# Promoting the Re-Vegetation Of Disturbed Pit and Quarry Soil Using Grodan® Rockwool Treated with Wastewater as a Nitrogen and Phosphorus Source

by

Joanne Mary Elizabeth Garnett

A Thesis

presented to

The University of Guelph

In partial fulfillment of requirements
for the degree of

Master of Science
in
The School of Environmental Science

Guelph, Ontario, Canada

# ABSTRACT

## PROMOTING THE RE-VEGETATION OF DISTURBED PIT AND QUARRY SOIL USING GRODAN® ROCKWOOL TREATED WITH WASTEWATER AS A NITROGEN AND PHOSPHORUS SOURCE

Joanne M. E. Garnett
University of Guelph, 2018

Advisor:
Dr. Susan Glasauer

The disturbed soils of former aggregate mining sites in Ontario can vary in texture, moisture retention, and fertility complicating efforts for re-vegetation. Grodan® rockwool, untreated (GRW) or treated with wastewater (GRW-AWW), was installed in stony and silt-loam soil, to determine if this amendment could help maintain moisture, provide plant-available nitrogen (N) and phosphorus (P) to perennial ryegrass (*Lolium perenne* L.), and reduce N and P leaching. A zone of GRW in stony soil demonstrated a mean higher volumetric water content percent (VWC%) than the control. The mean length of perennial ryegrass, grown in stony soil treated with GRW-AWW, were 0.99 cm longer than the control. A zone of saturated GRW was found to significantly reduce ammonium leaching in silt-loam soil. The rate of GRW's degradability was undetermined. Wastewater-treated GRW may be useful as a soil amendment, however, more research is needed to establish GRW's persistence and interaction with soil properties.

**Abbreviations:**

| | |
|---|---|
| Grodan® rockwool (GRW) | Untreated GRW (GRW) |
| Artificial wastewater (AWW) | Saturated GRW (GRW-0) |
| Artificial rainwater (AR) | Artificial wastewater treated GRW (GRW-AWW) |
| Silt-loam soil (Elora) | Non-specific nitrogen (N) and phosphorus (P) |
| Stony soil (MAC) | Field trial soil (Bovey) |

**Key words**: Grodan rockwool, wastewater, pit and quarry soil, re-vegetation, soil amendment.

**Acknowledgements**

I sincerely want to thank the following people, without whom, this thesis and research would not have been possible. Thank you to:

- Dr. Susan Glasauer, my advisor, for her guidance, positivity, and humour.
- Dr. Ivan O'Halloran (adv. committee), for his insights, suggestions, and passion to teach.
- Dr. Youbin Zheng (adv. committee), for his enthusiastic discussions about my research.
- The late, Dr. Irina Solntseva, for so much including the hands-on experience in the lab.
- Dr. Michelle Edwards and Ms. Lucia Costanzo, for their invaluable statistics assistance.
- Dr. John Lauzon, for his expertise and always having time for my questions.
- Stephen Wilson, machine shop technician, who always knew where to find things for me.
- Jill Filmer, for helping me harvest grass blades from gravel for 12 hours.
- Ron Dutton, Bovey greenhouse manager, for his kindness and problem-solving ability.
- My husband Brian, for his unwavering love and support throughout this endeavor.

I would also like to thank the late, Mr. Dave McKenzie, President of McKenzie Brothers Ltd., Guelph, Ontario, for sharing his insights into the aggregate mining industry and for generously donating the stony soil used throughout this project.

Finally, I gratefully acknowledge the financial support for this research from the Southern Ontario Water Consortium (SOWC) and Terratec Environmental Ltd., Hamilton, Ontario (Mark Janiec, Land Application Manager).

## Table of Contents

## List of Tables

## List of Figures

## List of Appendices

# Chapter 1 – General Introduction

## 1.1 Introduction

Aggregate production in Ontario continued to increase in 2015 for the third straight year, with almost 150 million tons excavated (TOARC, 2016). In 2004, there were more than 5,300 active aggregate mining sites in Ontario, Canada, with an average size of 12-15 hectares (Corry et al., 2008). The Aggregate Resources Act (ARA), instituted in 1990, requires the aggregate company restore the site progressively, during the pit or quarry operational lifetime (OSSGA, 2010). The disturbed soils of post-aggregate mining can vary in texture, moisture retention, and fertility, complicating efforts for re-vegetation. Stony soil commonly overlies sand and gravel deposits reducing the soil volume for plant roots requiring moisture and nutrients (Mackintosh and Mozuraitis,1982; SAROS, 2009). The sites frequently have shallow soil or overburden to plant in, and only natural precipitation to rely on, resulting in losses of transplanted vegetation. Commercial fertilizers are not typically used because of budgetary restrictions and because there is a high potential for nutrient leaching into water reservoirs due to the typical location of aggregate sites (personal conversation with D. McKenzie, McKenzie Bros. Ltd., Guelph, ON).

The aggregate mining industry has other site rehabilitation concerns. Prior to 1990, unregulated aggregate production left thousands of sites unrestored across Ontario referred to as legacy pits and quarries (TOARC, 2016). The Management of Abandoned Aggregate Properties (MAAP) program is mandated to restore them and continues to look for new and innovative ways to deal with the challenges of pit and quarry restoration whereby minimal intervention causes a site to naturalize faster than it would have on its own (TOARC, 2016).

A few studies have been conducted using greenhouse waste-rockwool as a soil amendment for soils damaged from mining (Gilewska, 2006) or from tillage (Reynolds et al., 2003). Some studies have also mixed fresh rockwool into soilless media to modify characteristics (Fonteno, and Nelson, 1990) and as a conditioner to boost water retention in sandy soils (Bussell and Mckennie, 2004). But no previous studies were found using rockwool treated with wastewater as a soil amendment. This thesis aims to investigate the potential for wastewater-treated fresh rockwool, used as a soil amendment supplying N and P fertilizer, to promote the re-vegetation of disturbed soils associated with aggregate mining.

## 1.2 Goal

➢ To provide scientific insight into the use of wastewater-treated Grodan rockwool as a soil amendment and fertilizer for promoting the re-vegetation of disturbed pit and quarry soil.

## 1.3 Objectives

1) Determine the persistence of Grodan rockwool (GRW) in the soil environment.
2) Determine if a subsurface zone of GRW can prolong moisture retention in the top 5 cm of stony and agricultural soil.
3) Determine if a subsurface zone of GRW, treated with wastewater, can provide plant-accessible P and N and diminish P and N leaching.
4) Determine the effect of wastewater-treated GRW on soil microbial growth as evidenced by $CO_2$-C respiration and SEM-detected biofilm.

## 1.4 Thesis Format

This thesis is composed of seven chapters. The first chapter introduces the issues associated with aggregate mining site re-vegetation along with the goal and objectives of the project. The second chapter provides a review of the scientific literature and previous research relevant to the four objectives of the thesis research. Chapters 3 – 6 present the methodology, results, and discussion of a series of separate but related experiments pertaining to the four objectives. Chapter 3 presents the results of two separate laboratory experiments undertaken to determine how long the Grodan® rockwool (GRW), which is ~ 46% silicon dioxide ($SiO_2$), will persist in the soil environment through pH and silicon dissolution (Si) analyses. Chapter 4 presents results of an investigation assessing the potential for a subsurface zone of saturated GRW (GRW-0) to maintain soil moisture in the top 5 cm of two soils, a stony soil (MAC) and a silt-loam agricultural soil (Elora). The results of microbial $CO_2$-C respiration in response to the soil treatment are also presented. Chapter 5 presents results from a leaching experiment using MAC and Elora soils, to assess the capacity of GRW-0 and GRW-AWW to reduce nitrogen (N) and phosphorus (P) leaching. Chapter 6 presents the results of a greenhouse experiment using both soils, as well as a field trial with stony soil in Guelph, Ontario. The goal was to assess the potential of GRW treated with artificial wastewater (GRW-AWW) to provide plant-available N and P to perennial ryegrass (*Lolium perenne* L.). The results of microbial $CO_2$-C respiration and Scanning Electron Microscope (SEM) findings, in response to the soil treatments, are also presented. The seventh and final chapter consists of a brief summary, overall conclusions, and recommendations for future research.

# Chapter 2 – Literature Review

## 2.1 Rehabilitation of Disturbed Aggregate Mining Sites

Mineral aggregate producers in Ontario supply sand, gravel, and crushed stone products to various industries. Aggregates are the main ingredients in a number of manufactured products including glass, pharmaceuticals, fertilizer, (OMNR, 2010) building materials, and rockwool to name a few. Mineral aggregate deposits tend to be found in outwash plains, limestone plains, and glacial deposits. These landforms commonly feature water resources, wetlands, woodlands, and agriculture. In 2004 there were more than 5,300 active aggregate mining sites in Ontario, Canada with an average size of 12-15 hectares (Corry et al., 2008). In addition to active sites, there were 3,666 licenses issued for pits and quarries on private land and 2,644 aggregate permits issued for Crown land in 2015 (TOARC, 2015). Typical aggregate extraction sites operate 30 to 40 years, after which, the final rehabilitation is expected to restore the site to the pre-mining state or to a function compatible with the surrounding land (Corry et al., 2008; OSSGA, 2010). The Aggregate Resources Act (ARA), instituted in 1990, requires the aggregate company restore the disturbed land progressively during the pit or quarry operational lifetime (OSSGA, 2010). This means areas of the site, where extractions are finished, undergo rehabilitation while extractions continue in another area. Re-vegetation in particular can be challenging in disturbed sites because they often have shallow soil or merely overburden to plant in and only natural precipitation to rely on. Also, commercial fertilizers are not an option because of the high risk of nutrient leaching into reservoirs and the expense would exceed a typical restoration budget of 25 cents per extracted tonne of aggregate (personal conversation with D. McKenzie, McKenzie

Bros. Ltd., Guelph, ON) . The progressive and final site restorations are deemed acceptable by the Ministry of Natural Resources and Forestry (MNRF) (OSSGA, 2010).

Since 1997, under the administration of the MNRF and directed by the Ontario Aggregate Resources Corporation (TOARC, 2016), the Management of Abandoned Aggregate Properties (MAAP) program has been mandated to research and facilitate the rehabilitation of 'abandoned' pits and quarries in Ontario. The unregulated aggregate extraction on these legacy sites typically took place prior to the ARA legislation of 1990 and involves properties now owned by individuals, corporations, or municipalities. In 2016, with the permission of owners, the MAAP program conducted rehabilitative activities on 36 of these sites. However, there remains 2919 known sites in Ontario requiring restoration (TOARC, 2016).

The cost to rehabilitate the average legacy site (1.59 ha) has been approximately $11,500/ ha which is not paid by the property owner (TOARC, 2016). Funding for the MAAP program is derived from the aggregate producer's licensing fee at a rate of 0.5 cent per tonne of aggregate (TOARC, 2016). On many legacy sites, re-vegetation is sparse or dominated by species that are not native to the area (Corry et al., 2008; OSSGA, 2010). The MAAP program gives priority to sites with a lack of vegetation or are at risk for erosion with safety or topography issues (TOARC, 2016).

The rehabilitation of disturbed sites usually includes grading slopes using pre-existing topsoil and subsoil (OSSGA, 2010). Stony soils overlying sand and gravel deposits is a common problem hindering mechanized cultivation and reducing soil volume for plant roots requiring moisture and nutrients (Mackintosh and Mozuraitis,1982; SAROS, 2009). The importation of large quantities of quality topsoil is not practical, physically possible, or economically feasible.

Therefore alternative schemes are considered such as procuring a thin layer of organic soil from an adjacent undisturbed area to spread over the barren site (TOARC, 2016).

The MAAP program continues to support research to develop new and innovative ideas to deal with the challenges of legacy pit and quarry restoration whereby minimal intervention causes a site to naturalize faster than it would have on its own (TOARC, 2016). The idea suggested by this study, is to utilize a readily available material, such as rockwool or even greenhouse waste-rockwool, treated with wastewater, as a soil amendment for promoting the re-vegetation of the post-extraction aggregate mining sites.

## 2.2 Previous Research Using Rockwool as a Soil Amendment

Few papers were found evaluating the use of fresh or greenhouse waste-rockwool as an innovative soil amendment to promote re-vegetation and soil reclamation. One study, for example, investigated a landfill in Poland that received ash and slag from a coal-burning power plant for decades. In an effort to reclaim the land, Gilewska (2006) dosed 1000 $m^2$ plots with 400 $m^3$ $ha^{-1}$ of waste-rockwool and 100 -300 kg N $ha^{-1}$over a four year period and observed the growth of winter wheat (*Triticum aestivum* L.). The results demonstrated the rockwool-treated soil had better drainage, less surface water pooling, 5-6 day earlier germination, and higher yields than the control treated with only N. Gilewska (2006) determined using the waste-rockwool was the cheapest and simplest method to deal with the accumulation of non-biodegradable waste-rockwool and achieve soil reclamation as well. The Jaroszuk-Sierocinska et al., (2014) study documented the deterioration of rockwool post-production in terms of structure, air-water properties, and bulk density. They found the properties of rockwool for horticultural use should not be considered representative of rockwool in the soil environment.

However, even in its deteriorated state, waste-rockwool would be effective in improving air-water properties of heavy or compacted soils (Jaroszuk-Sierocinska et al., 2014). Somewhat agreeing, Reynolds et al., (2003) found waste-rockwool, shredded to pass through a 6mm sieve, did not improve the plant-available water capacity but did improve the air capacity in a clay loam soil. Fonteno and Nelson's (1990) study found a similar result comparing the water holding capacity of rockwool to that of sand. However, its high porosity characteristic worked similarly to peat moss in soilless container mixes. On the other hand Bussell and McKennie (2004) found granular rockwool could boost the water holding capacity of sandy soil.

### 2.3 Grodan® Rockwool

Grodan® rockwool (GRW) was developed for use as a horticultural substrate more than 30 years ago. In 2012 the Grodan Group opened North American's first production line of Grodan® rockwool products at their sister company, Roxul Inc. in Milton, Ontario, Canada. The GRW is a fibrous material made from 60 percent basalt rock, 20 percent limestone, and 20 percent coke (Raviv and Lieth, 2008) melted at approximately1600$^{o}$C and spun at high speed to extrude fine fibres from the molten mixture (De Rijck, and Schrevens, 1998; Resh, 2004). Fibre diameters can vary from 2 to 6 μm (Canadian Environmental Protection Act, 1993) with the average approximately 4 μm (Bougoul et al., 2005). Moderate amounts of amorphous particulate (shot) (Fig. 2.1) is also produced during the manufacturing process averaging 20 to 50 percent of the material's weight (Canadian Environmental Protection Act, 1993). While cooling, the fibres are treated with a binder, allegedly urea-phenol-formaldehyde based, (Vantsi, O. and Karki, T., 2014) and an unnamed wetting agent, to reduce surface tension (Resh, 2004) and impart hydrophilic properties (da Silva et al., 1995; Urrestarazu et al., 2007; Raviv and Lieth, 2008)

respectively. The fibres are pressed loosely into mats, re-heated at 260°C to harden them (De Rijck, and Schrevens, 1998), then cut into various sizes such as slabs or cubes for greenhouse horticultural applications including plant propagation and crop production. Strategically, the fibres are assembled in a horizontal or vertical orientation, depending on the GRW product, to control drainage (Bussell and Mckennie, 2004; Bougoul et al., 2005; grodan101.com/faq).

Rockwool is inert with no cation exchange capacity (CEC) and almost no electrical conductivity (EC= 50 – 100 µS/cm) (De Rijck, and Schrevens, 1998). The composition of rockwool can be seen in Table 2.1. The pH of rockwool has been as high as 7 - 8.5 but 6 - 6.5 is presently the industry standard (Raviv and Lieth, 2008). Rockwool does not have a pH buffering capacity so the pH is easily manipulated when using nutrient solutions (Resh, 2004). Rockwool in general has a low bulk density (BD) ranging from 0.05 to 0.1 g $cm^3$ and a pore volume between 92 – 98 % (Resh, 2004; Raviv and Lieth, 2008) with an 80 % water-holding capacity (Resh, 2004).

Table 2. 1 The composition of Grodan® Rockwool (left) (adapted from grodan101.com) as compared with generic rockwool (right) (adapted from Vantsi and Karki, 2014).

| **Grodan® Rockwool** | |
|---|---|
| Element | % mass |
| Si | 46 |
| Ca | 16 |
| Al | 14 |
| Fe | 8 |
| Na | 2 |
| Ti | 1 |
| Mg | 1 |
| K | 1 |
| Mn | 1 |
| Total | 90% |

| **Rockwool** | |
|---|---|
| Oxide | % mass |
| $SiO_2$ | 46.43 |
| CaO | 17.89 |
| $Al_2O_3$ | 11.42 |
| MgO | 9.24 |
| FeO | 4.72 |
| $Fe_2O_3$ | 4.41 |
| $Na_2O$ | 3.07 |
| $TiO_2$ | 1.47 |
| $K_2O$ | 1.01 |
| MnO | 0.23 |
| BaO | 0.11 |
| Total | 100% |

Fluid is retained in variable sized pores of the rockwool by the action of capillary forces (Bougoul et al., 2005). The density of rockwool varies between products and can vary within the same rockwool slab. Consequently, the hydraulic conductivity (K) is anisotropic; changing in value in different directions, and influenced by the fibre orientation (Bougoul et al., 2005). The water holding capacity of rockwool is low (da Silva et al., 1995; Raviv and Lieth, 2008). For example, the K at saturation in a 7.5 cm thick slab is 4.6 cm $min^{-1}$ but the K value decreases sharply as water tension (suction) increases in small increments (da Silva et al., 1995) with free drainage. This property results in discrepancies in moisture and air volume between the upper and lower areas of the rockwool slab (Raviv and Lieth, 2008).

The intense heat during manufacturing renders the rockwool sterile, an invaluable attribute for the greenhouse industry. Even though the potential life span of rockwool for horticultural use can be up to 5 years, each slab is more likely used for only one (Acuna et al., 2013) to four cropping seasons (Dias et al., 2017; Resh, 2004) due to the potential for disease transmission and deteriorating quality including compaction, altered K (Wever, and Kipp, 1998), and reduced water holding capacity (Jaroszuk-Sierocinska, et al., 2014).

Figure 2. 1 Light microscopy image (400x) of Grodan® rockwool fibres showing glass-like translucency and some amorphous bodies called ‘shot’. (Image by J. Garnett, 2017)

### *2.3.1 Rockwool Biodegradability in the Environment*

Knowing how long Grodan® rockwool will persist in the soil environment would be useful for determining how long the material would be effective as a soil amendment. However, Grodan rockwool's persistence and biodegradability in the environment is unclear. An extensive literature search found no studies of the decomposition of rockwool, or similar mineral fibres, in the soil environment. A few papers, however, did mention rockwool was not biodegradable (Allaire et al., 2004; Gilewska, 2006; Acuna et al., 2013; Jaroszuk-Sierocinska et al., 2014) but there was no research to substantiate the claim.

Grodan does imply on their website (grodan101.com) that rockwool products will degrade under certain circumstances. For example, Grodan posted a 2011 recycling brochure on their website depicting waste-rockwool as a soil amendment, for sand or clay soils, that would be effective for 5-9 years. Also, the Grodan website cautions consumers not to use pre-conditioning or nutrient solutions lower than pH 5 because rockwool will "start to dissolve". Unfortunately, no references were given to justify the 5-9 year timeline or the concerns about degradation at low pH.

Information available, under the ecological subtitle of the Grodan® MSDS (2014) for mineral wool, states the persistence and degradability in the environment is not known. It further states that, although mineral wool (which includes rockwool) it is not classified as environmentally hazardous, "...this does not exclude the possibility that large or frequent spills can have a harmful or damaging effect on the environment". This is an interesting statement since Canadian greenhouses' most popular growing medium, rockwool, was routinely disposed of in landfills (Papadopoulos and Gosselin, 2007). Disposal in landfills is not environmentally sustainable; not only because rockwool is considered non-biodegradable (Allaire et al., 2004;

Gilewska, 2006; Acuna et al., 2013; Jaroszuk-Sierocinska et al., 2014) but it is bulky and there is a potential for high volumes. For example, the main substrate used in greenhouse vegetable production in developed countries is rockwool (Allaire et al., 2004). It has been estimated that 125 $m^3$ of waste-rockwool is produced per hectare of plant production (Raviv and Lieth, 2008). The Grodan company suggests 50 $m^3$ of rockwool will grow 350 metric tonnes of tomatoes (Rockwool, 2015). .In 2015, Ontario produced 183,823 metric tonnes of greenhouse tomatoes (Agriculture and Agri-Food Canada, 2016c) using rockwool which, by Grodan parameters, estimates to be at least 525.2 $m^3$ rockwool waste annually just for tomatoes. However, the total annual amount of post-production or waste- rockwool in Ontario is likely higher since Ontario represents 69% of Canada's 14 million square metres, of greenhouse vegetable production (Agriculture and Agri-Food Canada, 2016a). To address this mounting issue of waste-rockwool, Grodan began developing rockwool recycling programs about10 years ago (Raviv and Lieth, 2008) to facilitate its end-of-life disposal (Rockwool, 2015). Programs include recycling post-production or waste-rockwool into new rockwool products or construction bricks.

This controlled study required fresh Grodan rockwool in the experiments for material consistency. However, post-production or waste-rockwool from a greenhouse may have been considered a feasible and cheaper alternative.

### *2.3.2 Rockwool/Basalt Weathering in the Environment*

Rockwool is 60% amorphous basalt rock, a silicate mineral which is approximately 46 % silica. This study considered the dissolution of rockwool in the soil environment may be analogous with the natural processes of silicate mineral weathering.

Silicon (Si) readily bonds covalently with oxygen to form silicate tetrahedra ($SiO_4^{4-}$); the basic building block for silicate minerals that make up 90% of the earth's crust (Lutgens, and Tarbuck, 2015). Silica ($SiO_2$), also known as silicon dioxide, is constantly dissolving and precipitating in the earth's crust (Iler, 1979). Soluble silica ($Si(OH)_4$) results from the natural weathering of silicate minerals (Iler, 1979) by acidic plant root exudates (Jin et al., 2008), humic acids from decaying organisms, and water. Lichens secreting acids can cause the dissolution of silica from rock (Iler, 1979). In one study, the rate of basalt dissolution increased by a factor of three where vegetation was present (Drever, 1994). Temperature, moisture, and the amount of vegetation influences the rate of silica weathering.

