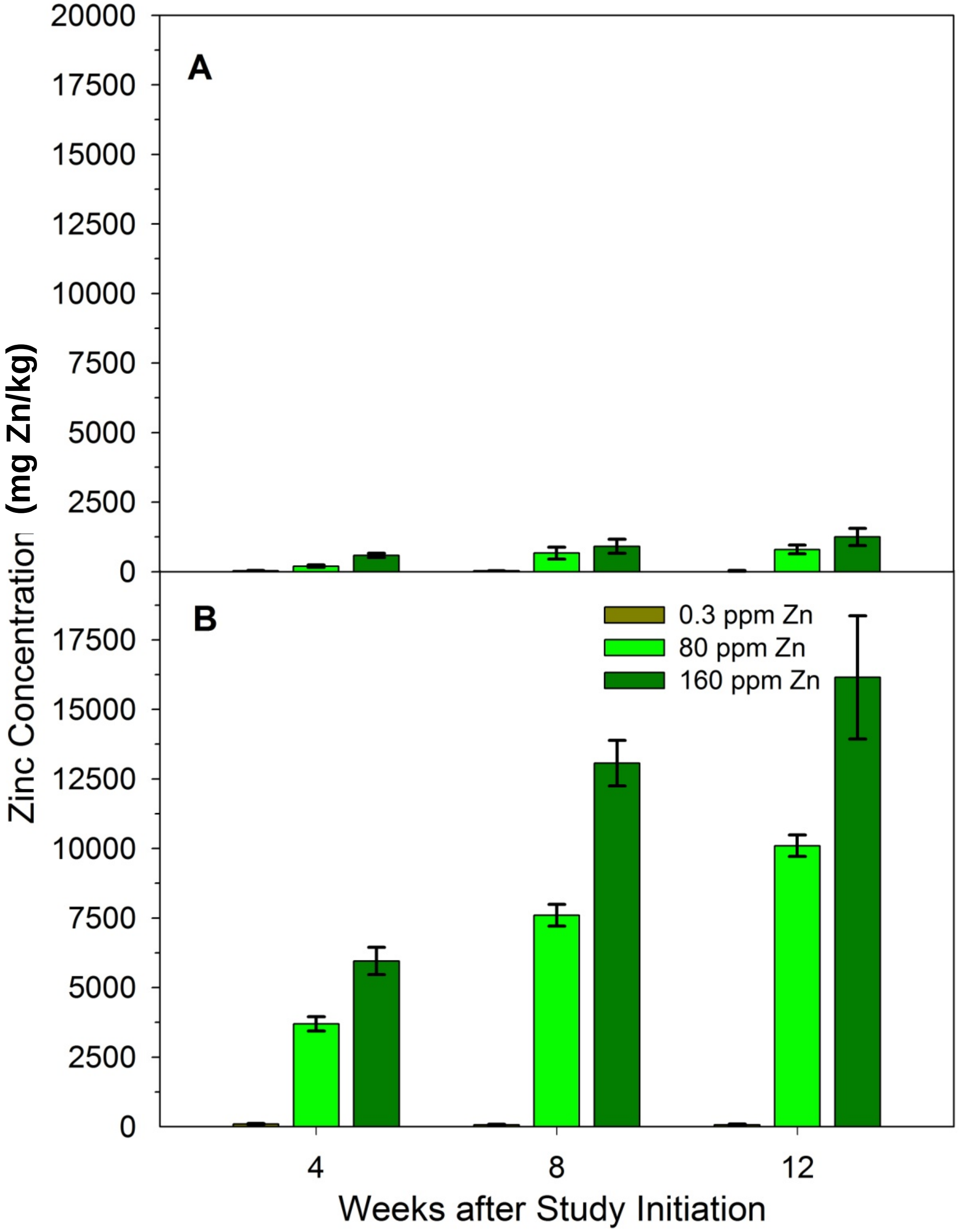
App. Fig. C4. Comparison of average root dry mass of *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a growth chamber environment.



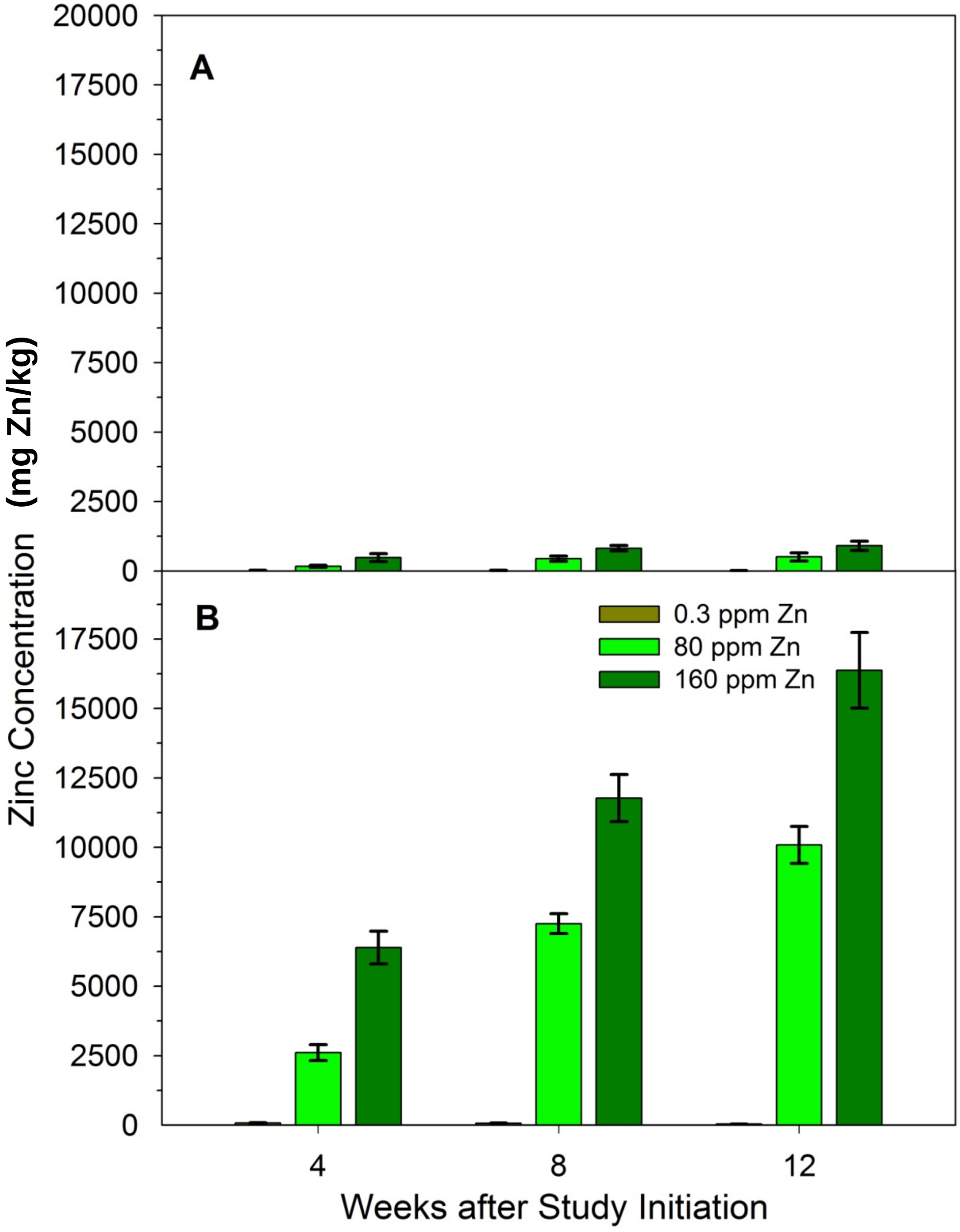
App. Fig. C5. Comparison of average shoot Zn concentration in *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a greenhouse environment.