Plants and their associated microbiota affect silicate mineral weathering by modifying pH through the production of $CO_2$, organic acids, and by changing the physical properties of the soil by binding small particles and altering soil hydrology (Drever, 1994). The dissolution rates of silicate minerals are unaffected by neutral pH, but increase with decreasing pH, and increase again in the alkaline region resulting in a U-shaped trend line. However the transition pH value from acid to neutral to alkaline is specific to the silicate mineral (Drever, 1994). Basalt dissolution and subsequent Si release exhibits a similar U-shape variation with pH however temperatures above $50^oC$ can slow down the Si release (Gudbrandsson, et al., 2011).

A study conducted by White and Brantley (2003) found weathering rates of silicate minerals were inversely proportional to the surface area where ion exchange reactions occurred. They developed an equation for the average silicate weathering rate for four silicate minerals including plagioclase, a mineral component of basalt. The equation for the average rate was $R= 3.1 \times 10^{-13} \times t^{-0.61}$ ( $mol\ m^{-2}\ s^{-1}$). Interestingly, they concluded the lab environment with small, fresh silicate minerals observed over short intervals, could not reproduce adequately the

natural environment for silicate weathering. Natural weathering rates are influenced by water exposure and flow patterns, variations in climate and biological activity, and ultimately, time (White and Brantley, 2003).

Time dependency of silicate weathering may lead us to conclude the environmental persistence of rockwool, which as previously mentioned is mostly basalt rock, may not be accurately determined through laboratory methods and would be better investigated in a long-term field study.

### *2.3.3 Rockwool Safety and Rockwool Dissolution in vitro Studies*

In 1988, rockwool was classified by the International Agency for Research on Cancer (IARC) as a Group 2b meaning it was 'possibly carcinogenic to humans' (Campopiano, et al., 2014). In 2001, with new data, the IARC re-classified rockwool as a Group 3 meaning 'there is inadequate evidence in humans, and limited evidence in experimental animals, to declare rockwool carcinogenic (IARC, 2002; Campopiano, et al., 2014). Health Canada (2006) however states there is some evidence of a link between working with rockwool and lung cancer but the studies are not conclusive.

The Grodan® mineral fibre Material Safety Data Sheet (MSDS) of 2014 refers to the composition of the mineral wool (rockwool) as "man-made synthetic vitreous silicate fibres with no crystalline silicate". Non-crystalline or amorphous is an important distinction since crystalline silica (quartz, cristobalite and tridymite) dust is a IARC Group 1 carcinogen known to cause lung-scarring silicosis and/or lung cancer. Reports addressing the possible link between cancer and man-made vitreous fibres (MMVF) such as rockwool have been widely published (Canadian Environmental Protection Act 1993; IARC, 2002; Health Canada, 2006). The MMFV

fibres have physical and aerodynamic properties similar to asbestos; a known carcinogen. However, unlike asbestos, the MMVF fibres on average, have a much wider diameter and tend to break transversely rather than down the long axis of the fibre (IARC, 2002). Fibres >20 μm long (IARC, 2002) are generally believed to increase incidence of fibrosis or cancer because they cannot be fully engulfed by macrophages and persist in the lung (Christensen et al., 1994; Campopiano, et al., 2014).

The biopersistance and biosolubility of mineral fibres including rockwool have been thoroughly researched in *in vitro* dissolution experiments (Scholze and Conradt, 1987; Christensen et al., 1994; Campopiano, et al., 2014). Hydrolysis is the process by which dissolution of rockwool and other MMVF fibres occurs (Schott and Oelkers, 1995). Water molecules attacking the fibre surface has the largest impact on fibre surface morphology (Lund and Yue, 2008). Water and amorphous silica, found in rockwool, are very much alike in that they both consist mainly of oxygen atoms with the smaller hydrogen or silicon atoms in the adjoining spaces. In amorphous silica there are 1.17 g $cm^3$ of oxygen whereas in water there is 0.89 g $cm^3$ (Iler, 1979). .The dissolution rate is largely due to the fibre's composition (IARC, 2002).

Most dissolution studies measure the loss of Si from the silicon-rich fibres and the subsequent Si concentration in solution, which is both easy and valid (de Meringo et al., 1994). Using a pH 7.6 solution, simulating the extracellular fluid in the lung, Scholze and Conradt (1987) measured the loss of Si from 1 μm diameter rockwool fibres at 37$^{o}$ C in a flow-through 120 day experiment. Their results indicated the rockwool fibre in a lung would dissolve completely in 1.2 to 2 years. Campopiano, et al. (2014) conducted a similar study but included pH 4.5 which would represent the acidic environment in the macrophages of the lung. Their results show rockwool's dissolution rate (weight loss /surface area / hour) would be 9.9

ng/cm$^2$/hr in pH 7.4 and 17 ng/cm$^2$/hr in pH 4.5. In one other study it was determined the dissolution rate of rockwool at pH 4.5 increases as the calcium oxide content increases and decreases as the silicon dioxide content increases (Christensen et al.,1994).

For decades several laboratories around the world have measured mineral fibres in human lung tissue but there has been no report of a substantial pulmonary concentration of MMVF fibres which may also be an indication of a low biopersistence (Sebastien, 1994).

### *2.3.4 Rockwool Binders and Wetting Agents*

Another health concern with rockwool (personal communication with the Ministry of the Environment and Climate Change, Ontario, Canada) is related to Grodan's undisclosed binder resin and wetting agent chemicals, touted as naturally derived and deemed a trade secret (personal email with Brett.Cherniack @grodan.com, Grodan Product Specialist). The binder resin and wetting chemical agents are not listed in the 2014 Grodan MSDS (Grodan, 2014). However a binder resin was mentioned in the Rockwool Int. 2008 Growth Substrate Patent (WO 2008009465 A1) as being either phenol formaldehyde or urea formaldehyde. Grodan's mineral wool 2005 MSDS (Grodan, 2005) refers to the resin as urea-phenol based. Both phenol and formaldehyde are toxic to humans and the environment under certain conditions. Apparently, modern phenol-formaldehyde resins do not release phenol or formaldehyde in amounts exceeding some safety limits (Vantsi and Karki, 2014).

In order to assess the safety of applying Grodan rockwool to soil in our field study (Chapter 6), samples of soil from the site were taken from each of the four replicate plots at a depth of 0-15 cm and composited. They represented soil exposed to untreated Grodan rockwool for seven months along with the control soil. The samples were sent to the ALS Environmental

laboratory in Waterloo, Ontario, Canada to be tested for phenol, formaldehyde, and volatile organic chemicals (VOC). The results confirmed the GRW-exposed soil did not contain elevated levels of the aforementioned chemicals as compared to the control soil. The ALS laboratory results can be viewed in Appendix 3 (Fig. A3.1 – A3.5).

## 2.4 Soil Phosphorus, Nitrogen, and Leaching

As a possible soil amendment promoting re-vegetation, the wastewater-treated Grodan rockwool (GRW-AWW) was expected to release plant-available N and P. It is the intent of this study to investigate whether the GRW-AWW also reduced N and P leaching in the process.

Nitrogen and P are essential macronutrients for plant growth. Depending on a number of factors, including soil texture and hydrology, both nutrients can leach downward in the soil solution or be lost in overland flow and enter waterways (Eghball et al., 1996; Brady and Weil, 2008; Wang et al., 2012, Drury et al., 2016). Over time, this can result in eutrophication and hypoxia of aquatic ecosystems. In Ontario, lakes and rivers with a total P concentration ranging from 0.035 to 0.10 mg $L^{-1}$, are considered eutrophic (Environment Canada, 2004). Phosphorus concentrations exceeding 0.065 mg $L^{-1}$ are sufficient to support algal growth in many water bodies when nitrate-N concentrations exceed 10 mg $L^{-1}$ (Haney et al., 2015). Elevated nitrate ($NO_3^-$) in surface water is also acidifying and can be toxic in drinking water (Kreyling et al., 2015).

Nutrient losses through run-off and leaching as well annual applications of N and P fertilizer, can be a costly, ongoing expense for some producers. Studies have demonstrated that nutrient leaching can be more profound in coarse-textured sandy soils (Eghball et al., 1996; Wang and Alva ,1996) as compared to clay or silty loam soils. In one study, more than 88% of

the total N was leached when soluble $NH_4NO_3$ was applied to sandy soil receiving intermittent precipitation (Wang and Alva ,1996). Eghball et al. (1996) found P originating from applied manure moved deeper into the soil profile than P from conventional fertilizer in sandy soil which increased the potential to contaminate shallow, groundwater tables.

Nitrogen in the forms of $NO_3^-$ and ammonium ($NH_4^+$) move through the soil by a combination of mass flow and diffusion (Richardson et al., 2009). However, $NH_4^+$ is less mobile than $NO_3^-$ in soil solution since it readily adsorbs to the anion exchange sites present in most soils contributed by clay minerals and organic matter. Plant uptake of $NO_3^-$ and $NH_4^+$ is facilitated by their concentrations in the soil and soil solution, and by soil water content, plant growth rate, and root distribution (Richardson et al., 2009). Comparatively, in sandy soils receiving 100 – 120 cm annual rainfall, N uptake efficiency by plants is 20% less than those grown in loam soil (Wang and Alva, 1996). In general, sandy soils have a reduced ability to provide adequate nutrients to young plants due to high macroporosity, low organic matter and low clay content required for nutrient retention (McClellan et al., 2007).

In soil solution, inorganic P is generally at low concentrations and considered a limiting factor for plant growth. The concentration of P in soil solution rarely exceeds 0.1 – 1ppm (Paul and Clark, 1989). Few unfertilized soils can release P at a rate required by crop plants to achieve desired yields (Schachtman, et al. 1998) therefore P fertilizer must be applied. Even then, plants may only access 15 – 20% of the applied P fertilizer in the year of application because the nutrient's movement is limited and it is quickly adsorbed by soil colloids (Smil, 2000; Debicka et al., 2015). Since approximately 80% of the applied P may not be plant accessible, P application rates may increase thereby increasing the potential for excess applied P to enter waterways through run-off or leaching (Eghball et al., 1996; Debicka et al., 2015).

Adsorption and precipitation reactions are the main processes by which P is removed from soil solution (Tunesi et al., 1999). The variability of P speciation in soil solution occurring with precipitation and dissolution, and adsorption and desorption, is influenced by pH, concentrations of anions competing with P for ligand exchange, and the concentration of calcium (Ca), iron (Fe), and aluminum (Al) metals. Plant-available P in soil, predominantly $H_2PO_4^-$ and $HPO_4^{2-}$ anions, is usually highest in the pH range of 6.0 to 7.0. At higher pH levels (>7.5), Ca and magnesium (Mg) cations can precipitate with orthophosphate ($PO_4^{3-}$) to form salts with low solubility. At lower pH levels (pH<6.0), iron ($Fe^{3+}$) and aluminum ($Al^{3+}$) cations react with the phosphate to form insoluble compounds (Tunesi et al., 1999; Brady and Weil, 2008).

The mobility and the concentration of P in soil solution is controlled by the solubility of P compounds and the adsorption of phosphate ions on the surface of soil particles (Brady and Weil, 2008). Some factors limiting the availability of P to plants are moisture and temperature. Phosphorus requires moisture for movement along a concentration gradient (diffusion) moving only a few millimeters in the subsoil per year (OMAFRA, 2006). However, excessive moisture could result in low oxygen which reduces microbial activity needed for organic P mineralization and solubilization. Cold air and soil temperatures also reduce microbial activity lowering organic P mineralization.

Debicka et al. (2015) suggests the soil organic matter (SOM) plays a major role in P availability. Humic molecules from the SOM compete with P for adsorption sites and may entrap reactive Fe and Al cations in stable organic complexes potentially increasing inorganic P availability for plants (Brady and Weil, 2008; Debicka et al., 2015). Sandy soils, commonly low in SOM, are more prone to P (as well as N) leaching when commercial fertilizer is applied.

However, the type of fertilizer and rate of application influences nutrient leaching in sandy soil as well (Weaver et al., 1988).

### *2.4.1 Wastewater-sourced N and P Fertilizer*

Conventional, on-site septic systems discharge wastewater from the septic tank directly to a leach field consisting of a tile bed or sand filter where it will slowly percolate down through the soil into the ground water. Essentially the soil becomes the final wastewater treatment medium, which may or may not have the properties to effectively immobilize and prevent the leaching of nutrients such as N or P. An Ontario-based company conducted a pilot project modifying an on-site septic system, using rockwool as a filter, in an attempt to reduce P leaching. Investigating the potential for the spent, rockwool filter, as a soil amendment to stimulate plant growth, became the purpose of this research.

By-products of municipally treated sewage have been land-applied for decades by companies like Terratec Environmental in Ontario and across Canada as a fertilizer for agriculture, reforestation, and brownfield reclamation to name a few (C.C.M.E., 2012). The wastewater by-products, called biosolids or wastewater residuals, can be solid, liquid, or pelletized. Biosolids are abundantly produced in Ontario. For example, between 2014 – 2016 wastewater treatment plants commonly generated 1000 – 5000 $m^3$ of liquid biosolids and around 5000 tonnes dewatered biosolids per year (Jin and Parker, 2017). Generally high in organic matter, wastewater residuals contain several nutrients required by plants including broad ranges of N (1 – 10.8% dry weight) and P (0.7 – 7.5% dry weight) depending on the influent quality and the sewage treatment process (C.C.M.E., 2012). All forms of N can be present in residuals

including organic N, $NO_3^-$, nitrite ($NO_2^-$) and ammonia ($NH_3$). Phosphorus can be present in the form of orthophosphate, polyphosphates and organic P (Loganathan et al., 2014).

Similar to commercial fertilizer, mineralization and plant-availability of the N and P from wastewater residuals ultimately depends on the inherent soil characteristics (texture, structure, moisture, pH), plant species/requirements, placement, timing, temperature, and precipitation. For example, wastewater-sourced fertilizer applied on stony soil followed by an intense rain event, may have increased leaching of P (Wang et al., 2012) and N, rendering the nutrients unavailable to plants and more likely to enter groundwater. In this case, a soil amendment that improves moisture retention would be beneficial by keeping dissolved nutrients in soil solution accessible to plants and by reducing nutrient leaching into waterways.

### *2.4.2 Benefits of N and P Sourced from Wastewater*

In 2015, N accounted for 70% and P 21% of the fertilizer used for agriculture in Canada (Agriculture and Agri-Food Canada, 2016b). Canada is a major producer of N (Agriculture and Agri-Food Canada, 2016b) but does not have a natural source of P so it must be imported (Bailey and Grant, 1990). However, the long-term supply of P may be in jeopardy since the foreign rock phosphate mines could be depleted of ore in 50 to 100 years at the current rate of extraction (Smil, 2000; Guan, et al., 2013; Loganathan, et al. 2014). Between 2010 - 2011 the price per tonne of mono-ammonium phosphate (MAP) fertilizer, a popular P fertilizer, increased 32% in Ontario (Agriculture and Agri-Food Canada, 2012). Nitrogen and P sourced from wastewater would provide economic as well as an environmental benefit because it is sustainable, renewable, and locally sourced. Fertilizer obtained from wastewater would reduce importation

costs, fuel consumption and associated $CO_2$ emissions, and environmental damage associated with P mining.

Wastewater fertilizer would also improve soil productivity and soil properties in by increasing organic matter (C.C.M.E., 2012; Loganathan et al., 2014), potentially lowering pH in alkaline soils (Bravo-Martin-Consuegra et al., 2016; C.C.M.E., 2012), and increase beneficial microbial populations.

## 2.5 Soil Microbial $CO_2$-C Respiration

The soil $CO_2$ respiration was assessed in this study as a means of determining changes in microbial biomass and activities as a result of various treatments.

There are billions of organisms in a handful of soil with a diversity greater than the total life forms on the surface of the earth (Brady and Weil, 2008). This community of micro and macro organisms are responsible for several soil functions including decomposition, carbon and nutrient cycling including the mineralization of organic N and P, and the support of plant growth to name a few (Brady and Weil, 2008).

The microbial decomposition of soil organic matter (SOM) is a source of $CO_2$ released from the soil surface. Roots, fauna, and dissolved carbonates also contribute to soil's $CO_2$ respiration (Rochette et al., 1997; Yiqi and Zhou, 2006; USDA, 2009). Dupuis and Whalen (2007) found soil properties such as texture, organic matter, and pH affect the activity and distribution of microbial biomass in the soil. Temperature, moisture, resource availability, as well as previous land management, including disturbances, can also affect soil microbial biomass accumulation and activity (Dupuis and Whalen, 2007).

### *2.5.1 The Solvita® $CO_2$ Burst Test*

Soil respiration can be used to assess biological activity in the soil (Doran et al., 1997; Yiqi and Zhou, 2006; Haney et al., 2012). Rochette et al. (1997) suggests soil respiration is a sensitive indicator of essential ecosystem processes including soil metabolic activity and the conversion of soil organic carbon to atmospheric $CO_2$. Studies have shown a strong flush of $CO_2$ respiration occurs after soil drying and re-wetting events (Franzluebbers et al., 2000; Chowdhury et al., 2011). Franzluebbers et al. (2000) concluded the $CO_2$ flush was associated with the soil's microbial biomass and the total carbon and N pools as well.

Historically, the soil $CO_2$ produced by microbial respiration was measured with an infrared gas analyzer (IRGA) or alkali trap. A disadvantage of the IRGA method is that it requires bulky, expensive equipment along with good weather because it is usually done *in situ*. The alkali trap method, on the other hand, is conducted in a lab using simple titration glassware. However, besides being time consuming, Yiqi and Zhou (2006) point out the alkali trap method is not standardized so results may vary between laboratories. The Solvita® $CO_2$ Burst Test is a standardized, colorimetric system using $CO_2$ – sensitive gel paddles (Woods End Laboratory, ME., USA) that measure $CO_2$ released from soil 24 hours after rewetting and incubation. Haney et al. (2008) conducted research that compared soil $CO_2$ measuring methods and concluded the Solvita system was highly correlated ($r^2$= 0.83) to the results with titration and infrared gas analysis. Goupil and Nkongolo (2014) assessed soil respiration rates using the Solvita $CO_2$ Burst Test after soil amendments were implemented on a mining site restoration in Sudbury, Ontario. They found the Solvita system was not only reliable but also cost effective. The Solvita $CO_2$ Burst Test kit (Fig. 2.2) has a Digital Colour Reader (DCR) that quantifies the colour of the

paddle after 24 hour exposure to soil-respired $CO_2$ by using white, LED light to excite the photons in the coloured gel.

Figure 2. 2 The Solvita $CO_2$ Burst Test ® kit including $CO_2$ sensitive paddle and Digital Colour Reader (DCR). Image adapted from solvita.com.

## 2.6 Perennial Ryegrass

Perennial ryegrass (*Lolium perenne* L.), a popular turfgrass, was chosen for this study because of its adaptability and potential to establish quickly in poor soil. Perennial ryegrass is often used for dairy cattle forage in temperate climates (Burkitt et al., 2007) and has been used for reducing soil erosion, recycling nutrients from manure and biosolids, wildlife feed, and silage (Hannaway et al., 1999). Perennial ryegrass can grow 30 to 90 cm per year for 3-4 years and is competitive and aggressive when established (OMAFRA, 2012). It is adaptable, tolerating both acidic and alkaline soil with a pH range of 5.1 to 8.4, and is suitable for well to poorly drained soils (Hannaway et al., 1999).

Perennial ryegrass has fine, 2-6 mm wide leaves that are 5-15 cm long. The root system is shallow and fibrous without rhizomes. A study by Lyons et al.(2008) found the root length of turfgrass could be altered with the placement of a low-dose P fertilizer deeper in the root zone.

They found it reduced grass shoot growth but promoted deeper rooting, thereby improving heat tolerance, drought resistance, and discouraged shallow-rooted weeds. These attributes would be invaluable for plant re-establishment in disturbed pit and quarry soils in remote locations.

With optimal moisture and temperatures (20 – 30°C) perennial ryegrass germination occurs in 5 – 7 days. Dormant seeding, an alternative sowing method done in late fall or early winter, delays germination until the soil warms up in the spring (OMAFRA, 2008) taking advantage of ample spring moisture.

Typically turfgrass tissue contains 2.0 to 5.0 g P per kg (0.2 – 0.5 % dry matter) (Fulkerson, 2007; Soldat and Petrovic, 2008) and 3.9 g N per kg of dry matter (Fulkerson, 2007). It is important to consider a study conducted by Bailey (1991) which found that the chemical analysis of ryegrass shoot tissue may not be sufficient to determine the P status of the whole plant since the P requirements of the roots are not clear.

# Chapter 3 – Estimating the persistence of Grodan® rockwool in the soil environment using the rate of silicon (Si) released in pH-adjusted rainwater.

## 3.1 Introduction

The research described in this chapter was focused on the persistence of Grodan rockwool (GRW) in the soil environment in order to predict the long-term effectiveness of treated rockwool as a soil amendment.

The biosolubility of mineral fibres, including rockwool, have been thoroughly researched in *in vitro* dissolution experiments (Scholze and Conradt, 1987; Christensen et al., 1994; Campopiano, et al., 2014). However, the biodegradability (broken down by microorganisms) or degradability (chemical decomposition) of Grodan® rockwool in the environment, particularly the soil environment, is unclear. Under the ecological information subtitle, the Grodan® Mineral Fibre MSDS (2014) refers to the persistence and degradability of rockwool as both "unavailable" or "not known" in water or the environment. However, Grodan's 2011 Recycling Options brochure, found under the Literature and Brochures tab on their webpage (grodan101.com), gives some insight into rockwool's degradability in garden soil. It mentions the fibres will separate over time but never disappear completely and will keep the soil light and easy to work with for 5 to 9 years. Unfortunately, there was no research cited to substantiate this claim.

An extensive literature search found no studies regarding rockwool or mineral fibre decomposition in the soil environment. A few papers did indicate rockwool is not biodegradable (Allaire et al., 2004; Gilewska, 2006; Acuna et al., 2013; Jaroszuk-Sierocinska et al., 2014) but no research was referenced to substantiate the claim.

To investigate the breakdown of rockwool, two different laboratory experiments were conducted using pH adjusted artificial rainwater (AR) (Table 3.1 ) and GRW. The breakdown was assessed by measuring the amount of silicon (Si) released from the GRW into solution. The first experiment, Silicon Shaker Experiment, took place over 24 hrs on an elliptical shaker with AR solutions adjusted to a wide range of pH values (pH 3, 5, 7, 8.5, 10). The second experiment, Silicon Mesh Lid Experiment, was conducted over 63 days using a range of pH values (pH 5.5, 7, 8, 8.5) more likely encountered in the soil environment and involved no mechanical agitation.