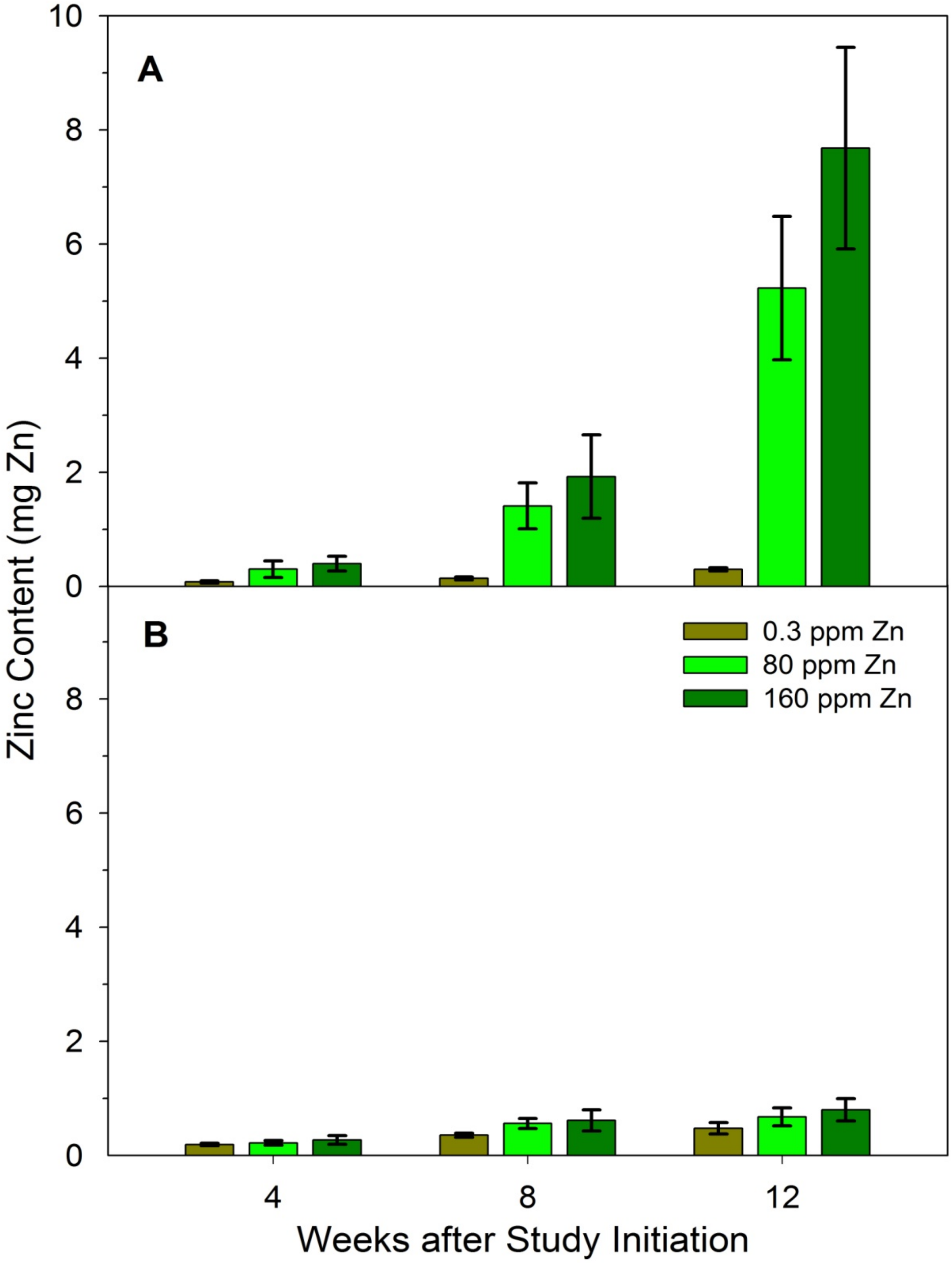
App. Fig. C6. Comparison of average shoot Zn concentration in *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a growth chamber environment.