## 3.2 Materials and Methods

Unless otherwise mentioned, artificial rainwater was used in order to simulate and include rainwater's effect on Si dissolution from GRW.

### *3.2.1 Artificial Rainwater (AR)*

All laboratory chemicals and equipment were obtained from the Fisher Scientific Company (Ottawa, ON, Canada) unless otherwise stated. The artificial rainwater (AR) solution was prepared according to Anderson et al. (2000) (Table. 3.1). The reagents and concentrations for the AR stock solution can be found in Table 3.1 The calcium chloride was obtained from Sigma-Aldrich Canada Company (Oakville, ON, Canada). Four litres of AR solution was prepared at a time. Forty milliliters of the [100x] stock solution was added to 3960 mL of deionized water, stirred with a magnetic stir bar (Isotemp Basic Magnetic Stirring Hotplate) for two minutes, and then stored in a polyethylene container with a screw-top plastic lid at room temperature. Nutrient analysis of the AR presented by Anderson et al (2000) included potassium (K) 0.39 mg/L, and $NH_4^+$ and $NO_3^-$ at 0.18 and 0.62 mg/L respectively.

Table 3. 1 Composition of artificial rainwater (Anderson et al., 2000). A final [1x] concentration included 10 mL of [100x] solution, diluted to 1L with deionized water, having a pH of 5.6.

| Component | | MW g/mol | Conc. in stock mol/L | stock g/100 mL | mL stock/L* |
|---|---|---|---|---|---|
| NaCl | Sodium chloride | 58.44 | 0.01 | 5.84 | 20 |
| KCl | Potassium chloride | 74.55 | 0.001 | 0.75 | 10 |
| $NH_4Cl$ | Ammonium chloride | 53.49 | 0.001 | 0.53 | 10 |
| $MgCl_2$ x $6H_2O$ | Magnesium chloride hexahydrate | 203.3 | 0.001 | 2.03 | 25 |
| $CaCl_2$ | Calcium chloride | 110.99 | 0.001 | 1.11 | 25 |
| $NaNO_3$ | Sodium nitrate | 84.99 | 0.001 | 0.85 | 10 |
| $Na_2SO_4$ | Sodium sulfate (anhydrous) | 126.04 | 0.001 | 1.26 | 25 |

* Diluted to 1L with deionized $H_2O$ = [100x]

### *3.2.2 Grodan Rockwool (GRW)Cubes*

Grow-Cubes® , 1cm square cubes of Grodan Rockwool (GRW) (Fig. 3.1), used throughout this investigation, were obtained in 28.3 L bags (Rockwool B.V., Roermond, The Netherlands) and acquired through the Grodan Group (Grodan Inc., Milton, ON, Canada). The GRW cubes were pre-conditioned prior to use, as recommend online by the manufacturer, to remove lime dust that occurs during manufacturing which could potentially increase the pH of a solution. The pre-conditioning for GRW in this study included a 30 minute soak in pH 5.5 deionized water (DI), drained for 5 minutes, and oven dried at 60°C unless otherwise stated.

Figure 3. 1 Grodan 1 cm Grow-Cubes® (Rockwool B.V., Roermond, The Netherlands) used in this thesis study. (Photo: joeshydro.com)

### *3.2.3 Silicon Shaker Experiment*

Three hundred grams of GRW, placed in a 11.3L plastic bin (Rubbermaid Manufacturing Company, Georgia, USA), was pre-conditioned in 5L of pH 5.6 AR. The GRW was drained for 5 minutes and placed in a 60$^{o}$C oven (Isotemp Oven, 120 V, 60 Hz, model 625G, Fisher Scientific, Ottawa, ON, Canada) to dry. One gram of the pre-conditioned GRW was then placed in a 125 mL high density polyethylene (HDPE) bottle followed by 50 mL of pH adjusted AR. Using a pH meter and probe (Accumet Basic AB15), AR was adjusted to either pH 3.0, 5.0, 7.0, 8.5, or 10.0 using aliquots of 1M hydrochloric acid (HCl), or 1M sodium hydroxide (NaOH).

The wide range of pH values were chosen to explore their potential to alter the integrity of the GRW cubes and release Si from the fibres. There were three treatment replicates and duplicate controls (no GRW) for each pH level for a total of 25 units. All bottles were sealed closed with parafilm and secured onto a C1 platform shaker (New Brunswick Scientific Classic Series, New Jersey, USA) for 24 hours at 25 strokes per minute. Shaking was chosen to maintain consistent contact of the solution with the GRW and facilitate Si solubilization.

After 24 hours the solutions were removed and filtered through Whatman #42 ashless filter paper, placed in clean HDPE bottles, preserved by acidifying to 1% $HNO_3$ (APHA, 1999), and stored at 4$^{o}$C. Analysis for silicon (Si), aluminum (Al), iron (Fe), calcium (Ca), and total P (TP) was conducted using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Varian Vista-Pro CCD Simultaneous ICP-OES, Varian Inc., CA., USA) at the University of Guelph Lab Services (Peter Smith) Guelph, ON, Canada.

### *3.2.4 Silicon Mesh-Lid Experiment*

A 25 mm diameter opening was cut into the 38 mm diameter lid for a 125 mL HDPE bottle using a metal lathe at the University of Guelph (Guelph, Ontario). A fine, polypropylene mesh measuring 43 x 43 holes per inch with 0.0159 inch openings (McMaster-Carr Company, Ohio, USA), was cut into a 28 mm diameter circle using scissors and secured to the inside of the lid using a mini hot glue gun (Model JY-2009, 60 HZ, 10W) and a hot-melt, 7mm adhesive stick. Two lids were modified this way in order to have separate strainers for the control solutions and the GRW-treated solutions (Fig. 3.2). The straining lid was implemented to prevent the passage of the GRW material into the solution during replacement thereby maintaining the initial 1g GRW for the duration of the experiment.

Figure 3.2 The modified lids of a 125 mL polypropylene bottle for the Silicon Mesh-Lid Experiment. The mesh inserts had 43 x 43 holes per inch with 0.0159 inch openings which acted as a strainer holding back the 1g GRW as the artificial rainwater solution was changed. (Photo: J. Garnett, 2017)

The GRW pre-conditioning and procedure to adjust the AR pH was the same as in the Silicon Shaker Experiment. One gram of GRW was placed into a 125 mL HDPE bottle along with 50 mL of AR adjusted to either pH 5.5, 7.0 , 8.0, or 8.5. The pH levels were chosen

because they could realistically be found in a natural soil environment. There were three treatment replicates and duplicate controls (no GRW) for each pH level for a total of 20 units. All bottles were kept at room temperature and lightly agitated (inverted five times) by hand after solutions were changed. All solutions were collected and replaced once a day for the first 35 days and then once a week for the next four weeks (63 days total). It was expected that the daily and weekly extractions along with the varied pH levels would indicate a trend for each of the elements analyzed.

Extractions selected for analysis were filtered through Whatman #42 ashless filter paper, placed in clean HDPE bottles, preserved by adding the appropriate amount of $HNO_3$ to achieve a 1% concentration (APHA, 1999), and stored at $4^oC$. Analysis included Si, Al, Fe, Ca, and TP using ICP-OES at the University of Guelph Lab Services (Peter Smith), Guelph, ON.

### 3.3 Statistical Analysis

The Silicon Shaker experiment was a factorial design and the Mesh-Lid experiment was a repeated-measures factorial design. Statistical analysis was conducted using SAS ( SAS Inc. Toronto, Canada), version University Edition (Red Hat 64 bit, Linux), and run through visualization software Oracle VM Virtual Box. The *Proc Glimmix* procedure for mixed models was used for each element analyzed and included a Tukey pair-wise comparison of means for pH and days. ANOVA tables were generated using *Proc Anova*. A series of residual plots were generated and examined to evaluate the validity of the assumptions for the model chosen (Bowley, 2015). Fit statistics including Shapiro-Wilk test of normality and '-2 log likelihood' were compared between model modifications to ensure the best model fit. Finally, the Pearson chi-square/df statistic was monitored as a means of measuring residual dispersion and fit

(Bowley, 2015). The best model representing the data was chosen to determine if the effects were significant at a type 1 error rate of $p < 0.05$.

The four regression equations for the Silcon Mesh-Lid experiment, representing rockwool Si dissolution over time, were generated using SAS (Bowley, 2015). The quadratic equation and coefficients were selected based on significance error rate of $p < 0.05$. Finally, the Efron's Pseudo $R^2$ was more appropriate than $r^2$ to verify the fit of the regression equation since it was non-linear (Bowley, 2015).

## 3.4 Results and Discussion

### *3.4.1 Silicon Shaker Experiment*

*Experimental summary*: *The timeline was 24 hours on an elliptical shaker. There were three replicates with two controls per pH value of 3, 5, 7, 8.5, and 10.*

Analysis using ICP-OES, for elements Al, Ca, Fe, P, and Si, was conducted on the filtered solutions including two controls for each pH value. Other than trace amounts of Si, mean < 0.02, no other analyzed elements were found in the controls. ANOVA tables can be found in Appendix 4 (Table A4.1). Table 3.2 illustrates means and pairwise comparisons with letters indicating significance. No P was detected with analysis. In general, there was a significant difference in the means of Al, Ca, Fe, and Si in solution for acidic pH 3 and alkaline pH 10 as compared to the more neutral pH 5, 7, and 8.5. Artificial rainwater solutions of pH 5, 7, and 8.5 consistently showed no significant difference between means for all elements.

The Si in solution at pH 3 and pH 10 was the highest at 10.74 mg/L and 2.67 mg/L respectively, whereas pH 5.0, 7.0, and 8.5 were not significantly different with <1.3 mg/L Si. This can be viewed as a U-shaped variation (Fig.3.3) where the extreme acidic and alkaline pH

produce the highest Si dissolution and the more neutral pH had the lowest, a finding previously documented in Si dissolution experiments using pH and silicate minerals (Drever, 1994; Gudbrandsson, et al., 2011).

Table 3. 2 The Silicon Shaker Experiment mean (mg/L) results for Al, Ca, Fe, P, and Si.. A 50 mL artificial rainwater solution containing 1g Grodan® rockwool, adjusted to either pH 3, 5, 7, 8.5 or 10, was placed on an elliptical shaker at 25 rpm for 24 hours. No P was detected in the analysis. Pairwise comparisons were conducted between pH values to determine statistically significant differences for Al (A), Ca (B), Fe (C) and Si (D).

**A**

| pH | Element | Mean mg/L | Standard Error | Significant Grouping* |
|---|---|---|---|---|
| 3 | Al | 4.73 | 0.162 | a |
| 5 | Al | 0.19 | 0.162 | c |
| 7 | Al | 0.50 | 0.162 | c |
| 8.5 | Al | 0.15 | 0.162 | c |
| 10 | Al | 1.38 | 0.162 | b |

**B**

| pH | Element | Mean mg/L | Standard Error | Significant Grouping* |
|---|---|---|---|---|
| 3 | Ca | 10.9 | 0.167 | a |
| 5 | Ca | 1.45 | 0.167 | bc |
| 7 | Ca | 1.73 | 0.167 | bc |
| 8.5 | Ca | 1.12 | 0.167 | c |
| 10 | Ca | 2.19 | 0.167 | b |

**C**

| pH | Element | Mean mg/L | Standard Error | Significant Grouping* |
|---|---|---|---|---|
| 3 | Fe | 3.28 | 0.059 | a |
| 5 | Fe | 0.32 | 0.059 | c |
| 7 | Fe | 0.41 | 0.059 | c |
| 8.5 | Fe | 0.24 | 0.059 | c |
| 10 | Fe | 0.56 | 0.059 | b |

**D**

| pH | Element | Mean mg/L | Standard Error | Significant Grouping* |
|---|---|---|---|---|
| 3 | Si | 10.74 | 0.262 | a |
| 5 | Si | 0.78 | 0.262 | c |
| 7 | Si | 1.29 | 0.262 | c |
| 8.5 | Si | 0.65 | 0.262 | c |
| 10 | Si | 2.67 | 0.262 | b |

*pH treatments with the same letter are not significantly different at $p < 0.05$

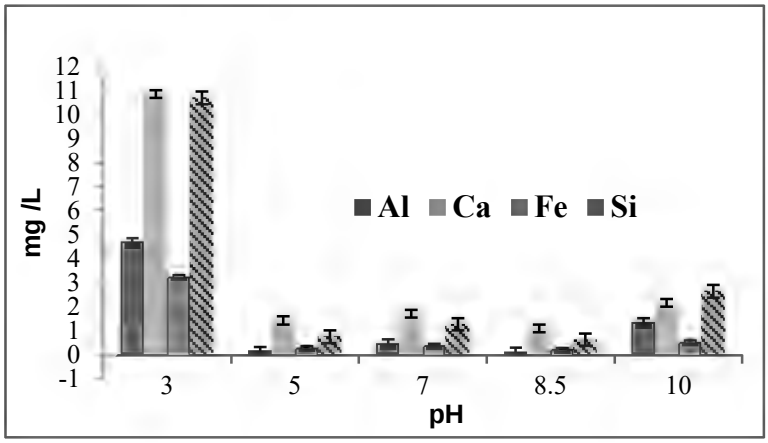



Figure 3. 3 The Silicon Shaker Experiment mean (mg/L) results for Al, Ca, Fe, and Si. No P was detected in the analysis. A 50 mL artificial rainwater solution containing 1g Grodan® rockwool, adjusted to either pH 3, 5, 7, 8.5 or 10, was placed on an elliptical shaker at 25 rpm for 24 hours. Error bars indicate a 95% CI.

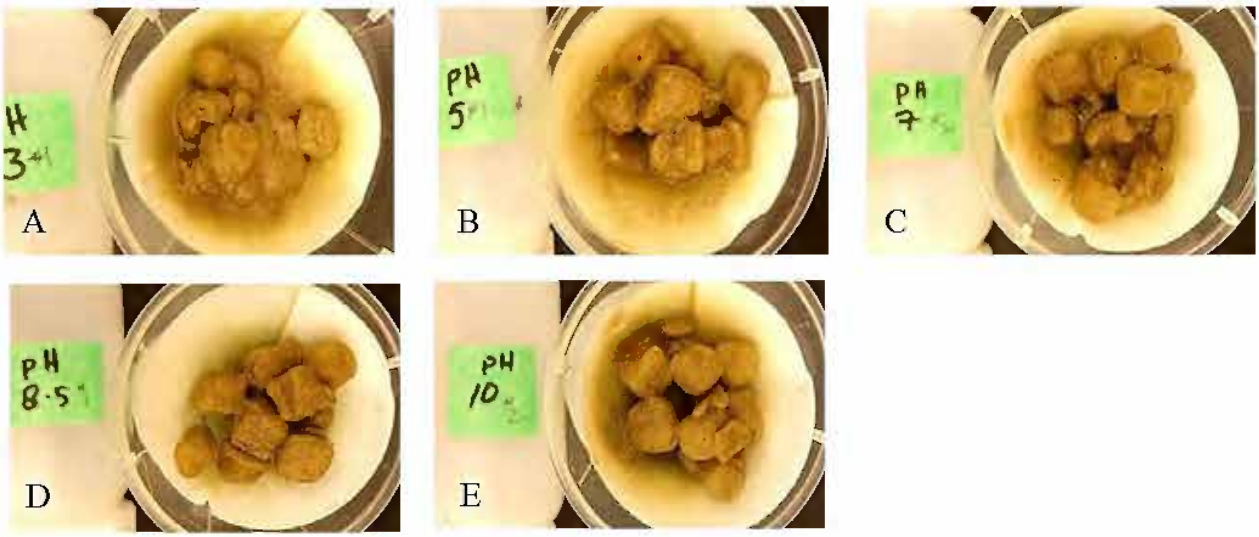



Figure 3. 4 Silicon Shaker Experiment. Photographs of 1g GRW recovered from 50 mL of pH-adjusted artificial rainwater at pH 3 (A), pH 5 (B), pH 7 (C), pH 8.5 (D), and pH 10 (E), after 24 hours on an elliptical shaker at 25 rpm. The images were taken after the material drained on filter paper for 24 hours. (Images by J. Garnett, 2017).

Images in Figure 3.4 show the extent of GRW degradation from the pH treatments with the greatest loss of structure produced by AR adjusted to pH 3. These findings indicate that GRW, consisting of 60% basalt, is degradable under pH extremes similar to previous studies of silicate minerals which may be useful information for future rockwool degradation studies.

### *3.4.2 Silicon Mesh-Lid Experiment*

*Experimental summary: The timeline was 63 days with no agitation. There were three replicates and two controls per pH value of 5.5, 7, 8, 8.5. Analysis was conducted on five, randomly selected days.*

Analysis using ICP-OES, for elements Al, Ca, Fe, P, and Si, was conducted on the filtered solutions for each pH value. The mean of three replicates for the five days analyzed for Al, Ca, and Fe can be found in Appendix 1 (Table A1.2). No P was detected with the analysis.

To reduce costs, only solutions from day 2, 7, 21, 43, and 63 were analyzed. Other than trace amounts of Si, mean <0.05, no other analyzed elements were found in the controls. ANOVA tables can be found in Appendix 4 (Table A4.2).

Table 3. 3 Silicon Mesh-Lid mean Si recovered from a 50 mL artificial rainwater solution adjusted to either pH 5.5, 7, 8, or 8.5, containing 1g Grodan® rockwool. The solution was replaced daily for the first 35days and then once a week to day 63. The means (mean of 3 replicates per pH, per day) represent randomly chosen days 2, 7, 21, 43, and 63.

| pH | Element | Mean mg/L | Standard Error | Significant Grouping* |
|---|---|---|---|---|
| 5.5 | Si | 0.44 | 0.037 | ab |
| 7 | Si | 0.37 | 0.009 | b |
| 8 | Si | 0.47 | 0.014 | a |
| 8.5 | Si | 0.55 | 0.033 | a |

*pH treatments with the same letter are not significantly different at $p < 0.05$

Table 3.3 indicates the mean Si recovery at pH 8.5, 8, and 5.5 was not significantly different at 0.44, 0.47 and 0.55 mg/L respectively, whereas pH 7.0 was significantly lower than pH 8 and 8.5 with a mean Si of 0.37 mg/L. The mean Si results re-produce the U-shaped variation of Si dissolution (Drever, 1994; Gudbrandsson et al., 2011) previously mentioned and seen in the Silicon Shaker experiment even though the pH range for the Silicon Mesh-Lid experiment was not as extreme. This finding also confirms that Si dissolution from GRW occurs at neutral pH.

Table 3.4 illustrates the mean Si (mg/L) recovered for each of the pH treatments for the five days analyzed. Day 2 and 7 were found to have the highest mean Si per pH treatment. This

finding is likely due to the pH-adjusted AR reacting with abundant, loosely-held external rockwool fibres or silicon dust from manufacturing. However, the mean Si dissolution seemed

Table 3. 4 Silicon Mesh-Lid Si (mean of three replicates) recovered per day analyzed from a 50 mL artificial rainwater solution adjusted to either pH 5.5, 7, 8, or 8.5, containing 1g Grodan® rockwool. The solution replaced daily is represented by days 2, 7, and 21 and then replaced once a week represented by days 43 and 63.

| | Si (mg/L) | | | | Significant Grouping* |
|---|---|---|---|---|---|
| Day / pH | 5.5 | 7 | 8 | 8.5 | |
| 2 | 0.66 | 0.35 | 0.87 | 0.79 | a |
| 7 | 0.35 | 0.50 | 0.52 | 0.45 | ab |
| 21 | 0.44 | 0.35 | 0.40 | 0.64 | b |
| 43 | 0.40 | 0.32 | 0.41 | 0.41 | b |
| 63 | 0.35 | 0.34 | 0.49 | 0.44 | b |
| Total | 2.20 | 1.86 | 2.70 | 2.73 | |

*Days with the same letter are not significantly different at $p < 0.05$

to stabilize for all treatments after day 21 with similar mean Si recovered. This stable but low Si recovery may be due to the high density of the GRW fibre network impairing the release of dissolved Si into solution. This may also be an indication that an equilibrium, ranging from 0.34 to 0.49 mg/L Si in the 50 AR solution, was consistently attained regardless of the solution being replaced daily or weekly.

In general, the mean Si amounts for the Mesh-Lid experiment recovered per day from 1g of GRW, was <1 mg/L per treatment. Likely the pH was not extreme enough (acidic or alkaline) to cause extensive Si dissolution as seen in the first Silicon Shaker experiment with pH 3 or 10 having a mean Si of 10.74 mg/L and 2.67 mg/L respectively (Table 3.2). Also, the agitation of the Shaker Experiment may have increased the Si into solution by loosening the fibres in the

GRW cubes, exposing more fibre surface to the AR, and facilitating the dissolved Si to exit the dense fibre network.

The Si recovered from the neutral or near neutral pH of the Mesh-Lid and Shaker experiments confirms that the GRW rockwool will degrade in neutral, aqueous solution albeit, more slowly than at acid or alkaline pH values. Hydrolysis, particularly at neutral pH, was likely the main degradative process whereby water molecules were involved in breaking chemical bonds, particularly Si-O bonds, on the surface (Schott and Oelkers, 1995; Lund and Yue, 2008) of the rockwool fibres. However, this may be offset with some of the Si in solution re-crystalizing, through chemical affinity, (Schott, and Oelkers, 1995) back onto the rockwool fibre surface resulting in low Si recovery and producing a Si equilibrium in solution.

White and Brantley (2003) developed an equation for the average silicate weathering rate for four silicate minerals including plagioclase, a mineral component of basalt. Their equation: weathering rate = $3.1 \times 10^{-13} \times t^{-0.61}$ (mol $m^{-2}$ $s^{-1}$), does not take soil pH into consideration which plays a role in mineral weathering. It is important to keep in mind that disturbed soils of former pit and quarries may have variable pH due to previous management, stoniness, or organic matter content for example. Campopiano et al. (2014) conducted an *in vitro* study for silicate mineral fibre dissolution that included pH and produced this equation set (weight loss /surface area / hour) 9.9 ng/$cm^2$/hr in pH 7.4 and 17 ng/$cm^2$/hr in pH 4.5. There are similarities between these two equations but it is clear that incorporating pH into the equation affects the rate of Si dissolution.

We used analytical software SAS (University Edition) to generate coefficients in an attempt to develop four quadratic equations representing aqueous Si dissolution rates at the four pH values (Fig.3.5). However, the Efron Pseudo $R^2$ values are $\leq 0.36$, which indicates more

research is needed to produce equations more representative of the Si dissolution rates, and ultimately, the breakdown rate of the rockwool. Particularly of concern is the serpentine-trend associated with the Si mean values on days 2, 7, and 21 (Fig. 3.5) which may require the development of a secondary equation. Also, day 43 and 63, representing solutions changed once a week, may require an adjustment to the equations unless the Si equilibrium hypothesis is verified. Figure 3.5 also shows the equations may have a predicted trend towards increasing

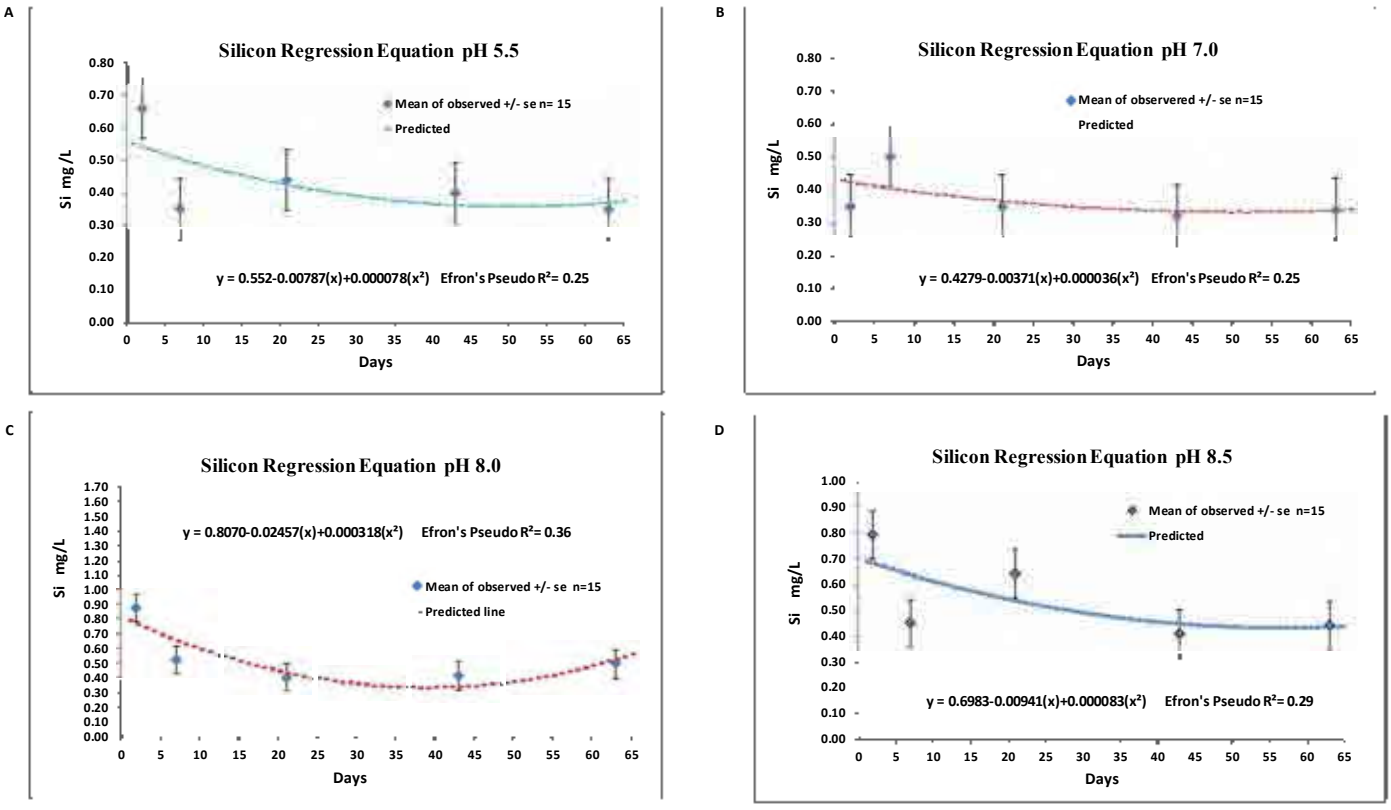



Figure 3.5 Silicon Mesh-Lid experiment. Data points represent the mean Si (mean of 3 replicates) recovered from 50 mL of artificial rainwater containing 1g Grodan® rockwool, adjusted to either pH 5.5, 7, 8, or 8.5. The solution was changed daily represented by day 2, 7, and 21; then changed once weekly represented by day 43 and 63. Si regression quadratic equations for pH 5.5 (A), pH 7 (B), pH 8 (C), and pH 8.5 (D), were determined using SAS (University Edition). The Efron's Pseudo $R^2$ values indicate the fit of the equation to the mean of the observed and predicted values. Error bars represent 95% CI.

mean Si after 43 days. Although the rate of Si dissolution may increase as more GRW fibres become exposed, there is likely a downward trend at some point, not shown in Figure 3.5, due to depleted Si reserves especially if the Si solution is being regularly removed. More research would be required to determine when a downward trend occurs.

### 3.5 Conclusions

This study was able to demonstrate that GRW will degrade, evident by the mean Si in solution, when exposed to water adjusted to a wide range of pH values including neutral. It was clear that AR with values below pH 5 or greater than pH 8.5, increased the Si dissolution rate from GRW. Also, for most pH values, the highest Si recovered in solution was within the first 21 days. This study, however, was not able to establish a definitive rate of Si dissolution from GRW in order to determine the length of time GRW would remain in the soil environment. Although information obtained from previous studies investigating Si dissolution rates from silicate minerals appears to be similar to the Si dissolution from GRW in pH-adjusted AR, it may not be representative of the Si dissolution from GRW in the soil environment. Factors in the soil environment affecting the rate of Si dissolution may include duration of water exposure and flow patterns, variations in climate and biological activity, and ultimately, time. This suggests a long-term field study investigating soil conditions that can accelerate or decrease Si dissolution could produce more suitable data to determine the longevity of GRW in the soil environment.

# Chapter 4 – Using a subsurface zone of Grodan® rockwool to maintain moisture in the top five centimeters of stony and silt-loam soil.

## 4.1 Introduction

Former pit and quarry sites often have shallow soil or overburden for re-vegetation and only natural precipitation to rely on (personal conversation with D. McKenzie, McKenzie Bros. Ltd., Guelph, ON), increasing the potential losses of transplanted vegetation. The goal of this study was to determine if a subsurface zone of GRW-0 could help maintain moisture in the top 5 cm of stony and silt-loam soil. Soil moisture maintained in this zone would promote seed germination as well as support seedling roots especially in a non-irrigated, remote site. Moisture is also required by the soil microbes for survival (Chenu et al., 2001). We hypothesized that the GRW-0 could also help support microbial activities by maintaining moisture. Soil $CO_2$ respiration can be used to assess the biological activity in the soil (Doran et al., 1997; Yiqi and Zhou, 2006; Haney et al., 2012; Goupil and Nkongolo, 2014). The Solvita $CO_2$ Burst Test was used in this study to quantify the impact of GRW-0 on soil microbial activity. This study also investigated the rehydration of a subsurface zone of GRW-0 and its effect on moisture retention in the top 5 cm of soil.

There have been studies utilizing fresh or waste-rockwool to amend soil structure, moisture, and drainage. Gilewska's (2006) study demonstrated soil, treated with greenhouse waste-rockwool, had better drainage, less surface water pooling, 5-6 day earlier seed germination, and higher crop yields than the control treated with only N. Jaroszuk-Sierocinska et al., (2014) concluded greenhouse waste-rockwool was more compacted and retained more water than fresh rockwool and, as a soil amendment, could improve the aeration of compacted

soils and increase field water capacity. In contrast, Reynolds et al., (2003) found waste-rockwool did not improve the plant-available water in clay loam. Fonteno and Nelson's (1990) study somewhat agreed with Reynolds et al., suggesting that the water-holding capacity of rockwool was comparable to sand.

In this study, four separate greenhouse pot experiments (Sensor 1,2, 3 and rehydration of Sensor 1) were conducted utilizing a subsurface zone of GRW-0 introduced in Chapter 3. The volumetric water content percent (VWC%), in the top 5 cm of potted stony and silt-loam soil, was monitored with sensors, while the soil dried, to determine the treatment efficacy.

## 4.2 Materials and Methods

### *4.2.1 Time-Domain-Reflectometry (TDR) Sensors*

The moisture or volumetric water content (VWC%) of the top 5 cm of the two soil types, stony and silt-loam, was determined using time-domain-reflectometry (TDR) two-pronged sensors (Decagon EC-5, Hoskin Scientific, Burlington, ON, Canada). TDR sensors assess soil moisture by measuring the dielectric constant of the soil, an electrical property that is highly dependent on the moisture content. The hand-held data logger automatically converts the output data to VWC%. All 16 sensors were calibrated in water to determine individual variability. All were found to be within ± 3% as noted in the manufacturer's manual. The sensors were horizontally positioned in the pot (Fig. 4.1) to facilitate the measurement of moisture in the soil located 3 cm above, 2.5 cm below, 3.25 cm to the sides, and 1cm from the tip of the sensor. The manufacture's manual indicated the volume of soil measured by each sensor was approximately 0.2 L.

### *4.2.2 Soil Types and Characteristics*

Two different soil types were obtained for this study. A summary of the soil characteristics can be found in Table 4.1 and 4.2. The stony soil, referred to as *Mackenzie* (MAC, Mac or M), was a Grade A gravel with fines and ½ inch stones. It was obtained in 2016-2017 from a gravel pit operated by Mackenzie Brothers Guelph Ltd., site ID: 15338, south of Cooks Mill Road, in Guelph, ON, Canada (43$^{o}$ 32.621 ‘ N, 80$^{o}$ 11.065’ W, Elevation 315 m). The agricultural soil, referred to as *Elora* (Elora or E), was a silt-loam collected in 2015 from a field associated with the Elora Research Station in Ariss, ON, Canada (43$^{o}$ 39.031 ‘ N, 80$^{o}$ 23.612’ W, Elevation 382 m).

Sand, silt, and clay particle size distribution (Table 4.1) was determined from 40g (dry weight) sieved to 2mm for the Elora and MAC soils using the hydrometer method (Kroetsch and Wang, 2008). Particle sizes for MAC (Table 4.2) were determined with a shaker (Retsch AS200) and sieve stack made up of the following sieve identification numbers and grid openings: 7/16 (11.2 mm), 1/4 (6.3 mm), 5 (4.0 mm), 10 (2.0 mm), 18 (1.0 mm), 35 (0.5 mm), 60 (0.25 mm), 100 (0.150 mm), and pan. The shaker frequency was set at 60rpm and operated for 5 minutes.

#### *4.2.2.1 Soil Analysis Methods*

Laboratory Services Agriculture and Food Laboratory (AFL) in Guelph, Ontario, conducted soil analysis using the following methods (with references) unless otherwise stated: (i) Soil pH was determined using saturated paste, or on the soil sample if sufficiently moist. (Hendershot et al., 1993); (ii) Elements K, Mg, Ca, and Na (extractable) were determined with ICP-OES on soil samples that were extracted using 1.0 M ammonium acetate solution (Simard,

1993); (iii) Soil P or the Olsen P, was conducted on soil samples extracted with a 0.5M sodium bicarbonate and determined colourimetrically using a Seal AA3 (Reid, 1998); (iv) The Elementar Vario Macro Cube (Elementar Analysensysteme GmbH, Germany) measured the total carbon and total N content in soil samples by combustion (950 °C). Organic carbon was calculated by subtracting the inorganic carbon from the total carbon; (v) Nitrate and ammonium was determined using the autoanalyzer (Seal AQ2) from soil samples which were first extracted with 2 M KCl (Maynard and Kaltra, 1993) prior to analysis.

Table 4. 1 Characteristics of silt-loam soil, Elora, and stony soil Mackenzie (MAC), sieved to 2mm prior to analysis. Laboratory Services Agriculture and Food Laboratory in Guelph, Ontario, conducted the soil analysis for total nitrogen (TN), phosphorus (Olsen P), extractable potassium (K), total carbon (TC) and pH. Soil texture was determined using hydrometer method without chemically removing the organic fraction.

| Soil | TN g/kg | Olsen P mg/L | K mg/L | TC g/kg | pH | % sand | % silt | % clay |
|---|---|---|---|---|---|---|---|---|
| Elora | 1.70 | 11.0 | 54.0 | 20.4 | 7.3 | 48.75 | 45.00 | 6.25 |
| MAC | <0.5 | <0.85 | 14.0 | 64.2 | 8.3 | 86.87 | 8.75 | 4.37 |

Table 4. 2 Stony soil Mackenzie (MAC) particle sizes and percentage of total sample weight. Particle sizes for MAC were determined with a shaker (Retsch AS200) and sieve stack with grid openings: 7/16 (11.2 mm), 1/4 (6.3 mm), 5 (4.0 mm), 10 (2.0 mm), 18 (1.0 mm), 35 (0.5 mm), 60 (0.25 mm), 100 (0.150 mm), and pan. The shaker frequency was set at 60rpm and operated for 5 minutes.

| Sieve / Particle size mm | % of total sample weight |
|---|---|
| > 11.20 | 1.3 |
| > 6.30 < 11.20 | 16.6 |
| > 4.00 < 6.30 | 16.0 |
| > 2.00 < 4.00 | 13.8 |
| < 2.00 | 52.2 |

### *4.2.3 Greenhouse Details*

Pot experiments were conducted in a curved roof, ridge and furrow, multi-room greenhouse with a galvanized steel truss system and single glass panes located at the University of Guelph, Guelph, ON, Canada (43$^{o}$ 31.613 ‘ N, 80$^{o}$ 13.718’ W, elevation 333 m). The greenhouse room dimensions were 20 ft long x 25 ft high, with 12ft sidewalls and 4ft curtain walls. A centralized boiler system produced heat through radiant pipes along the inside of the curtain wall during colder temperatures. The temperature was also moderated with an automated shade system on the sidewall and a peaked, flat, truss to truss system for the roof.

The roof vents and evaporative pad cooling system were operative with warmer temperatures. The control system monitoring (Argus Sensor Model AMWBDB-2.0/C, the Argus Session Manager, and the Argus Control Systems software 2014 1.0.0.14 , Argus Control Systems Ltd, British Columbia, Canada) included indoor and outdoor temperatures ($^{o}$C), humidity (Rh%), sunlight (W/m$^{2}$), and rainfall (mm).

The 14ft long x 4 ft wide greenhouse bench (Paul Boers Ltd, Vineland Station, ON, Canada) consisted of six five-inch-wide aluminum troughs, spaced 2.5 inches apart on a 32 inch tall, galvanized metal frame. The front of the bench was located 8.5 ft from the west-facing sidewall window and curtain wall that housed the heating and cooling apparatus. The room temperature was maintained at 22.0 $^{o}$C ± 5.0 $^{o}$C. No supplemental light or humidity was used for any of the greenhouse experiments. Six inch pots were spaced 12 cm apart in the trough on the bench for all experiments.

### *4.2.4 Sensor Experiments #1 – 3 and Rehydration Sensor #1*

The two soils, MAC and Elora, described earlier, were used in this series of greenhouse pot experiments. Moisture content of the soil was determined before the commencement of the experiments. The greenhouse environment was unchanged from the previous description. The assembly of the pots containing soil, GRW-0, and a sensor (Fig.4.1) for the four sensor experiments was the same. Empty pots, sensors, and GRW-0 were weighed individually, prior to commencing the experiment, so that dry soil weights could be determined.

In a six-inch 1.67 L polypropylene nursery pot (ITML Inc., Brantford, ON, Canada), aluminum screening (New York Wire, IWM International, PA.,USA) with 13 x 18 openings per inch, was cut to 10 cm diameter and placed over the drainage holes.

Due to MAC's textural heterogeneity, establishing a consistent bulk density for the soil was unachievable. Therefore, to be consistent, both soil volumes were measured with the same technique. Soil poured into a glass beaker was settled to the desired 200 or 300 mL by firmly hitting the bottom of the beaker with an open palm 5 times. Once the measurement was obtained, it was deposited into the pot. Once the pot was assembled it was firmly tapped 5 times again.

Dry, unconditioned GRW cubes, either 1g, 5g, or 10 g, was placed in a 17.7 x 18.8 cm, medium double zipper Ziploc® bag (S.C. Johnson Co., WI., USA) and saturated for 48 hours in 500 mL of DI. The amount of DI absorbed by the GRW was determined by weighing the GRW-0 after draining in a metal strainer for two minutes. The mean amounts of deionized water absorbed by 1g, 5g, and 10g GRW can be viewed in Appendix 1 (Table A1.1).

The GRW-0, identified by its dry weight of 1g, 5g, or 10g, was placed in a layer on top of 200 mL of soil in a 6 inch pot, then covered with an additional 300 mL soil. At that soil height a

hole was drilled in the side of the pot using a DeWalt 12 V Max drill (Stanley Black and Decker Inc., CT., USA) and a 21/64th drill bit. An EC-5 5cm soil moisture sensor with 3.5 mm stereo plug (Hoskin Scientific, Burlington, ON, Canada) was fixed into position by threading the cord through the hole starting with the stereo plug end. The sensor was stabilized by taping the sensor cord to the outside of the pot. A final addition of 300 mL of soil covered the sensor which remained undisturbed for the duration of the experiment. Pots without sensors, including controls (no GRW), were assembled and weighed in the same manner.

After the bottom of the pot was tapped 5 times with an open palm, it was set into an extra-large (33 x 39.6 cm), clear, freezer bag with a double zipper seal (President's Choice, Loblaw Companies Ltd., ON, Canada). Each pot was watered slowly with DI from the top until saturation—when a shallow pool of water formed in the bottom of the bag. The bags were then sealed for 48 hrs to allow for even moisture dispersion throughout the soil. Pots were then removed from the bags and allowed to drain for 2 hours before the first weights and sensor readings were taken. The pots were weighed and sensor readings were taken almost daily for the first 10 days and then two or three times a week as the soil and GRW-0 air-dried.

Rewetting the pots for the Rehydration of Sensor 1 experiment involved the same procedure for Sensor 1 saturation previously described. Pots were considered re-saturated when the same starting weight as Sensor 1 was achieved. Pots were sealed in freezer bags for one week to allow for even moisture dispersion throughout the soil. Pots were then removed from the bags and allowed to drain for 2 hours before the first weights and sensor readings were taken.

Sensor 1, 2, and 3 experiments ended when the pots' weight remained constant which indicated the moisture loss had arrested. The weight loss also acted as a check for changes in the

sensor readings. The rehydration experiment was stopped on day 12 when MAC pots' weight became constant.

The Sensor Experiment was repeated four times (Sensor 1, 2, 3, and rehydration Sensor 1) however, the number of observations (n) differed between experiments largely due to the number of sensors available. Sensor #1 and the Rehydration of Sensor #1 consisted of 10g, 5g, 1g GRW-0 treatments and a control (no GRW-0) using 12 pots of MAC soil and 12 pots of Elora soil. Two sensors were installed in the 10g, and 5g treatments, but only one sensor was available for one 1g and one control. The Sensor #2 experiment was identical to Sensor #1 except 4 additional EC-5 sensors were acquired allowing two sensors in each treatment. Finally, the Sensor #3 experiment consisted of 12 pots of MAC soil, containing the same aforementioned treatments in triplicate, with a sensor in each pot. The remaining 4 sensors were placed in 4 pots of Elora soil consisting of two controls and two, 10g GRW-0 treatments.

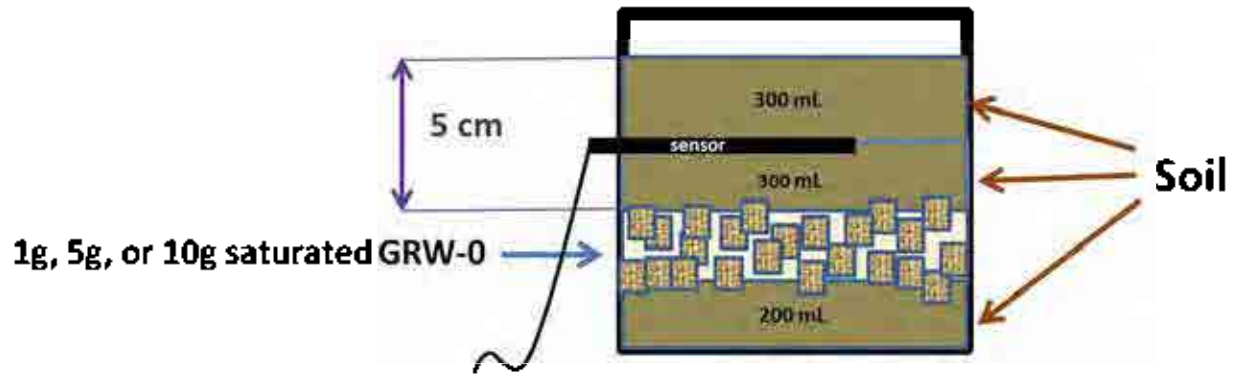



Figure 4.1 An illustration of the soil and GRW-0 layering in the 6 inch-1.67 L pot for Sensor 1, 2, 3, and rehydration Sensor 1 experiments. The TDR EC-5 5cm sensor, horizontally positioned, had a 3.5 mm stereo plug (not shown) at the end of the cord which connected to a Pro-Check Digital / Analog Reader (also not shown) (Hoskin Scientific, Burlington, ON, Canada).

### *4.2.5 Solvita CO2 Burst Test*

Soil samples were sieved to 2mm and oven dried at 40°C for 24 hours as per manufacturer's instructions for the Solvita $CO_2$ Burst Test (Woods End Laboratory, ME., USA). A 40 g sample of dried soil was placed in a 50 cc beaker specifically for the Solvita tests. Following the $CO_2$ Burst protocol, the beaker was tapped on the counter 3-4 times to settle the soil. A chart in the Solvita manual was consulted to determine how much distilled water (DI) to add based on the volume the soil settled to. An amount of 5 mL DI was slowly added to 25 cc of MAC soil and 10 mL of DI was added to 35cc of the Elora soil using a micropipette. The beaker of soil was then placed in a 250 mL mason jar along with the Solvita $CO_2$-sensitive-gel paddles and sealed gas-tight with a rubber gasket-lined lid. The jars were incubated (Model #1545, VWR International Ltd., ON, Canada) at 22.0$^{o}$C ± 1 $^{o}$C for 24 hours.