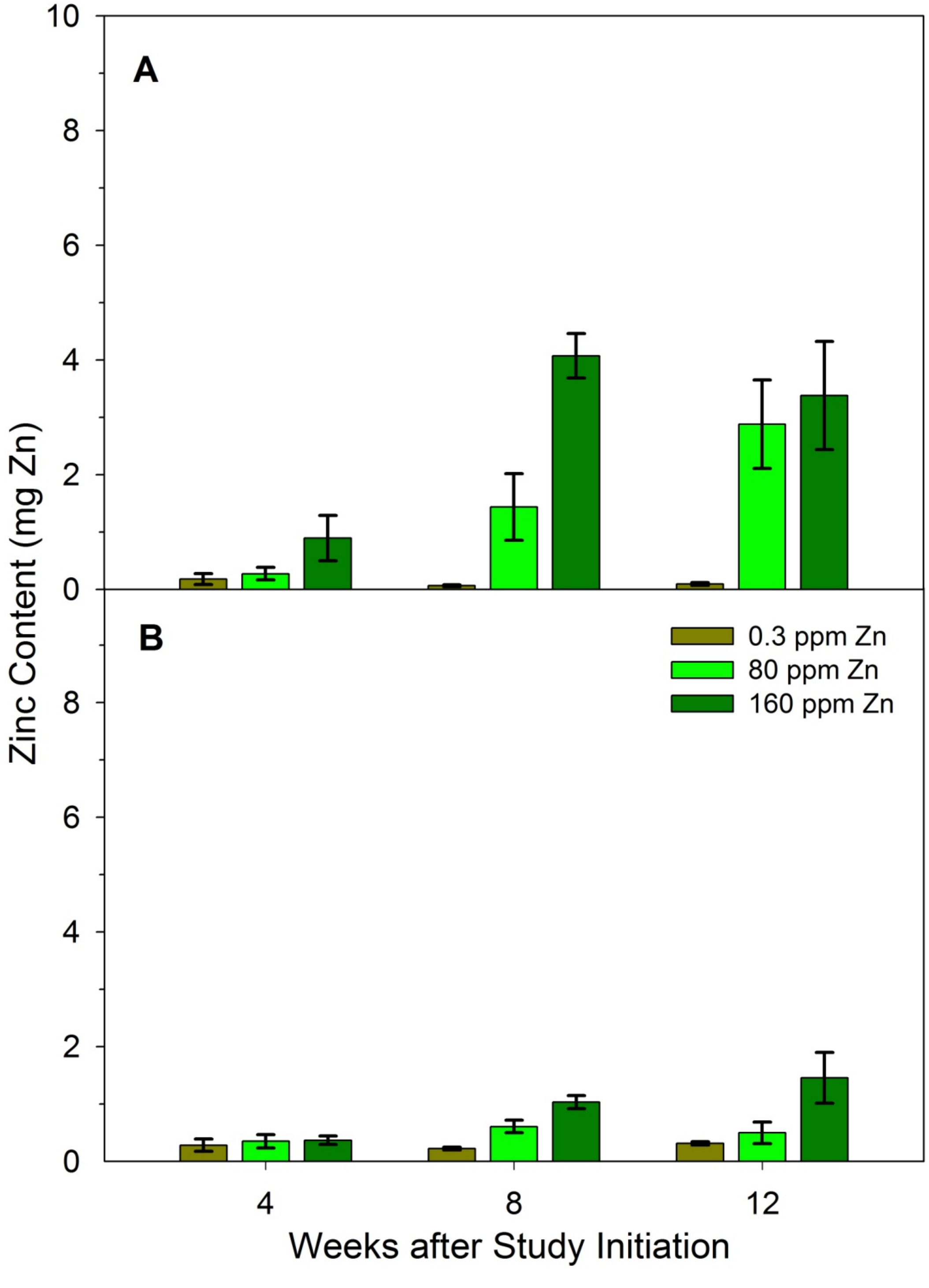
App. Fig. C7. Comparison of average root Zn concentration in *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a greenhouse environment.