After 24 hours the paddles were removed one at a time and analyzed for $CO_2$-C values using the Solvita Digital Colour Reader (DCR) (Fig. 2.1) which had been calibrated using the standard 'grey' chip provided. The colorimetric system has a colour range from 1 – 6, which coincides with the level of $CO_2$-C (mg/kg or ppm), and equates to the potential N mineralization (none to high) as well. The DCR's colour accuracy is "up to 0.2 colour units" according to the manual.

## 4.3 Statistical Analysis

The Sensor 1, 2, 3, and Rehydration Sensor 1 were repeated measures designs. The statistical analysis was conducted using SAS ( SAS Inc. Toronto, Canada) version University Edition (Red Hat 64 bit, Linux) run through visualization software Oracle VM Virtual Box. Tukey pair-wise comparisons of means between soil-type, GRW-0 treatments, and days were

conducted using the *Proc Glimmix* procedure quadrature method with a beta distribution. Importantly, the VWC% data were converted to decimal prior to SAS analysis. For the Solvita $CO_2$-C analysis a Tukey pair-wise comparisons of means was conducted between the soil-types, GRW-0 treatments, and microbial $CO_2$-C using *Proc Glimmix* with a Gaussian distribution. ANOVA tables were generated using *Proc Anova.*

A series of residual plots were generated and examined to evaluate the validity of the assumptions for the model(s) chosen (Bowley, 2015). Fit statistics including the Shapiro-Wilk test of normality and '-2 log likelihood' were compared between model modifications to ensure the best model fit. Finally, the Pearson chi-square/df statistic was tracked as a means of measuring residual dispersion and fit (Bowley, 2015). The best model representing the data was chosen to determine if the effects were significant at a type 1 error rate of $p < 0.05$. A likelihood ratio test was conducted and determined the relative humidity of the greenhouse (Rh %) did not have an effect or influence the VWC% results.

Finally, since the repeated measures were unequal time spacings, a direct variable was defined so that the interval spacing could be specified along with the covariance structure Spatial Power (sp(pow)) (Bowley, 2015).

### 4.4 Results and Discussion

The following results refer to a mean VWC%, representing all the days, detected in the top 5 cm of soil at $p \leq 0.05$ significance unless otherwise indicated. Graphs of mean VWC% for Sensor # 1, Rehydration Sensor #1, Sensor #2, and Sensor #3 are shown in Appendix 1 (Fig. A1.1 – A1.3). ANOVA tables for all the Sensor experiments can be found in Appendix 4, (Table A4.4).

### *4.4.1 Sensor #1*

*Experimental Summary: The timeline was 27 days of which 16 days were documented using 12 sensors. The GRW-0 treatments were 10g, 5g, 1g, and a control with no GRW.*

The Elora soil treated with 10g GRW-0 had a mean moisture content significantly lower than the control. The mean moisture content for treatments 5g and 1g were not significantly different from each other, however, they were significantly lower than the control.

The MAC soil treated with 10 g GRW-0 had a mean moisture content that was higher than the control. The mean moisture content for treatments 5g and 1g were not significantly different from each other, however, they were significantly lower than the control (Table 4.3A).

### *4.4.2 Sensor #2*

*Experimental Summary: The timeline was 25 days of which 19 days were documented using 16 sensors. The GRW-0 treatments were 10g, 5g, 1g, and a control with no GRW.*

For the Elora soil, there was no significant difference in moisture retention between the control and the 5g treatment overall. Treatments 1g and 10g were not significantly different from each other; however, they were significantly different from control and the 5g GRW-0 treatment which had higher moisture detected. For the MAC soil, there were no significant differences between any of the treatments (Table 4.3B).

Statistical analysis did not detect an effect of the greenhouse relative humidity (Rh%) on the soil VWC %. However, the rate of evaporation from the soil in the pots is controlled by the steepness of the vapor pressure gradient from the evaporating surface to the surrounding air (Mastalerz, 1977). Therefore fluctuations in the greenhouse Rh% , which was low (<32%) for

several days during the Sensor 2 experiment, likely played a role in accelerating the moisture evaporation from the soil during this study.

### *4.4.3 Sensor #3*

*Experimental Summary: The timeline was 22 days of which 18 days were documented using 16 sensors. In MAC soil, the GRW-0 treatments were 10g, 5g, 1g, and a control with no GRW. The Elora soil had only the 10 g GRW-0 treatment and a control.*

The Elora soil treated with 10g GRW-0 had a mean moisture content significantly lower than the control. This finding was consistent with Sensor 1 and 2 experiments.

For the MAC soil, the 5g and 10g GRW-0 treatments had similar moisture content between them but they had significantly more moisture than the control. The 1g GRW-0 treatment had significantly more moisture than the control as well (Table 4.3C).

### *4.4.4 Rehydration of Sensor #1*

*Experimental Summary: The timeline was 12 days of which 8 days were documented using 12 sensors. The GRW-0 treatments were 10g, 5g, 1g, and control with no GRW.*

For the Elora soil, there were no significant differences between the 1g, 5g, and 10g treatments. They all had significantly higher moisture than the control (Table 4.3D). This is contrary to what was consistently observed in Sensor 1, 2 and 3 experiments, particularly with the Elora soil treated with 10g GRW-0. This may be due to changes in the structural properties of the GRW-0, incurred from water and soil exposure causing compaction altering the hydraulic conductivity (Wever, and Kipp, 1998), and increasing the water-holding capacity (Jaroszuk-Sierocinska, et al., 2014). It is possible the pores of the GRW-0 had become compressed and

clogged with clay particles as well. On the other hand, the MAC soil, which has a lower clay content than the Elora soil (Table 4.1), had significantly higher moisture than the control when treated with 10g GRW-0; a finding consistent with two of the three previous sensor experiments.

Table 4. 3 Mean VWC% results in the top 5 cm of stony (MAC) and silt-loam (Elora) soils. The experiments represented are Sensor #1 (A), Sensor #2 (B), Sensor #3 (C), and Rehydration Sensor #1 (D). The stony MAC and silt-loam Elora soil were treated with a 1g, 5g, or 10g (dry weight) zone of saturated rockwool (GRW-0). Tukey pairwise comparisons were conducted between treatments.

**A**

| Soil | Sensors per trmt | Rockwool dry wt (g) | Mean VWC% | Standard Error | Significant Grouping * |
|---|---|---|---|---|---|
| Elora | 1 | Control | 17.02 | 0.024 | a |
| Elora | 1 | 1g | 14.32 | 0.027 | b |
| Elora | 2 | 5g | 14.38 | 0.018 | b |
| Elora | 2 | 10g | 12.17 | 0.023 | c |
| MAC | 1 | Control | 7.20 | 0.033 | g |
| MAC | 1 | 1g | 9.15 | 0.029 | e |
| MAC | 2 | 5g | 8.20 | 0.022 | f |
| MAC | 2 | 10g | 10.96 | 0.019 | d |

*Treatments with the same letter are not significantly different to p< 0.05.

**B**

| Soil | Sensors per trmt | Rockwool dry wt (g) | Mean VWC% | Standard Error | Significant Grouping * |
|---|---|---|---|---|---|
| Elora | 2 | Control | 8.70 | 0.052 | a |
| Elora | 2 | 1g | 6.50 | 0.067 | b |
| Elora | 2 | 5g | 8.70 | 0.057 | a |
| Elora | 2 | 10g | 6.50 | 0.070 | b |
| MAC | 2 | Control | 7.80 | 0.048 | ab |
| MAC | 2 | 1g | 7.30 | 0.049 | ab |
| MAC | 2 | 5g | 7.60 | 0.049 | ab |
| MAC | 2 | 10g | 6.90 | 0.051 | b |

* Treatments with the same letter are not significantly different to p< 0.05.

**C**

| Soil | Sensors per trmt | Rockwool dry wt (g) | Mean VWC% | Standard Error | Significant Grouping * |
|---|---|---|---|---|---|
| Elora | 2 | Control | 14.43 | 0.019 | a |
| Elora | 2 | 10g | 12.47 | 0.021 | b |
| MAC | 3 | Control | 7.13 | 0.019 | e |
| MAC | 3 | 1g | 7.80 | 0.018 | d |
| MAC | 3 | 5g | 9.28 | 0.016 | c |
| MAC | 3 | 10g | 8.72 | 0.017 | c |

* Treatments with the same letter are not significantly different to p< 0.05.

**D**

| Soil | Sensors per trmt | Rockwool dry wt (g) | Mean VWC% | Standard Error | Significant Grouping * |
|---|---|---|---|---|---|
| Elora | 1 | Control | 22.53 | 0.027 | b |
| Elora | 1 | 1g | 25.92 | 0.026 | a |
| Elora | 2 | 5g | 26.10 | 0.012 | a |
| Elora | 2 | 10g | 26.10 | 0.011 | a |
| MAC | 1 | Control | 11.99 | 0.035 | d |
| MAC | 1 | 1g | 12.50 | 0.034 | d |
| MAC | 2 | 5g | 12.20 | 0.020 | d |
| MAC | 2 | 10g | 15.75 | 0.016 | c |

* Treatments with the same letter are not significantly different to p< 0.05.

In Table 4.4, the mean VWC% of the treatments in the first 12 days were compared between the Sensor #1 and the subsequent Rehydration of Sensor #1. This was to determine if the mean VWC % response to the treatments had changed as a result of the drying and subsequent rehydration of the rockwool. In general, the differences were subtle except for the Elora 10g GRW-0 treatment being higher than the Elora control in the rehydration experiment as compared to the original Sensor #1. The lower VWC% for the control could be due to a hydrophobic response subsequent to drying that some soils are known to exhibit. The higher

mean VWC% of the 10g GRW-0 in the rehydration experiment may indicate that a threshold amount of rockwool exists whereby greater amounts of the material may actually impair drainage in silt-loam soil especially after it has become condensed or clogged with clay.

Table 4. 4 Mean VWC% in the top 5cm of soil the Sensor #1 (A) and the Rehydration of Sensor #1 (B). The stony MAC and silt-loam Elora soil were treated with a 1g, 5g, or 10g (dry weight) zone of saturated rockwool (GRW-0). The mean VWC% represents 12 days of drying from saturation.

**A**

| Sensor #1 | | | |
|---|---|---|---|
| Soil | Sensors per trmt | Rockwool Dry Wt (g) | Mean VWC% |
| Elora | 1 | Control | 24.3 |
| Elora | 1 | 1g | 25.6 |
| Elora | 2 | 5g | 25.4 |
| Elora | 2 | 10g | 22.9 |
| MAC | 1 | Control | 11.9 |
| MAC | 1 | 1g | 13.2 |
| MAC | 2 | 5g | 13.2 |
| MAC | 2 | 10g | 16.1 |

**B**

| Rehydration Sensor #1 | | | |
|---|---|---|---|
| Soil | Sensors per trmt | Rockwool Dry Wt (g) | Mean VWC% |
| Elora | 1 | Control | 22.5 |
| Elora | 1 | 1g | 25.9 |
| Elora | 2 | 5g | 26.1 |
| Elora | 2 | 10g | 26.1 |
| MAC | 1 | Control | 12.0 |
| MAC | 1 | 1g | 12.5 |
| MAC | 2 | 5g | 12.2 |
| MAC | 2 | 10g | 15.8 |

The discrepancy between the two soils, in response to the 10g GRW-0 in particular, may be partially explained by soil hydraulic conductivity (K), which probably played a key role in water movement and retention between the GRW and soil.

Soil water moves naturally from high potential to low potential aided by gravitational forces through pores. Pore sizes vary between soil textures, so MAC, which has a higher sand content, has larger pores compared to Elora, a silt-loam soil, which generally has small pores. When comparing hydraulic conductivity, the MAC soil, being mostly sand and gravel, is between 80 – 100 m/day whereas the Elora (silt-loam) is in the 0.1 – 1.0 m/day range (Fetter, 2001). Rockwool on the other hand, has mostly large pores with a hydraulic conductivity between 127 – 724 m/day depending on the Grodan rockwool product and the orientation of the

fibres (Bougoul et al., 2005). Water in saturated soil moves downward with gravity and suction gradients, referred to as gravity drainage or redistribution, and can persist for days depending on the soil structure (Hillel, 1982, Fetter, 2001). The water moves out of the largest pores first, such as those associated with sand or GRW, due to weak adhesion forces. Smaller pores, such as those in the Elora soil, are more resistant to water movement due to strong adhesion forces that can hold water more effectively against the force of gravity. It is therefore feasible, that when gravitational forces began to pulling water down and out of the GRW-0, a suction was created that strongly pulled the moisture out of the smaller pores of the Elora soil layer located above the GRW-0, resulting in a lower mean VWC% than the control. However, because the MAC soil pores were similar in size to the GRW-0, the suction created by gravity pulling water from the GRW-0 would not be as strong, leaving moisture in the MAC soil layer above the GRW-0 and maintaining the mean VWC%. In the rehydration experiment, the higher VWC% in the Elora soil treated with 10g GRW-0, relative to the control, is likely due to the GRW-0 becoming compressed or clogged with clay thereby reducing the large pore spaces and reducing the suction effect on the Elora soil.

Moisture evaporating from the soil surface causes water to be drawn upward through interconnected capillary pores from the zone of saturation (Fetter, 2001). The VWC% discrepancy, between the Elora control and the Elora GRW-0 treated soil, may also be explained as a disconnect in the capillary pores caused by the GRW-0 impairing the upward movement of water from the soil below the GRW-0 layer. Moisture retention in the MAC soil, on the other hand, may also be explained by the heterogeneous distribution of stones. Stones embedded in the soil surface can reduce evaporation like a mulch (Poesen et al., 1990; Cousin et al., 2003).

Stones both on the surface and in the soil have also been found to cause breaks in pore connectivity slowing gravitational drainage (Poesen et al., 1990; Katra, et al., 2008).

Table 4. 5 The Solvita $CO_2$-C for Sensor #1 and Rehydration Sensor #1. Day 1 and day 27 represent the first and last day of Sensor #1 respectively. Day 40 represents the last day of the Rehydration of Sensor #1. Potted soil was treated with a subsurface zone of 1, 5, or 10g of GRW-0 (saturated) which was allowed to dry to a consistent pot weight. Treatments with the same letter are not significantly different at $p < 0.05$.

.

| Soil | Day | Treatment | Mean CO2-C mg/kg | Standard Error | Significant Grouping * |
|---|---|---|---|---|---|
| Elora | 1 | Control | 45.99 | 1.752 | a |
| Elora | 1 | 1g GRW-0 | 45.99 | 1.752 | a |
| Elora | 1 | 5g GRW-0 | 45.99 | 2.478 | a |
| Elora | 1 | 10g GRW-0 | 45.99 | 2.478 | a |
| Elora | 27 | Control | 37.08 | 1.752 | ab |
| Elora | 27 | 1g GRW-0 | 37.08 | 1.752 | ab |
| Elora | 27 | 5g GRW-0 | 37.08 | 2.478 | ab |
| Elora | 27 | 10g GRW-0 | 37.08 | 2.478 | ab |
| Elora | 40 | Control | 32.07 | 2.478 | ab |
| Elora | 40 | 1g GRW-0 | 31.05 | 2.478 | ab |
| Elora | 40 | 5g GRW-0 | 30.02 | 1.752 | b |
| Elora | 40 | 10g GRW-0 | 30.66 | 1.752 | b |
| MAC | 1 | Control | 4.79 | 0.287 | c |
| MAC | 1 | 1g GRW-0 | 4.79 | 0.287 | c |
| MAC | 1 | 5g GRW-0 | 4.79 | 0.406 | c |
| MAC | 1 | 10g GRW-0 | 4.79 | 0.406 | c |
| MAC | 27 | Control | 5.38 | 0.287 | c |
| MAC | 27 | 1g GRW-0 | 4.47 | 0.287 | c |
| MAC | 27 | 5g GRW-0 | 4.98 | 0.406 | c |
| MAC | 27 | 10g GRW-0 | 5.35 | 0.406 | c |
| MAC | 40 | Control | 4.61 | 0.406 | c |
| MAC | 40 | 1g GRW-0 | 4.32 | 0.406 | c |
| MAC | 40 | 5g GRW-0 | 5.03 | 0.287 | c |
| MAC | 40 | 10g GRW-0 | 4.46 | 0.287 | c |

* Treatments with the same letter are not significantly different to p= <0.05.

### *4.4.5 Solvita $CO_2$ Burst Test Sensor #1 and Rehydration Sensor #1*

The Solvita $CO_2$-C ANOVA table for Sensor # 1 and Rehydration Sensor #1 can be found in Appendix 4 (Table A4.7). For the MAC soil, there was no significant effect of the

GRW-0 1g, 5g, or 10g treatment on the microbial respiration or $CO_2$-C concentrations after the Sensor #1 or Rehydration of Sensor #1 experiments (Table 4.5). This was not completely unexpected since the initial $CO_2$-C concentration in MAC soil was so low (4.79 mg/kg) to begin with, indicating a very small microbial population, therefore changes in the $CO_2$-C concentration would likely have been small as well. For the Elora soil, there was no significant effect of treatment either, but there was a significant effect of day in that there was a decline in the $CO_2$-C concentration from the first day (Day 1) of the Sensor #1 experiment (45.99 mg/kg ) to the last day (Day 40) of the Rehydration of Sensor #1 (30.66 mg/kg). This reduction in the $CO_2$-C concentration may be an indication of a decline in the microbial biomass caused by the two extended periods of drying which can have a significant and long-lasting impact on microbial activities (Chowdhury et al., 2011). The microbial $CO_2$-C decline for all GRW-0 treatments may also indicate the GRW-0 did not provide additional moisture to support the microbial population.

## 4.5 Conclusions

The Sensor Experiments did consistently demonstrate 10 g (dry weight) GRW-0 in MAC soil maintained a mean higher VWC% in the top 5 cm, as the soil dried, than the control whereas the opposite was observed with the Elora soil. However, when the Elora soil, containing the 10g GRW-0, was rehydrated and dried again, the mean VWC% was higher than the control. This would indicate GRW is a good candidate as a soil amendment capable of maintaining moisture in the top 5 cm of stony soil and may also be effective for moisture retention in agricultural soil. However, the discrepancy in the moisture retention between the two soils, in response to the subsurface zone of GRW-0, requires more research to clarify how GRW-0 impacts soil properties.

# Chapter 5 – The impact of wastewater-treated Grodan® rockwool on phosphorus and nitrogen leaching in stony and silt-loam soil.

## 5.1 Introduction

Nitrogen and P are essential macro nutrients for plant growth. Depending on a number of factors, including soil texture and hydrology, both nutrients can leach downward with the soil solution or be lost in overland flow and enter waterways (Yeates and Clarke, 1993; Wang et al., 2012, Drury et al., 2016). Over time, this can result in eutrophication and hypoxia of aquatic ecosystems. As well, elevated nitrate levels in surface water is acidifying and can be toxic in drinking water (Kreyling et al., 2015). Soils with a higher sand content, in particular, can lose more P (Yeates and Clarke, 1993) and N to leaching (Wang and Alva, 1996) as compared to other soil-types. In one study, more than 88% of total N was leached when soluble $NH_4NO_3$ was applied to sandy soil receiving intermittent precipitation (Wang and Alva ,1996). Nutrient leaching and subsequent N and P applications of commercial fertilizer can be an ongoing expense for some producers.

In this study GRW treated with artificial wastewater (GRW-AWW) was used as an N and P source. Actual wastewater was not used because the laboratory was not permitted to handle septic material. The leaching solution was the artificial rainwater (AR) introduced in Chapter 3 (Table 3.1). The two soils, MAC and Elora, described earlier, were used in this two-part leaching experiment (Table 4.1 and 4.2). The first part, Leaching Experiment #1, had columns with the four experimental controls of GRW-0, GRW-AWW, as well as MAC and Elora soil which were leached with AR to determine existing leachable amounts of Al, Fe, K, Mg, P, S, $NH_4^+$, and $NO_3^-$. There were also two columns each with 82 mg monoammonium phosphate

(MAP) incorporated into MAC and Elora soil. The 82 mg of MAP per 400 mL of soil was based on a typical 200 kg per hectare agricultural field application (personal communication with Dr. J. Lauzon).

For the second part, Leaching Experiment #2, columns of MAC and Elora soils were combined with a subsurface zone of 1g and 5g GRW-0 as well as 1g and 5g GRW-AWW and leached with AR. The leachates were analyzed for Al, Fe, K, Mg, TP, S, $NH_4^+$, and $NO_3^-$ and compared to Leaching Experiment #1.

The goal of this study was to determine if a subsurface zone of GRW-AWW reduced the amount of N and P leaching in stony and silt-loam soil.

## 5.2 Materials and Methods

### *5.2.1 Artificial Wastewater (AWW)*

All laboratory chemicals and equipment used to make the artificial wastewater (AWW) were obtained from the Fisher Scientific Company (Ottawa, ON, Canada) unless otherwise stated. The AWW solution was prepared according to Pell and Nyberg (1989). The reagents and concentrations for the AWW stock solution can be found in Table 5.1. The calcium chloride, casein hydrolysate, and meat extract were obtained from Sigma-Aldrich Canada Company (Oakville, ON, Canada). A balance (Denver Instuments, New York, USA) was used to weigh the appropriate amount of each chemical. The stock [50x]/L solution was stored in a 1 L nalgene container, wrapped in foil to minimize light exposure, and stored in a 4$^o$C cooler. The solution was considered expired after 1 month when it became odorous and precipitates formed. The AWW [50x]/L stock solution was remade only once during this study.

The final AWW solution was prepared by measuring 200 mL of the [50x]/L stock solution and diluting with deionized water to 10L. Five hundred grams of GRW was placed in a 38L plastic bin (Sterilite Corporation, Massachusetts, USA) and soaked for 48 hours in 10L AWW adjusted with HCl to pH ~7.2 (Pell and Nyberg, 1989). The GRW treated with AWW (GRW-AWW) was drained, in increments of ~100 g, for 5 minutes each using a 12 inch diameter polyethylene sieve (2mm). Unabsorbed AWW was collected and measured to determine the quantity of AWW absorbed by the GRW cubes.

Table 5. 1 Composition of [50x]/L* artificial wastewater (Pell and Nyberg, 1989).

| **Component** | **g** |
|---|---|
| $NaHCO_3$ | 26.25 |
| Casein hydrolysate | 26.25 |
| Meat extract | 17.50 |
| Urea | 4.55 |
| NaCI | 1.05 |
| $CaCl_2$ | 0.53 |
| $MgSO_4$ X 7 $H_2O$ | 0.35 |

*Diluted to 1L with deionized $H_2O$

The GRW-AWW was then placed in a 50$^{o}$C - 60 $^{o}$C oven (Isotemp Oven, 120 V, 60 Hz, model 625G, Fisher Scientific, Ottawa, ON, Canada) to dry for several days. The low temperature was selected to minimize GRW fibre degradation and volatilization of ammonia.

Laboratory Services Agriculture and Food Laboratory (AFL) in Guelph, Ontario, conducted wastewater analysis following the Toxi-24 method in AOAC 2011.14. Samples were

first microwave digested in a closed vessel using nitric acid and hydrochloric acid. Following digestion, the samples were brought to volume with Nano pure water and then analyzed using ICP-OES with a pneumatic nebulizer. Total N (TN) was determined using a Kjeldahl automated system. University of Guelph Laboratory Services (Peter Smith) also conducted ICP-OES analysis on filtered wastewater solutions with no additional preparation.

The AWW solution was analyzed (Table 5.2) before and after GRW exposure for Al, Ca, Cu, Fe, K, Mg, Mn, Ni, Na, TP, S, and Zn, as well TN, $NH_4$-N, and $NO_3$-N by methods previously described (University of Guelph Lab Services (Peter Smith) and Laboratory Services Agriculture and Food Laboratory, Guelph, ON). This analysis was done to see if we could use the information to estimate the amount of nutrients absorbed by the GRW from the AWW.

The alkalinity of the AWW before and after GRW exposure, 285.67 and 336.33 mg/L $CaCO_3$ respectively, was determined in triplicate 100 mL samples using the HACH® Digital Titrator Method 8203 specifically for wastewater (HACH Co., CO., USA).

The nutrients released from the GRW-AWW was determined by placing 1g and 5g of dried GRW-AWW in a 125 mL HDPE bottle with 100 mL of pH 5.5 AR . The bottles were placed on a shaker, set at 25 rpm for 24 hours, to maximize the exposure of the fibres to the solution. These samples were prepared in triplicate with three controls of AR only. The solutions were filtered through Whatman #42 filter paper. One gram GRW-AWW was analyzed for Al, Ca, Cu, Fe, K, Mg, Na, Ni, total P, S and Zn. One gram and 5g GRW-AWW were analyzed for $NH_4$-N and $NO_3$-N using an autoanalyzer Seal-AAIII and methods from the Seal Manual. To analyze for $NH_4^+$, the liquid sample is first reacted with salicylate and dichloro-isocyanuric acid to produce a blue coloured compound measured at 660nm. Nitroprusside is added as a catalyst. Method No. G-102-93 Rev.7 (multi test MT7/MT 8). To analyze for $NO_3^-$

the nitrate in the liquid sample is first reduced to nitrite by a copper-cadmium reductor at pH 8 then chemically changed to form a reddish purple azo dye measured at 550 nm. Method No. G-200-97 Rev.6 (Multi test MT7A/MT8A). (University of Guelph Lab Services (Peter Smith) and Laboratory Services Agriculture and Food Laboratory, Guelph, ON) (Table 5.2).

Table 5. 2 Nutrient content (mean of three replicates) of artificial wastewater (AWW) before and after 48 hours exposure to untreated Grodan rockwool (GRW). Analysis also included nutrients released by 1g and 5g of Grodan rockwool, treated with artificial wastewater (GRW-AWW), into 100 mL of artificial rainwater (AR) after 24 hours on a shaker at 25 rpm.

| | mg/L | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **NH4-N** | **NO3-N** | **TN** | **TP** | **K** | **Al** | **Ca** | **Cu** | **Fe** | **Mg** | **Na** | **Ni> 0.1** | **S** | **Mn** | **Zn** |
| **AWW (before GRW)** | 30.6 | 0.19 | 94.95 | 4.59 | 9.70 | * | 2.14 | <0.10 | 0.15 | 0.52 | 171.52 | 0 | 9.53 | <0.10 | < 1.0 |
| **AWW (after 48 hr GRW)** | 24.3 | 0.17 | 96.48 | 4.11 | 24.19 | * | 2.15 | <0.10 | 0.14 | 0.90 | 180.87 | 0 | 18.64 | <0.10 | < 1.0 |
| **1g GRW-AWW** | 0.30 | 0.43 | * | 0.42 | 3.80 | 0.82 | 1.11 | * | 0.16 | 0.76 | 21.22 | * | 2.57 | * | * |
| **5g GRW-AWW** | 1.73 | 0.45 | * | * | * | * | * | * | * | * | * | * | * | * | * |
| **AR control** | 0.10 | 0.22 | * | * | * | * | * | * | * | * | * | * | * | * | * |

* not requested

The MAC and Elora soil required for the leaching experiment was oven-dried (DKN912/535L, Yamato Scientific Co. Ltd, Tokyo, Japan) at 104$^{o}$C . This was done for two reasons. First of all, by removing the soil's existing moisture we could control the volume of the leachate produced during the leaching experiment. Secondly, high heat would reduce the microbial populations and minimize their potential to alter $NH_4^+$ and $NO_3^-$ in the soil and in the leachate.

### *5.2.2 Leaching Apparatus*


Figure 5.1 The 16 column leaching apparatus with two peristaltic pumps (A). The AR leaching solution tubing was stabilized at the top of the column (B) and collected in 250 mL mason jars with funnels (C). (Photo: J. Garnett 2017)

Two eight-column leaching apparatuses (modified from A. Bradley, University of Wisconsin-Madison, WI, USA) were built to accommodate the eight-channel manifold of two peristaltic pumps, Watson Marlow 205U and Watson Marlow 205S (Watson Marlow Fluid Technology Group, MA, USA). The leaching tubing (Tygon E-3603, McMaster-Carr Co., Ohio, USA), cut to 66.0 cm lengths, had a 1/16 inch inner diameter (ID) and an 1/8 inch outer diameter (OD), carried the AR solution from the 4 L reservoir to the manifold tubing and from the manifold to the top of the column. The 'yellow and blue' manifold tubing was 45 cm long and had a 0.060 inch ID (Fisher Scientific Co., Ottawa, ON). The connectors joining the manifold and leaching tubes were 1/16 x 1/16 inch barbed white plastic (Fisher Scientific Co., Ottawa, ON, Canada). A 1 x 2 cm binder clip clamped to the top of the column secured the leaching tubing. The delivery rate of the AR was set at 1 mL $min^{-1}$ on the pumps (8.7 rpm for the 205U and 8.75 rpm for the 205S) which ran for one hour each day for 2 weeks. The timer used was an Android Stopwatch Application (Version 1.41, Jupiter Apps, Dover, United Kingdom).

The 16 columns were white 'gas-vent' grade, polyvinyl chloride (PVC) with a 5.0 cm ID, 6 cm OD, (McMaster-Carr Co., Ohio, USA) cut to ~30.5 cm lengths with a band saw. Attached to one end was a 10 x 10 cm polypropylene mesh with 0.0469 inch openings, (McMaster-Carr Co., Ohio, USA) secured with an adjustable, 3 inch alloy metal hose clamp. A metal hose clamp, lined with a 1 cm x 1.5 cm x 17 cm rigid rubber strip, also encircled the centre of the column forming a ledge that supported the column's weight on the frame. The two, 10 x10 x 20 inch long wooden frames were built by fastening together 1x2 inch and 2x2 inch thick strips of pine wood.

### *5.2.3 Leaching Experiment #1 Column Composition*

Table 5.3 details the leaching column assembly for Leaching Experiment #1. The basis of the experiment was to determine the N and P leaching from the soils and GRW materials separately before combining them in Leaching Experiment #2. Due to the limits of the peristaltic pump manifold, two column assemblies, MAPMac and MAPElora, were replicated in duplicate. All other column assemblies were replicated in triplicate.

Dry, conditioned GRW (GRW-0) and GRW-AWW were lightly packed to measure 400 mL in a beaker, using finger pressure to align the cubes and reduce open spaces, and then weighed. Four hundred milliliters of both dry soils were also measured and weighed. The 400 mL volume was chosen for a few reasons. First, a higher volume of material did not leave enough room at the top of the column for swelling or AR accumulation. Secondly, 400 mL was exactly half of the soil volume being used in the greenhouse experiments, so correlations could easily be made. Finally, the density of 400 mL of material allowed the leaching solution to pass through the column contents in a reasonable amount of time.

The use of monoammonium phosphate (MAP), a common agricultural fertilizer with nutrient ratios 11-52-0, (Brussels Agromart Ltd, Brussels, ON, Canada) was chosen to demonstrate conventional fertilizer N and P leaching with and without a zone of GRW. Based on an area basis, the MAP was applied at an equivalent rate of 200 kg ha $^{-1}$ (82 mg per leaching column) typical of a field application (personal communication with Dr. J. Lauzon). The MAP calculations can be viewed in Appendix 1 (Equation A1.1). At this rate, increased amounts of P and N in the leachate would be attributed to the MAP applied since the soils were poor in P and N.

The MAP granules were pulverized with a ceramic mortar and pestle to facilitate even distribution in the column soil, and to accelerate dissolution with the AR. The MAP was weighed using an analytical balance (Sartorius T Basic B1205, Germany) and mixed into a small quantity of soil first before being mixed again into the final volume of soil and placed in the column.

The amount of DI needed to achieve moisture at 100% container capacity, in the columns containing the soils and GRW material, was predetermined by comparing the weight of 200 mL of dry GRW, MAC ,and Elora soil to the weight of 200 mL of each saturated but no longer dripping when draining. The weight difference was converted to milliliters and used as a container capacity reference. The soils and GRW were evenly hydrated for 24 hours before starting the leaching to prevent the possibility of uneven leaching through the materials. AR was not used to hydrate the soils and GRW since it contained trace amounts of nutrients that could prematurely be introduced. The appropriate volume of deionized water (DI) was slowly added to the soil from the top of the column to achieve 100% container capacity (no excess). The column was then enclosed in a 10lb-size Ronco poly-woven plastic bag (Ronco Co., ON. Canada) using

a binder clip for 48 hours for even moisture distribution. If a small amount of moisture was found in a bag after 24 hours, it was poured back into the column.

Table 5.3 Composition of the sixteen columns in the Leaching Experiment #1. The two peristaltic pumps, identified as 205U and 205S, pumped the artificial rainwater leaching solution 1 mL $min^{-1}$. The column contents included stony soil MAC, silt-loam Elora, saturated rockwool (GRW-0), artificial wastewater treated rockwool (GRW-AWW), and MAC and Elora soils treated with monoammonium phosphate fertilizer (MAP).

| Column content & sample # | Leaching column # | Volume of soil or GRW (mL) | Dry weight of material (g) | DI for 100% container cap. (mL) |
|---|---|---|---|---|
| MAC 1 | 205U 1 | 400 | 746.8 | 78.0 |
| MAC 2 | 2 | 400 | 746.9 | 78.0 |
| MAC 3 | 3 | 400 | 746.0 | 78.0 |
| Elora 1 | 4 | 400 | 431.5 | 200.0 |
| Elora 2 | 5 | 400 | 432.9 | 200.0 |
| Elora 3 | 6 | 400 | 431.7 | 200.0 |
| GRW-0 1 | 7 | 400 | 21.4 | 200.0 |
| GRW-0 2 | 8 | 400 | 21.3 | 200.0 |
| GRW-0 3 | 205S 9 | 400 | 21.4 | 200.0 |
| GRW-AWW 1 | 10 | 400 | 21.3 | 200.0 |
| GRW-AWW 2 | 11 | 400 | 21.4 | 200.0 |
| GRW-AWW 3 | 12 | 400 | 21.4 | 200.0 |
| MAPMac 1 | 13 | 400 | 746.1 | 78.0 |
| MAPMac 2 | 14 | 400 | 746.2 | 78.0 |
| MAPElora 1 | 15 | 400 | 431.9 | 200.0 |
| MAPElora 2 | 16 | 400 | 432.7 | 200.0 |

Both the GRW-0 and GRW-AWW were pre-moistened with DI in a beaker to ensure even wetting, then covered, for even moisture distribution for 24 hours. The pre-moistened GRW-0 was then placed into the columns and lightly packed with a metal ruler to align the cubes. The pre-moistened soils settled into the columns without requiring additional packing. A clear plastic sheet was draped over the leaching apparatus when it was not operating to reduce evaporation.

Leachate was collected in 250 mL, clear glass mason jars with a 70 mm diameter opening (Golden Harvest®, Newell Brands, NJ., USA) that accommodated a polypropylene 50 mL, 6 cm OD funnel positioned directly under the leaching column (Fig. 5.1C). The leachate was collected once a day for 14 days and measured with a graduated cylinder. It was then filtered using Whatman #42 filter paper, transferred into 50 mL falcon tubes, and stored at -10°C until analysis for Al, Fe, K, Mg, S, and TP using ICP-OES and $NH_4^+$, $NO_3^-$ using an autoanalyzer Seal-AA3 with methods previously described (University of Guelph Lab Services (Peter Smith).

### *5.2.4 Leaching Experiment #2 Column Composition*

The leaching apparatus for this experiment was assembled in the same manner as Leaching Experiment #1. This second experiment, Leaching Experiment #2, is considered an extension of the first separated only by two weeks. For this reason, column 16 in Table 5.4 is labelled MAPMac3, because samples MAPMac1 and MAPMac2 were in the Leaching Experiment #1 assembly. The contents of the columns overall differed in that 12 of the 16 columns contained 200 mL of soil above and below a middle zone of either 1g or 5g of GRW-AWW. The 1g or 5g were chosen due to the restricted space in the column and to demonstrate the effect of different amounts of GRW-AWW on the amounts of N and P leaching from the column. Locating the GRW-AWW between the soil layers was done to model the influence of GRW-AWW on N and P leaching from a seedling root zone approximately 5 cm below the soil surface. This placement corresponds to the 5-7cm depth at which the GRW-AWW was installed in both the greenhouse pot and field studies.

Three columns labelled Elora/MAP-GRW0-1g (Table 5.4 ) contained 82 mg of pulverized MAP mixed into the top 200 mL of soil only, separated by 1g of GRW-0 covering

200 mL of soil in the lower half of the column. This arrangement was to demonstrate the influence of 1g GRW-0 on N and P leaching from commercial fertilizer.

The pre-moistened GRW-0 and GRW-AWW were layered into the columns with the dry soil. Appropriate amounts of DI were then added slowly from the top of the column to achieve 100% container capacity. The columns were then enclosed in a 10 lb size Ronco plastic bag and

Table 5. 4 Composition of the sixteen columns in the Leaching Experiment #2. The column contents included stony soil MAC (Mac) or silt-loam Elora treated with either a 1g or 5g mid-zone of rockwool treated with artificial wastewater (GRW-AWW). The Elora/MAP-GRW0-1g treatment was 1g of saturated, untreated rockwool (GRW-0) in the mid-zone with 82 mg monoammonium phosphate (MAP) fertilizer mixed in the soil above the GRW-0. MAPMac was the 3rd replicate from Leaching #1 and contained 82 mg MAP mixed throughout MAC soil. The two peristaltic pumps, identified as 205U and 205S, pumped the artificial rainwater leaching solution at a rate of 1 mL $min^{-1}$ .

| Column contents & sample # | Leaching column # | Volume of soil (mL) | Dry weight of soil (g) | DI for 100% container cap. (mL) |
|---|---|---|---|---|
| Mac 1g GRW-AWW 1 | 205U 1 | 400 | 729.50 | 85 |
| Mac 1g GRW-AWW 2 | 2 | 400 | 729.70 | 85 |
| Mac 1g GRW-AWW 3 | 3 | 400 | 730.06 | 85 |
| Mac 5g GRW-AWW 1 | 4 | 400 | 734.63 | 85 |
| Mac 5g GRW-AWW 2 | 5 | 400 | 730.70 | 85 |
| Mac 5g GRW-AWW 3 | 6 | 400 | 729.57 | 85 |
| Elora 1g GRW-AWW 1 | 7 | 400 | 509.31 | 160 |
| Elora 1g GRW-AWW 2 | 8 | 400 | 508.40 | 160 |
| Elora 1g GRW-AWW 3 | 205S 9 | 400 | 508.18 | 160 |
| Elora 5g GRW-AWW 1 | 10 | 400 | 509.01 | 145 |
| Elora 5g GRW-AWW 2 | 11 | 400 | 506.90 | 145 |
| Elora 5g GRW-AWW 3 | 12 | 400 | 508.86 | 145 |
| Elora/MAP-GRW0-1g-1 | 13 | 400 | 506.73 | 165 |
| Elora/MAP-GRW0-1g-2 | 14 | 400 | 507.36 | 165 |
| Elora/MAP-GRW0-1g-3 | 15 | 400 | 508.05 | 165 |
| MAPMac3 | 16 | 400 | 734.56 | 80 |

secured with a binder clip for 48 hours for even moisture distribution. Excess moisture in the bag after 24 hours was poured back into the column once. A clear plastic sheet was draped over the leaching apparatus when it was not operating to reduce evaporation. Leachate was collected in the same way as previously mentioned in the Leaching #1 experiment. The leachate was also filtered, stored, and analyzed in the same way as the Leaching #1 experiment.

### 5.3 Statistical analysis

The experimental design is repeated measures for both Leaching #1 and #2. The statistical analysis was conducted using SAS ( SAS Inc. Toronto, Canada) version University Edition (Red Hat 64 bit, Linux) run through visualization software Oracle VM Virtual Box. Tukey pair-wise comparisons of means for soils, treatments, and days were conducted using the *Proc Glimmix* procedure. *Proc Anova* was used to generate ANOVA tables. A series of residual plots were generated and examined to evaluate the validity of the assumptions for the model(s) chosen (Bowley, 2015).

Fit statistics including Shapiro-Wilk test of normality and '-2 log likelihood' were compared between model modifications to ensure the best model fit. Finally, the Pearson chi-square/df statistic was tracked as a means of measuring residual dispersion and fit (Bowley, 2015). The best model representing the data was chosen to determine if the effects were significant at a type 1 error rate of $p < 0.05$.

## 5.4 Results and Discussion

### *5.4.1 Leaching Experiments*

*Experimental Summary: Leach #1 and #2 combined for a total of 11 treatments, leached at a rate of 1mL $min^{-1}$ AR, for one hour each day, for 14 consecutive days. The randomly selected days for analysis were day 2, 5, 10, and 14.*

Four days were randomly chosen for analysis in order to reduce costs. Leaching ANOVA tables are located in Appendix 4, (Table A4.3). Results for mean mg/L per day (mean of three replicates) for K, Al , Fe, Mg, and S, can be found in Table A1.3. The results for $NH_4^+$, $NO_3^-$, and TP were the focus of this investigation and discussed.

High amounts of $NO_3^-$ was observed on day 2 for the EloraMAPGRW-01g treatment at 476.33 mg/L but values were not significantly different from the Elora soil treated with 1g or 5g GRW-AWW at 388.67 and 386.67 mg/L respectively (Table 5.5). However, the $NO_3^-$ in these treatments were significantly higher than the Elora control at 145.57 mg/L. This finding indicates the 1g and 5g GRW-AWW treatments did not prevent the leaching of $NO_3^-$ in the Elora soil.

The $NO_3^-$ results from the MAC soil treated with 1g and 5g GRW-AWW were not significantly different when compared to the MAC control. The $NO_3^-$ contributed by these treatments were expected to be 0.43 and 0.45 mg/L respectively (Table 5.2). This finding may indicate that the GRW-AWW prevented the leaching of $NO_3^-$ in the stony soil.

Figure 5.2 illustrates the leachate amounts (mg/L) for day 2, 5, 10, and 14 specifically for $NH_4^+$ and TP for treatments involving MAC and Elora soil. For the MAC soil, the MAPMac treatment leached the most TP on day 2 but there was no significant difference between the

concentration of TP collected on different days. For the Elora soil the highest TP occurred for treatments on day 2 but there was

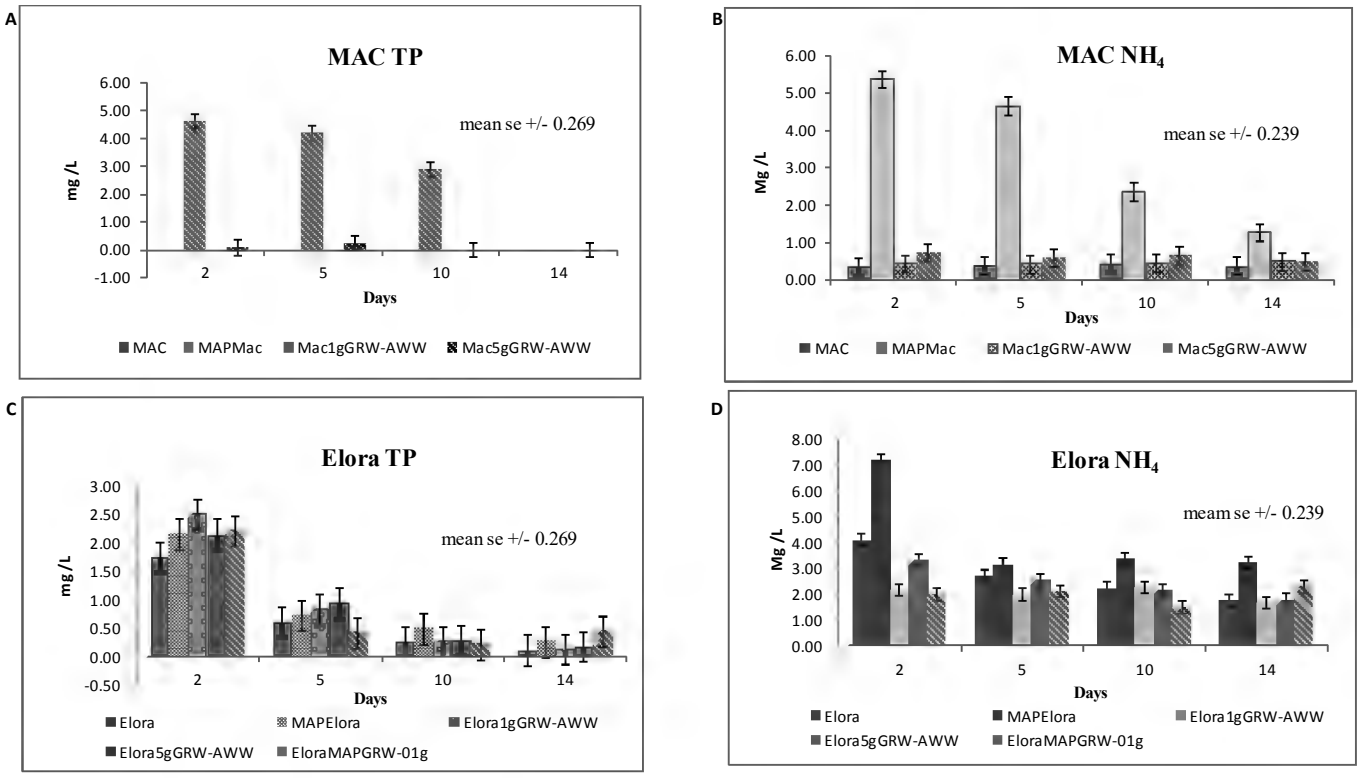



Figure 5.2 Total phosphorus (TP) and ammonium ($NH_4^+$) for stony soil MAC (A and B) and silt-loam soil Elora (C and D) for day 2, 5, 10 and 14. The column treatments included controls stony soil MAC and Elora, 1 g zone of saturated rockwool (GRW-0), 1g and 5g zones of artificial wastewater treated rockwool (GRW-AWW), and 82 mg monoammonium phosphate fertilizer (MAP) mixed throughout MAC and Elora soils or mixed into Elora soil above a 1g zone of GRW-0. The columns were leached at a rate of 1mL $min^{-1}$ with artificial rainwater, for one hour each day, for 14 consecutive days. Error bars represent 95% CI.

no significant difference between day 5, 10, and 14. This may indicate the majority of the applied P was removed from the soil solution by precipitating with Al, Fe, or Ca cations (Tunesi et al., 1999) around day 2. The $NH_4^+$ concentrations in the leachate from the MAPMac and MAPElora, were highest on day 2 at 5.36 and 7.15 mg/L respectively. However, for the other

treatments involving MAC and Elora soils, the $NH_4^+$ amounts per day were not significantly different.