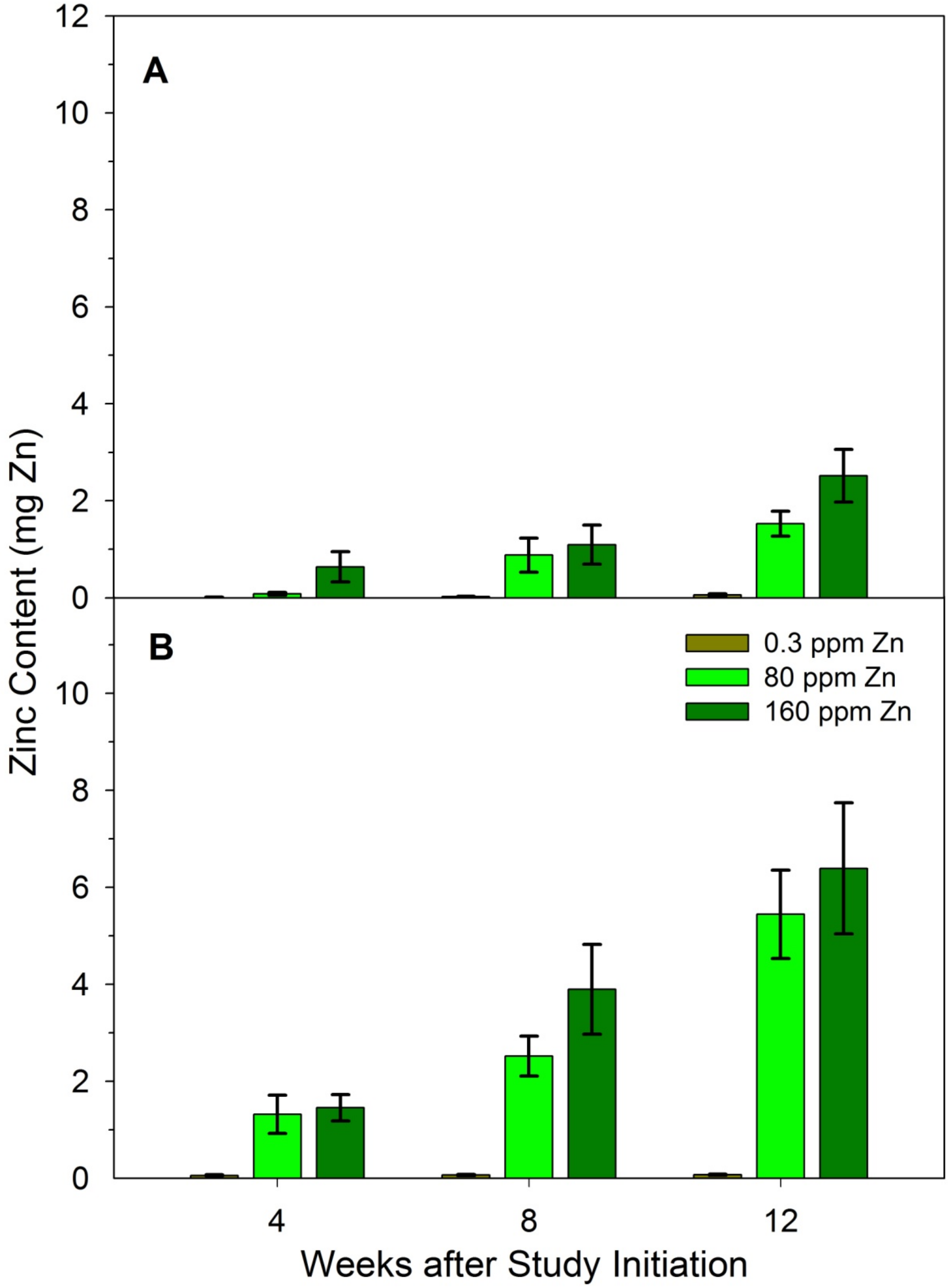
App. Fig. C8. Comparison of average root Zn concentration in *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a growth chamber environment.