Significant differences between treatments were determined by the overall mean (mg/L) amounts (3 replicates, four days) for this leaching study. It was not surprising that the MAC soil, treated with 82 mg MAP (MAPMac) fertilizer, leached more $NH_4^+$ (3.40) and TP (3.34) mg/L than the untreated, MAC control at 0.39 and 0.0 mg/L respectively (Table 5.5). The fast flow-rate of the AR solution and the capillary connectivity of the large pores of the stony soil, likely contributed to the leaching. Also, the MAC soil did not have abundant organic matter or clay to help retain nutrients. These characteristics and subsequent nutrient leaching is one reason why conventional commercial fertilizers are a poor choice for disturbed pit and quarry soils consisting of stony soil or overburden.

The leachate of the MAPElora also contained more TP (0.90) and $NH_4^+$ (4.20 mg/L) than the untreated, Elora control with 0.69 and 2.68 mg/L respectively. For the TP, this may indicate the soil in the MAPElora column had reached P saturation. The $NH_4^+$, on the other hand, may have leached more because of the high AR flow rate, since it is highly soluble. It is important to note that the microbes, required for nitrification, were intentionally reduced by drying the soil at 104$^o$C, which may have resulted in more $NH_4^+$ in soil solution.

Interestingly, the overall mean $NH_4^+$ leached by the Elora/MAP-GRW0-1g treatment was 1.95 mg/L as compared to the MAPElora at 4.20 mg/L. This may indicate the 1g GRW-0 provided a barrier that restricted the amount of $NH_4^+$ leaching, but was observed to be ineffective at preventing $NO_3^-$ leaching, as previously mentioned.

Table 5. 5 Leachate means (3 replicates, 4 days) for $NH_4^+$ (A), $NO_3^-$ (B), and TP (C). Tukey pairwise comparisons were conducted between treatments. Treatments with the same letter are not significantly different at $p < 0.05$.

**A**

| Treatment | Mean **$NH_4$** mg/L | Significant Grouping |
|---|---|---|
| MAPElora | 4.20 | a |
| Elora | 2.68 | b |
| E5g GRW-AWW | 2.43 | bc |
| E1g GRW-AWW | 2.00 | c |
| EMAPGRW-01g | 1.95 | c |
| MAPMac | 3.40 | a |
| M5g GRW-AWW | 0.60 | b |
| M1g GRW-AWW | 0.44 | b |
| MAC | 0.39 | b |

**B**

| Treatment | Mean **$NO_3$** mg/ L | Significant Grouping |
|---|---|---|
| EMAPGRW-01g | 130.81 | a |
| E5g GRW-AWW | 115.87 | a |
| E1g GRW-AWW | 99.72 | a |
| Elora | 38.62 | b |
| MAPElora | 16.63 | b |
| M5g GRW-AWW | 1.83 | a |
| MAPMac | 1.76 | a |
| M1g GRW-AWW | 1.11 | a |
| MAC | 0.45 | a |

**C**

| Treatment | Mean **TP** mg/L | Significant Grouping |
|---|---|---|
| E1g GRW-AWW | 0.93 | a |
| MAPElora | 0.90 | a |
| E5g GRW-AWW | 0.89 | a |
| EMAPGRW-01g | 0.82 | a |
| Elora | 0.69 | a |
| MAPMac | 3.34 | a |
| M5g GRW-AWW | 0.08 | b |
| MAC | 0.00 | b |
| M1g GRW-AWW | 0.00 | b |

The 82 mg of MAP (11-52-0) was estimated to supply 18 mg P and 9 mg N to the 400 ml of soil (MAC or Elora) in the MAP-treated columns. Calculations for N and P in MAP can be viewed in Appendix 1 (Equation A1.1). The 4-day total (mg) losses of N and TP (Table 5.6) in the leachate of MapElora ($NH_4^+$ 0.75, $NO_3^-$ 3.10, TP 0.16), and MAPMac ($NH_4^+$ 0.60, $NO_3^-$ 0.32 and TP 0.59), indicates both treatments leached less N and P than was supplied by the MAP fertilizer. This appears to show that most of the P was retained, in both the Elora and MAC soils, likely by precipitating with Al, Fe or Ca cations. Some $NH_4^+$ may have been retained as well by being adsorbed onto negatively charged soil colloids (Brady and Weil, 2008) or trapped in soil pores and not mobilized by the preferential flow in the leaching column.

One final observation was the incremental increase of $NH_4^+$ and $NO_3^-$ in the leachate of the MAC and Elora soils treated with 1g and 5g GRW-AWW (Table 5.5). Phosphorous concentrations did not appear to be similarly affected. This was also evident in Table 5.2 where the $NH_4^+$ and $NO_3^-$ released by 1g GRW-AWW as 0.30 and 0.43 mg/L respectively and 5g GRW-AWW was 1.73 and 0.45 mg/L respectively. This may indicate the GRW-AWW can release some nutrients relative to the amount of GRW-AWW applied. However, more research is needed to calibrate the nutrients released specific to the amount of GRW-AWW applied if it is to be used as a dependable fertilizer source.

Table 5. 6 Leaching experiment four-day total amounts (mg) of TP, $NH_4^+$, and $NO_3^-$ from leachates (volumes not shown) collected on day 2, 5, 10 and 14. The 11 column treatments included stony MAC (or Mac) and silt-loam Elora soils treated with a zone of 1g of saturated rockwool (GRW-0), 1g or 5g of artificial wastewater-treated rockwool (GRW-AWW), or 82 mg monoammonium phosphate fertilizer (MAP) mixed throughout the soil or mixed into the Elora soil above a 1g zone of GRW-0. The columns were leached at a rate of 1mL $min^{-1}$ with artificial rainwater, for one hour each day, for 14 consecutive days.

| | | mg | |
|---|---|---|---|
| **Treatment** | **TP** | **$NH_4$** | **$NO_3$** |
| 400 ml GRW-0 | 0.00 | 0.09 | 0.02 |
| 400 ml GRW-AWW | 0.21 | 0.33 | 2.06 |
| | | | |
| MAC | 0.00 | 0.07 | 0.08 |
| MAPMac | 0.59 | 0.60 | 0.32 |
| Mac1g GRW-AWW | 0.00 | 0.08 | 0.20 |
| Mac5g GRW-AWW | 0.01 | 0.10 | 0.32 |
| | | | |
| Elora | 0.12 | 0.48 | 7.22 |
| MAPElora | 0.16 | 0.75 | 3.10 |
| Elora/MAPGRW-01g | 0.15 | 0.35 | 24.17 |
| Elora1g GRW-AWW | 0.18 | 0.37 | 19.39 |
| Elora5g GRW-AWW | 0.16 | 0.43 | 20.88 |

## 5.5 Conclusions

There is some indication that the GRW-AWW can release $NH_4^+$ and $NO_3^-$ relative to the amount of GRW-AWW applied. However, more research is needed to calibrate the nutrients released specific to the amount of GRW-AWW if it is to be used as a dependable fertilizer source.

The highest (mg) $NO_3^-$ was found in the leachate of the Elora silt-loam soil treated with 82 mg MAP incorporated above a zone of 1g GRW-0. However, this same treatment leached less $NH_4^+$ than the MAPElora treatment indicating the 1g GRW-0 zone may have reduced $NH_4^+$ leaching in the silt-loam soil. A reduction in P leaching was not observed with this treatment.

This study did not include a leaching column similar to the EloraMAPGRW0-1g for stony soil MAC due to space restrictions. This treatment in stony soil could provide valuable information and should be investigated in a future experiment.

Phosphorous leaching was found to be highest (0.59 mg) in the stony MAC soil treated with 82 mg of the commercial fertilizer MAP. This observation demonstrated the need to find an alternative P application method for stony soils. One aspect of this study was to determine if GRW-AWW in a subsurface zone would both contribute soil P and reduce P leaching in stony soil. However, it is likely the P levels available in the GRW-AWW were too low to clearly demonstrate the effect of the treatment and requires further studies.

The findings from this leaching experiment warrant further research to explore GRW as a soil amendment and investigate its potential to reduce N and P leaching.

# Chapter 6 – Determining the efficacy of a subsurface zone of wastewater-treated Grodan® rockwool to provide plant-accessible phosphorus and nitrogen in nutrient-poor stony and silt-loam soil.

## 6.1 Introduction

Nitrogen and P are essential macronutrients for plant growth. The soils of disturbed pits and quarries can vary in texture, moisture retention, and fertility, complicating efforts for re-vegetation. Stony soil commonly overlies sand and gravel deposits reducing the soil volume for plant roots needing moisture and nutrients (Mackintosh and Mozuraitis,1982; SAROS, 2009). The sites frequently have shallow soil or overburden to plant in, and only natural precipitation to rely on, resulting in losses of transplanted vegetation. Commercial fertilizers are not typically used due to budgetary restrictions and there is a high potential for nutrient leaching into water reservoirs commonly located near aggregate sites (personal conversation with D. McKenzie, McKenzie Bros. Ltd., Guelph, ON). It is the intent of this study to determine the efficacy of a subsurface zone of wastewater-treated Grodan rockwool (GRW-AWW) to provide plant-accessible P and N in nutrient-poor stony and silt-loam soil. The wastewater would provide the N and P and potentially increase the soil organic matter with time (Bravo-Martin-Consuegra et al., 2016). This study was conducted in a greenhouse for 5 weeks, using MAC and Elora soils previously described in Chapter 4, and included a field trial in stony soil for 7 months in Guelph, Ontario.

Perennial ryegrass (*Lolium perenne* L.), was chosen for this study because of its adaptability and potential to establish quickly in poor soil. The fibrous root system tolerates both acidic and alkaline soil in a pH range of 5.1 to 8.4, and is suitable for well to poorly drained soils

(Hannaway et al., 1999). A popular turfgrass, perennial ryegrass has also been utilized for dairy cattle forage in temperate climates (Burkitt et al., 2007), and used for reducing soil erosion, recycling nutrients from manure and biosolids, wildlife feed, and silage (Hannaway et al., 1999). In a study by Lyons et al.(2008), it was observed the root length of turfgrass could be altered with the placement of a low-dose P fertilizer deeper in the root zone. This fertilizer placement reduced shoot growth but promoted deeper rooting, thereby improving heat tolerance, drought resistance, and reduced the establishment of shallow-rooted weeds. It was hoped that placing the GRW-AWW 5-7 cm below the soil surface would have a similar outcome which would be favourable for remote pit and quarry sites undergoing re-vegetation. Once plants become established, particularly in stony soils with high sand content, it is expected the macropore spaces will decrease with organic matter accumulation, reducing the leaching potential and improving the cation exchange capacity CEC and nutrient retention (McClellan et al., 2007).

Soil microbial biomass is responsible for several soil functions including nutrient cycling, such as the mineralization of organic N and P, to support plant growth. Soil $CO_2$ respiration can be used to assess the biological activity in the soil (Doran et al., 1997; Yiqi and Zhou, 2006; Haney et al., 2012; Goupil and Nkongolo, 2014) and has been widely used to quantify the impact of various treatments on soil microbial activity (Haney et al., 2008). A strong flush of $CO_2$ respiration, after soil drying and re-wetting events, is closely associated with microbial activities (Chowdhury et al., 2011) and the increase in soil microbial biomass (Funke and Harris, 1968; Franzluebbers et al., 2000).

The impact of GRW and GRW-AWW treatments on soil microbial biomass was assessed through the use of a scanning electron microscope (SEM) and by measuring soil $CO_2$ respiration with the Solvita $CO_2$ Burst Test. Dilution culture plating was used to establish pre-treatment,

non-quantitative microbial biomass in the MAC , Elora, and the field trial soil. Finally, SEM imaging was also used to assess changes in the GRW fibres before and after treatment.

## 6.2 Materials and Methods

### *6.2.1 Greenhouse Pot Study*

*Experimental Summary: The timeline of the greenhouse study was 5 weeks. Perennial ryegrass was grown in MAC (n=30) and Elora soil (n=30), treated with or without 5g GRW-AWW. Some analysis was conducted on combined samples (n=18).*

The greenhouse, the soils, the nursery pots, the GRW-AWW preparation and GRW source were the same for this experiment as previously described in Chapter 4. The pot assembly was also the same as shown in Figure 4.1 except this experiment did not use sensors or GRW-0. The greenhouse pot experiment used 5g (dry weight) GRW-AWW pre-moistened conservatively with 60 mL DI . The DI amounts were determined in a preliminary, moisture-retention-per-gram-GRW study, but were reduced slightly to minimize nutrient loss with excess moisture. The 5 grams of GRW-AWW was chosen because it covered almost 95 % of the soil surface in a single layer at approximately 5cm depth. As such, it coincided with the 5-7 cm depth installation of a single layer of GRW-AWW in the field study for this thesis.

Fifteen pots of MAC soil and 15 pots of Elora soil were assembled with a subsurface zone of 5g GRW-AWW. The pot drainage holes were covered with a 10 cm diameter polypropylene mesh with 0.0469 inch openings, (McMaster-Carr Co., Ohio, USA) instead of the aluminum mesh used previously. This was done to reduce the potential of losing plant-available P to aluminum phosphate ($AlPO_4$) formation. Each pot had a corresponding control (no GRW-0) bringing the total experimental units to 60 arranged in a complete block design. The pots were

brought to 100% container capacity with pH 5.6 ± 0.1 AR. The AR was chosen to simulate natural rainwater in producing optimal soil conditions for seed germination. Pots were bagged for 24 hours, then unbagged and allowed to drain for 24 hours under a loose fitting 54.5 x 28 cm clear plastic cover. The drainage was essential to re-establish open pore spaces in the soil for oxygen and aerobic microbial activities important for plant development.

Perennial ryegrass (*Lolium perenne* L.) has been successfully used in previous greenhouse pot studies and was selected for this study for a few reasons. First of all, this C3 grass is tolerant of alkaline soils, up to pH 8.4 (Hannaway, et al., 1999), which was the pH of the MAC soil. Secondly, perennial ryegrass is reasonably resistant to common greenhouse pests and disease. Finally, the germination rate is dependable and was found to be 95% in eight days. The perennial ryegrass variety 'Fiesta 4' (Pickseed Canada Inc., Winnipeg, MB, Canada) was seeded at a rate of 50 g/m$^2$. This was a slightly higher rate than OMAFRA's recommended rate of 40 g/m$^2$ to compensate for the low P values and the less-than-ideal soil texture of MAC. Also, 50 g/m$^2$ was the same seeding rate for the perennial ryegrass used in the field study discussed later on in this thesis.

The seeding rate per pot was calculated to be 0.6 g. The pots were weighed first, then seeds were weighed and evenly distributed on the soil surface by hand. The greenhouse roof shade curtains were kept closed to reduce potentially damaging sunlight. The clear plastic cover was replaced and left undisturbed until 90 % germination was evident. At that point the plastic lids were permanently removed and the shade curtains reset to operate automatically.

The pots were weighed daily to determine water loss. Separate mean water losses, calculated from the day of seeding for MAC and Elora, were determined using Microsoft Excel 2010 (Version 14.0.7184.5000, Microsoft Corp., USA). Each MAC pot received the same mean

amount of AR lost from MAC pots and each Elora pot received the same mean amount of AR lost from Elora pots. This watering regime was instituted to eliminate from the experiment the 'water-retention' characteristic of GRW and its effect on plant growth. The objective of the experiment was to determine the ability of GRW-AWW to supply N and P to support plant growth. Watering was done from the top and water did not leach out of the pot bottom due to the moderate quantities applied. Pots on the bench were rearranged at week two and early in week four to minimize the position-effect on plant growth such as sunlight exposure from the west window and the draft from the west side cooling vent.

At five weeks post germination, the perennial ryegrass was harvested with scissors and weighed. Twelve pairs of tillers equaling 24 grass blades were randomly selected from each pot harvested and measured. The grass was oven dried at 65$^{o}$C for two days, weighed and then analyzed for % dry weight of P, K, Mg, Ca and TN. Laboratory Services Agriculture and Food Laboratory, Guelph, ON, Canada, conducted plant tissue analysis for Ca, Mg, P, and K based on the USEPA Method 6020. The dried plant material was closed-vessel microwave digested with nitric acid and hydrochloric acid, brought to volume with Nanopure water, and then analyzed for Ca, Mg, P, and K using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The Elementar Vario Macro Cube was used to measure the total nitrogen (TN) (method previously described) content in the plant tissue. Soil subsamples from each pot in the study were sieved to 2 mm and frozen (-10 °C) until P, Mg, K, and TN analysis (methods previously described in Chapter 4) (Laboratory Services Agriculture and Food Laboratory, Guelph, ON, Canada).

Soil electrical conductivity (EC) was determined for MAC and Elora potted soil using the Thermo Scientific Orion Star A 212 Benchtop Conductivity Meter and electrode (Thermo Fisher Scientific Co., MA, USA) calibrated with a standard 100.01 µS/cm at 25ºC. The EC needed to

be assessed because the watering regime did not allow water to flow out from the pot, potentially causing toxic salt build-up from the AR irrigation and potentially reducing nutrient or moisture uptake by the perennial ryegrass.

### *6.2.2 Bovey Field Trial*

*Experimental Summary: The timeline of this field trial was 7 months. The field was divided into 12 plots consisting of 4 blocks with 3 treatments (control, GRW, GRW-AWW); each planted with perennial ryegrass.*

This field trial was installed in November, 2016 at the University of Guelph (43$^{o}$ 31.658 'N, 80$^{o}$ 13.748' W, elevation 332 m) and is referred to as Bovey for its proximity to the Bovey Greenhouses. The soil texture was sandy limestone gravel (Table 6.1, 6.2), more than 15 cm deep, which has been in place for more than 20 years (personal communication with Ron Dutton, Greenhouse Manager). The site was mostly in full sun however there was a 30 cm shadow cast on the south side of the plot, for a few hours each day in the early spring, from the greenhouse curtain wall. A 10 ft chain-link fence enclosed the site which had never been cultivated. The area has a north to south 2% slope and was lightly populated with various weed species that were treated with Round Up® (*Glyphosate*) twice each year (Monsanto Co., MO, USA). The most recent application was in July 2016. The site was chosen because it closely resembled the typical stony soil of overburden left exposed subsequent to pit aggregate mining, and it had a similar soil texture to the MAC soil (Tables 4.1, 4.2). Also, the site was protected by a fence preventing public and animal contamination for the duration of the field experiment.

Sand, silt, and clay particle size distribution (Table 6.1) was determined from 40g (dry weight) sieved to 2mm for the Bovey soil using the hydrometer method (Kroetsch and Wang,

2008). The Bovey Field Trial soil particle sizes were determined with a shaker (Retsch AS200) and sieve stack made up of the following sieve identification numbers and grid openings: 7/16 (11.2 mm), 1/4 (6.3 mm), 5 (4.0 mm), 10 (2.0 mm), 18 (1.0 mm), 35 (0.5 mm), 60 (0.25 mm), 100 (0.150 mm), and pan. Results are shown in Table 6.2.

Table 6. 1 Characteristics of Bovey Field Trial soil sieved to 2mm prior to analysis. Laboratory Services Agriculture and Food Laboratory in Guelph, Ontario, conducted the soil analysis for total nitrogen (TN), phosphorus (Olsen P), extractable potassium (K), total carbon (TC) and pH. Soil texture was determined using an hydrometer method without chemically removing the organic fraction.

| Soil | TN g/kg | Olsen P mg/L | K mg/L | TC g/kg | pH | % sand | % silt | % clay |
|---|---|---|---|---|---|---|---|---|
| Bovey | 0.50 | 3.1 | 14.0 | n/a | 8.6 | 86.63 | 10.25 | 3.13 |

Table 6. 2 Bovey Field Trial soil particle sizes and percentage of total sample weight. Particle sizes for Bovey were determined with a shaker (Retsch AS200) and sieve stack with grid openings: 7/16 (11.2 mm), 1/4 (6.3 mm), 5 (4.0 mm), 10 (2.0 mm), 18 (1.0 mm), 35 (0.5 mm), 60 (0.25 mm), 100 (0.150 mm), and pan. The shaker frequency was set at 60rpm and operated for 5 minutes.

| Bovey Particle Sizes | |
|---|---|
| Sieve/Particle size mm | % of total sample weight |
| > 11.20 | 23.5 |
| > 6.30 < 11.20 | 13.2 |
| > 4.00 < 6.30 | 9.8 |
| > 2.00 < 4.00 | 13.4 |
| < 2.00 | 40.1 |

The Bovey site was prepared and planted in mid-November 2016 during an unusually warm period (average high temperature of 17.5 $^{o}$C over four days). The complete block design consisted of 4 blocks with 3 treatments each. The 4.3 x 4.6 m total area was dug to 7 cm ± 2 cm depth with a spade (Fig. 6.1). The entire area was composited in a pile and then redistributed evenly with a rake and spade. Compositing the soil was required to ensure localized soil

attributes that could influence plant growth, such as organic matter, were homogenized within the entire research area. Twelve 1m$^2$ square plots were staked out and defined with 0.3 m foot-paths (Fig. 6.1). Plots were dug to 7 cm ± 2 cm depth including 4 control plots. Eight random

Figure 6.1 The Bovey Field Trial installed in November 2016. The 4.3 x 4.6 m total area was first homogenized and then redistributed. Twelve plots were dug to 7 cm ± 2 cm and 100 g (dry weight) of either GRW or GRW-AWW or control was placed by hand in 8 plots, then the soil was replaced on top. (Photo: J. Garnett, 2016)

plots received a single layer of 100 g (dry weight) GRW or GRW-AWW, placed by hand, at the 7 cm ± 2 cm depth and then covered with the stony soil. Four control plots were dug similarly but did not contain rockwool. The depth coincided with the root zone of most agricultural seedlings where nutrients such as N and P would be required. The depth was also consistent with the GRW-AWW and GRW placement in other experiments (greenhouse and leaching) previously discussed in this paper.