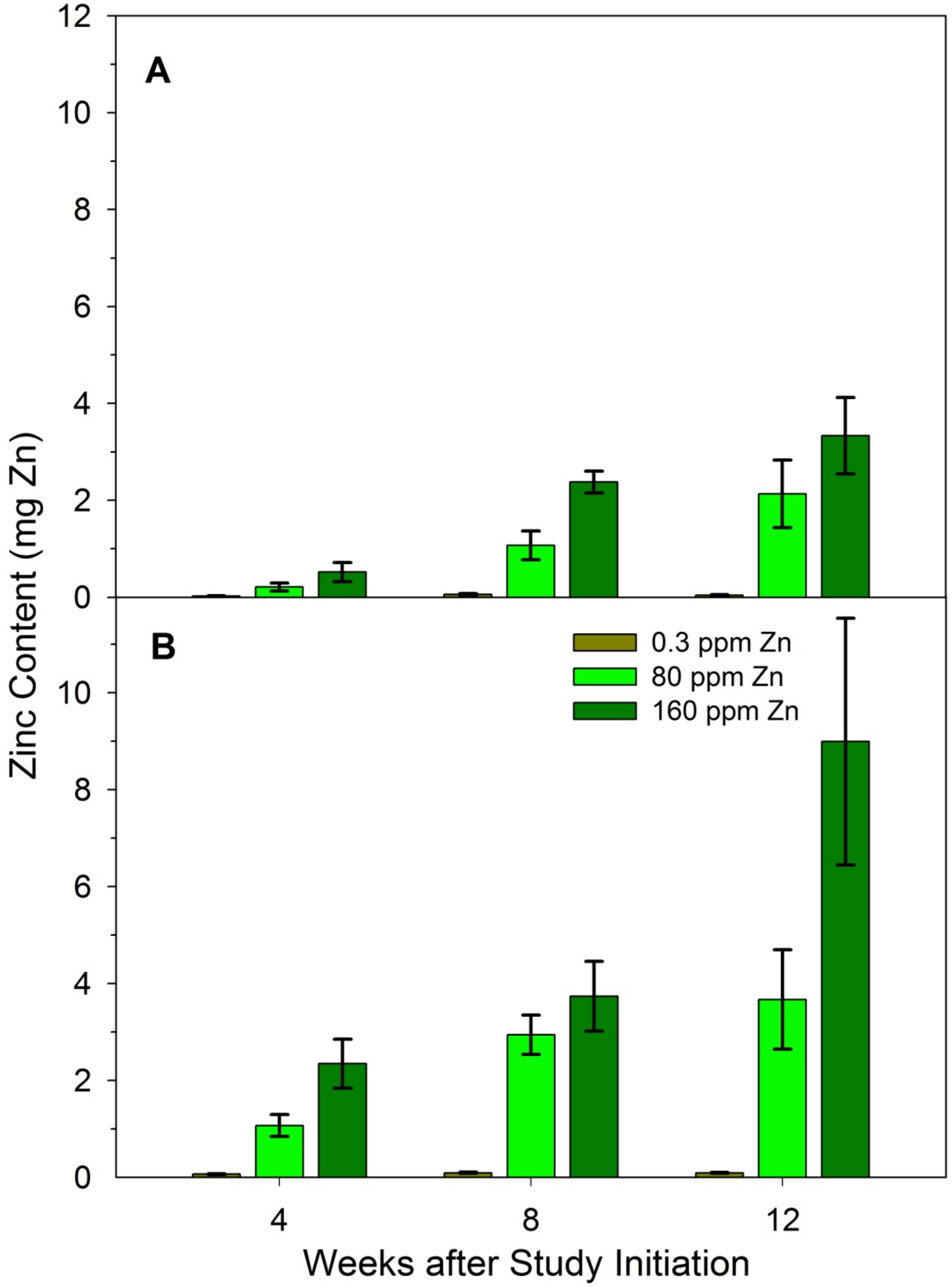
App. Fig. C9. Comparison of average shoot Zn content in *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a greenhouse environment.



App. Fig. C10. Comparison of average shoot Zn content in *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a growth chamber environment.



App. Fig. C11. Comparison of average root Zn content in *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a greenhouse environment.



App. Fig. C12. Comparison of average root Zn content in *S. kamtschaticum* for three Zn treatments (0.3, 80 and 160 mg Zn/L), grown for twelve weeks in (A) rooflite® and (B) glass bead substrates, in a growth chamber environment.

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