The plots were seeded with perennial ryegrass (*Lolium perenne* var. 'Karma') (Pickseed Canada Inc., Winnipeg, MB, Canada) which had a germination rate of 92% in seven days. A seeding rate of 50 g per m$^2$ was used, which was slightly higher than the OMAFRA (2008) recommendation of 40 g per m$^2$, to compensate for the poor growing conditions. It rained 7.6

mm the day after planting followed by freezing temperatures and snow. These were ideal conditions for dormant seeding whereby seeds were not expected to germinate until the following spring. Dormant seeding was chosen to take advantage of the moisture produced by melting snow since the Bovey site did not have direct water access.

After the mid-April germination, 25 grass blade lengths per plot were measured using a randomly placed 2 x 10 cm diameter PVC ring and a ruler at week 2, 5, and 7 post-germination. At post-germination week 4 and 8, digital photographs were taken with a Nikon 1 digital camera (Nikon Corp., ON., Canada) positioned 1m above the plot on a Velbon tripod (Velbon Corp., Tokyo, Japan). The images were viewed with GIMP software (GNU Image Manipulation Program 2.8 ©1995-2017) and Image J 1.46 Software (Public Domain) to assess percent greening of the plots as a measure of grass blade density in the plots.

The perennial ryegrass was harvested at week 8 post-germination using scissors. Ten centimeter wide frames were installed to prevent harvesting from the plot's edge, thereby minimizing the collection of plant material subjected to edge effects. Fresh weights per plot were recorded. The grass was oven-dried (Isotemp Oven, 120 V, 60 Hz, model 625G), at the recommended 65$^{o}$C for two days, weighed, and then analyzed for % dry Total N, P, K, Mg, and Ca. with methods described earlier in this chapter (Laboratory Services Agriculture and Food Laboratory, Guelph, ON, Canada). Soil from each plot was sieved to 2 mm, frozen (-10 °C), and analyzed for TN, P, Mg, and K with methods previously described in Chapter 4 (Laboratory Services Agriculture and Food Laboratory, Guelph, ON, Canada).

### *6.2.3 Solvita $CO_2$-C Burst Test for the Greenhouse Pot Study and Bovey Field Trial*

Material and methods for the Solvita $CO_2$-C Burst Test were the same as previously described in Chapter 4. MAC and Elora soil samples from the Greenhouse Pot Study, along with Bovey field soil samples, were analyzed for $CO_2$–C respiration.

### *6.2.4 Scanning Electron Microscope (SEM)*

The SEM was used to assess changes in the soil microbial biomass as well as changes in the GRW fibres, before and after treatment.

Fresh GRW, and post-treatment samples of GRW-0 and GRW-AWW cubes, along with MAC, Elora, and Bovey soil samples, were viewed with the SEM (FEI Quanta FEG-250), at the University of Guelph Imaging Facility. A 10 kV accelerating voltage was used to view changes in the microbial biomass and the rockwool fibres. Samples were mounted on an aluminum stub using double-sided carbon tape and sputter-coated in a vacuum chamber (Denton Vacuum Desk V, NJ, USA) with a standard metal, 60:40 gold-palladium alloy. An attempt was made to view the GRW without sputter-coating but the fibres appeared to melt soon after the electron beam was turned on. The SEM operation and image analysis for microbial biomass were assisted by the Imaging Lab technicians, Mr. Bob Harris, or Dr. Michaela Struder-Kypke.

### *6.2.5 Dilution plating: non-quantitative microbial cultures*

Non-quantitative microbial cultures representing the Bovey Field Trial soil as well as MAC and Elora soils (before GRW exposure) were prepared through dilution plating and examined. A non-selective media was prepared using Difco Tryptic Soy Agar (Sigma-Aldrich Canada Company, ON, Canada) autoclaved 121$^{o}$C for 20 minutes and placed in 100 x 15 mm

petri dishes under a Mott fume hood (Thermo Electron Corp., ON, Canada). Ten grams of fresh soil was placed into 95 mL nano- pure $H_2O$ (dilution $10^{-1}$ ) followed by subsequent dilutions of $10^{-3}$, $10^{-5}$, and $10^{-7}$. After agitation by hand, 1 mL of the dilutions was used to inoculate the prepared petri dishes. The petri dishes were incubated (Model #1545, VWR International Ltd., ON, Canada) at 25$^{o}$C for three days and then assessed for microbial growth.

### 6.3 Statistical analysis

The experimental design was a randomized complete block for the Greenhouse Pot Study and the Bovey Field trial. The statistical analysis was conducted using SAS ( SAS Inc. Toronto, Canada) version University Edition (Red Hat 64 bit, Linux) run through visualization software Oracle VM Virtual Box. Data that was percent dry weight was changed to a decimal prior to analysis. This included grass tissue analysis TN, P, K, Mg, Ca, and soil analysis TN.

Tukey pair-wise comparisons of means between soils, treatment, days, and microbial $CO_2$-C were conducted using the *Proc Glimmix* procedure. *Proc Anova* was used to generate ANOVA tables. A series of residual plots were generated and examined to evaluate the validity of the assumptions for the model(s) chosen (Bowley, 2015). Fit statistics including Shapiro-Wilk test of normality and '-2 log likelihood' were compared between model modifications to ensure the best model fit. Finally, the Pearson chi-square/df statistic was tracked as a means of measuring residual dispersion and fit (Bowley, 2015). The best model representing the data was chosen to determine if the effects were significant at a type 1 error rate of $p < 0.05$. All percent data was changed to decimal prior to analysis.

## 6.4 Results and Discussion

### *6.4.1 The Greenhouse Pot Study*

ANOVA tables for the Greenhouse Pot Study can be found in Appendix 4 (Table A4.5). The greenhouse experiment was terminated at 5 weeks when it appeared the perennial ryegrass, particularly in the MAC soil, was in distress. The leaf colour had become light green with red-brown stems potentially indicating nutrient deficiencies such as N and P respectively. In contrast, the perennial ryegrass growing in the Elora soil appeared generally healthy and dark green (Fig. 6.2 A). At 5 weeks, the perennial ryegrass in both soils had produced robust root systems that extended beyond the screening in the bottom of the pots for both the treatment and control (Fig. 6.2 B-E ).

Since the irrigation was restricted to eliminate water flow-through, the electrical conductivity (EC) was determined at the end of the experiment to rule out causal effect for the apparent declining plant vigor. The MAC potted soil (n=18), had an acceptable EC mean of 0.11 dS/m as did the Elora potted soil (n=18) with EC 0.28 dS/m at 25$^{o}$C ± 2 $^{o}$C; according to the Canadian Society of Soil Science (CSSS). CSSS suggests an EC of 0-2 dS/m would have a negligible effect on plant yield whereas 2-4 dS/m would reduce yield in sensitive crops (Carter and Gregorich, 2008). Interestingly, the optimum EC for greenhouse tomatoes and some horticultural plants is 2-4 dS/m (Resh, 2004).

The tissue analysis of the perennial ryegrass (*Lolium perenne var. 'Fiesta 4'*) was completed on combined samples (n=36) to reduce costs. There was no significant effect of treatment (5 g GRW-AWW) detected for the % dry weight concentrations of TN, P, K, Mg, or Ca in the perennial ryegrass tissue grown in Elora or MAC soil, as compared to the controls.


Figure 6.2 Image of the Greenhouse Pot Study (A) bench arrangement. At 5 weeks post-germination, the light green colour of the perennial ryegrass in MAC soil (front 5 rows) is contrasted with the dark green colour of the perennial ryegrass in the Elora soil (back rows). The root development in 5g GRW-AWW and control in MAC soil (B, C) respectively, was robust and indistinguishable from the treatment and control pots of Elora soil (D, E) respectively. (Photo: J. Garnett, 2017)

The soil analysis of the silt-loam Elora and stony soil MAC was also conducted on the same combination of samples (n=36). There was no significant effect of treatment (5g GRW-AWW) for concentrations of TN, P, or Mg for the Elora or MAC soil. There was a significant

effect for the mean concentration of K (Table 6.3) in both of the controls for Elora (37.78 mg/L) and MAC (16.22 mg/L) which had more K than their respective treatments. This may indicate that the perennial ryegrass grown in the 5g GRW-AWW treated soils were 'healthier' thereby taking more K from the soil (Table 6.3).

Table 6. 3 The Greenhouse Pot Study soil analysis for potassium (K) for MAC and Elora soil treated with or without (control) a subsurface zone of 5g GRW-AWW and perennial ryegrass for 5 weeks.

| Soil | Element | Treatment | Mean % Dry Wt | Standard Error | Significant Grouping* |
|---|---|---|---|---|---|
| Elora | K | Control | 37.78 | 0.283 | a |
| Elora | K | 5g GRW-AWW | 36.67 | 0.283 | b |
| MAC | K | Control | 16.20 | 0.283 | c |
| MAC | K | 5g GRW-AWW | 15.00 | 0.283 | d |

* Treatments with the same letter are not significantly different at $p < 0.05$.

Dry weight and blade lengths were measured on the uncombined samples (n=60). No significant effect of treatment (5g GRW-AWW) on the perennial ryegrass dry weight grown in either soil was found. However, there was a significant effect of treatment (5 g GRW-AWW) on the blade length of perennial ryegrass grown in the MAC soil. The mean blade length for the treatment was 0.99 cm longer than the mean blade length of the control (Table 6.4).

Table 6. 4 The Greenhouse Pot Study mean perennial ryegrass (*Lolium perenne* var.'Fiesta 4') blade lengths (L) and significance at 5 weeks post-germination (n= 360 per treatment).

| Soil | Treatment | Mean L (cm) | Standard Error | Significant Grouping* |
|---|---|---|---|---|
| Elora | 5g GRW-AWW | 13.66 | 0.021 | a |
| Elora | control | 13.64 | 0.021 | a |
| MAC | 5g GRW-AWW | 8.20 | 0.021 | b |
| MAC | control | 7.22 | 0.021 | c |

* Treatments with the same letter are not significantly different at $p < 0.05$.

It was curious that the increased blade length did not show up as increased dry weight for the perennial ryegrass grown in the 5g GRW-AWW MAC soil. A possible explanation may be due to the perennial ryegrass grown in MAC soil having a very low dry weight of 0.5g , on average per sample, whereby weight differences may not have been detected using a balance that was accurate only to two decimal places.

### *6.4.2 Solvita $CO_2$-C for the Greenhouse Pot Study*

The Solvita $CO_2$-C ANOVA table for the Greenhouse Pot Study can be found in Appendix 4 (Table A4.7). After exposure to 5g GRW-AWW and the root exudates of the perennial ryegrass, the microbial $CO_2$ respiration in the MAC soil increased slightly, but did not show a significant effect of treatment (Table 6.5). For the Elora soil, there was a significant effect of the 5g GRW-AWW treatment with the $CO_2$-C higher at 23.95 mg/kg as compared to the control at 22.11 mg/kg. The difference in response between the MAC and Elora soils may be partly due to the difference in the initial $CO_2$-C concentrations. MAC soil's $CO_2$-C of < 5 mg/kg, indicates a very small microbial biomass according to the Solvita manual. On the other

hand, the Elora soil's $CO_2$-C was > 20 mg/kg indicating a microbial biomass potentially more than 5 times that of MAC. As such, the rate of microbial growth and subsequent $CO_2$-C respiration in the Elora soil would be exponentially larger as compared to the MAC soil.

Table 6. 5 The Solvita mean $CO_2$-C and associated treatments for the Greenhouse Pot Study. The mean $CO_2$-C was determined for the MAC and Elora soil (n=36). Perennial ryegrass (*Lolium perenne* L.) was grown in both GRW-AWW treated and control soil for 5 weeks.

| Soil | Treatment | Mean CO2-C mg/kg | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| Elora | Control | 22.11 | 0.316 | b |
| Elora | 5g GRW-AWW | 23.95 | 0.318 | a |
| MAC | Control | 4.59 | 0.316 | c |
| MAC | 5g GRW-AWW | 4.66 | 0.318 | c |

* Treatments with the same letter are not significantly different at p <0.05.

### *6.4.3 Bovey Field Trial (BFT)*

The precipitation totals and temperatures for the Bovey Field Trial can be viewed in Appendix 2 (Table A2.1). The tissue analysis for the perennial ryegrass (*Lolium perenne* var. 'Karma') indicated no significant effect of treatment (control, GRW, GRW-AWW) on % dry weight concentrations of TN, P, K, Mg, and Ca. Also, there was no significant effect of treatment (control, GRW, GRW-AWW) on the perennial ryegrass dry weight or blade length. Analysis did indicate a significant effect of week (p=0.0366) in blade length but only for the controls. The perennial ryegrass control (without GRW treatment) were 0.675 cm longer in week 7 as compared to the mean blade length of the control in week 2 (Table 6.6).

Table 6. 6 Bovey Field Trial perennial ryegrass (*Lolium perenne* var. 'Karma') mean blade lengths with significance (n=100 per treatment). Each plot was dug to 7cm ±2 and had a subsurface layer of either 100 g (dry weight) of GRW or GRW-AWW.

| Week | Treatment | Mean L (cm) | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| 2 | Control | 1.53 | 0.139 | b |
| 2 | GRW | 1.63 | 0.139 | ab |
| 2 | GRW-AWW | 1.63 | 0.139 | ab |
| 5 | Control | 1.70 | 0.139 | ab |
| 5 | GRW | 1.65 | 0.139 | ab |
| 5 | GRW-AWW | 1.78 | 0.139 | ab |
| 7 | Control | 2.20 | 0.139 | a |
| 7 | GRW | 1.83 | 0.139 | ab |
| 7 | GRW-AWW | 2.15 | 0.139 | ab |

* Treatments with the same letter are not significantly different at $p < 0.05$.

There was no significant effect of treatment (control, GRW, GRW-AWW) on the percent green measured (data not shown) at week 4 and week 8 post germination. Finally, the soil analysis (data not shown) did not show any significant effect of treatment (control, GRW, GRW-AWW) on TN, P, K, and Mg concentrations.

For both the greenhouse and field study it appears that the nutrients TN, TP, K, and Mg, confirmed to leach from the 5g GRW-AWW by the leaching experiment (Chapter 5), were not found in the shoots of the perennial ryegrass or in the soil treated with the GRW-AWW. It is unlikely the nutrients leached during the greenhouse experiment because the watering was controlled so that no outflow occurred. Leaching was possible in the field trial, but the soil samples were taken from a 15 cm depth which should have revealed at least some N and P displacement. It is possible the nutrients were taken up by the perennial ryegrass root system but did not get translocated to the shoots and therefore were not accounted for. This has been demonstrated in previous studies of perennial ryegrass root systems, specifically regarding N (Bowman and Paul, 1988) and P uptake (Bailey, 1991). The roots would have had adequate

access to the GRW-AWW nutrients, in both soils, since GRW does not have a CEC to retain nutrients and the mechanism of nutrient movement is through matrix and preferential flow.

Future studies may want to consider tissue analysis of the whole plant, including the roots, in order to account for all the N and P taken up by the plant from the fertilizer source.

Additional analysis on soil samples from the Bovey Field Trial were conducted by an independent laboratory (ALS Canada Ltd., Waterloo, ON. Canada) which determined there were no elevated levels of formaldehyde, phenols, or volatile organic compounds found in the plots where Grodan® rockwool was incorporated 7 months earlier, as compared to the control plots. The certificate of analysis and report from ALS laboratory is shown in Appendix 3 (Fig. A3.1 – A3.5).

### *6.4.4 Solvita $CO_2$-C for the Bovey Field Trial*

The Solvita $CO_2$-C ANOVA tables for the Bovey Field Trial can be found in Appendix 4 (Table A4.7). It was surprising that after exposure to the GRW-AWW and the root exudates of the perennial ryegrass for several months, there was no significant effect of treatment on the microbial $CO_2$-C levels for the Bovey field trial. The mean microbial $CO_2$-C levels for all treatments decreased almost 50% from day 1 to day 212 (Table 6.7). The reason may be largely due to the initial soil disturbance during the homogenizing process when the field study was installed. Chenu et al. (2001) points out the location of microorganisms in the soil matrix is key to their survival and activity. The large, well connected pores of sandy soils, like the Bovey soil, can be considered to have one type of habitat, whereas soils with smaller pores, such as Elora, have several (Chenu et al., 2001). The large pores of sandy soils cannot retain moisture as efficiently as smaller pores which is why soil microbes are more likely to inhabit smaller pores

(Chowdhury et al.,2011). When the sandy soil of the Bovey Field Trial was composited the microbial populations and their habitat were likely disturbed and reduced. Also, similar to tillage damage, mycorrhizal hyphae connectivity was likely destroyed and oxygen was incorporated into anaerobic zones potentially accelerating the decomposition of the organic matter. This extent of soil disturbance could be representative of the soil damage one might find at a former aggregate mining site.

Another reason for the reduced $CO_2$-C concentrations when the field trial ended may have been due to the time needed for the recovery of microbial activity in the soil samples after the drying procedure. Chowdhury et al. (2011) observed that for some sandy soils, soil microbial activity may not be fully responsive for days after rewetting. Future field trials using sandy soils may need to consider conducting and comparing soil $CO_2$ respiration tests over a period of time in order to assess the full impact of the treatment.

Table 6. 7 The Solvita mean $CO_2$-C for the 7 month (212 day) Bovey Field Trial. Plots were treated with a subsurface layer of either 100 g (dry weight) of GRW or GRW-AWW or a control with no GRW. Perennial ryegrass (*Lolium perenne* L.) was grown on all plots.

| Day | Treatment | Mean CO2-C mg/kg | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| 1 | Control | 26.93 | 3.719 | ab |
| 1 | GRW | 28.71 | 3.719 | a |
| 1 | GRW-AWW | 22.14 | 3.719 | ab |
| 212 | Control | 15.74 | 3.719 | ab |
| 212 | GRW | 12.83 | 3.719 | b |
| 212 | GRW-AWW | 13.07 | 3.719 | b |

* Treatments with the same letter are not significantly different at $p < 0.05$.

### *6.4.5 Scanning Electron Microscope (SEM) and Dilution Plating*

After viewing more than 20 sample preparations, the SEM was unable to reveal any microbial colonies, before or after treatments, in the MAC or Elora soils or among the treated

rockwool fibres. Dilution plating of microbial cultures did, however, confirm their existence in the MAC and Elora as well as the Bovey soil (Fig. 6.3). A colony of cocci bacteria (Fig. 6.4) was found in the Bovey field soil sample but there was no indication that the GRW-AWW treatment promoted it. Cocci (spherical) are the second most populous morphological group of soil bacteria followed by spirillum (wavy chains) with the most populous being bacilli (rod-shaped) (Tantiado et al., 2016).

Apparently visually detecting microbial biomass in the soil environment with the SEM is difficult. Soil bacteria are not easily identified because their size and shape closely resemble the soil's clay particles (Chenu et al., 2001). Also, the minimal concentration of microorganisms required in soil for SEM detection is estimated to be between $10^7$ and $10^{10}$ cells per gram of soil (Hagen et al., 1968). The microbial biomass, especially for the MAC soil, was categorized as very low by the Solvita manual as evidenced by the $CO_2$-C of < 5.0 mg/kg which may have contributed to its lack of visual detection by the SEM.

The SEM was able to successfully image the GRW fibre surfaces before and after treatments (Fig.6.5). SEM images, from a previous basaltic mineral wool study by Lund and Yue (2008), compared the smooth surface of a fresh mineral wool fibre to a mineral wool fibre that was exposed to humidity for four weeks as well as a fibre exposed to water for four weeks (Fig. 6.5, top, Ia-IIIa). Lund and Yue (2008) refer to the bumps and scars, observed on the surface of both treated fibres, as "particle-like topographic elements".

The SEM images obtained for this 2017 study indicate a marked difference in the surface texture of the rockwool fibres after treatments as compared to the fresh fibre. The fibres, exposed to MAC, Elora, and Bovey soil, appear to have bumps and are flaking as compared the smooth surface of the fresh GRW fibre (Fig. 6.5, A - D).

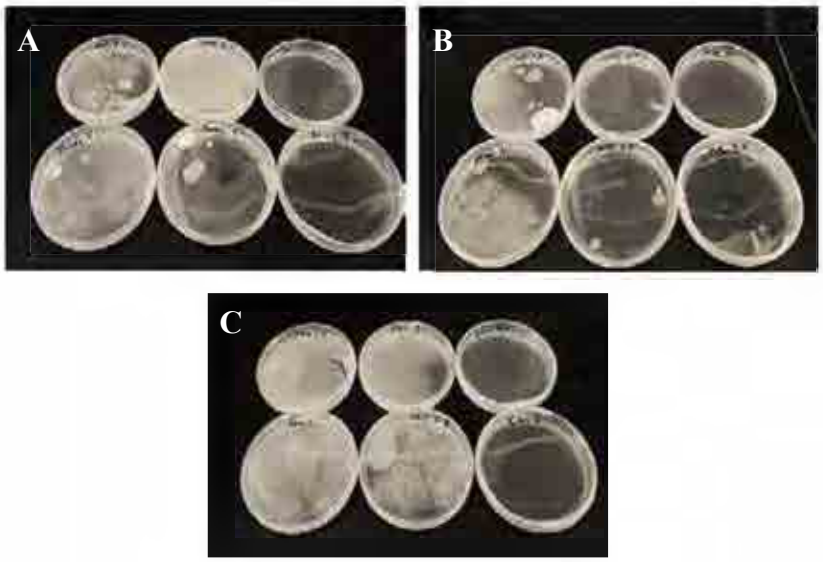



Figure 6.3 Non-quantitative dilution cultures of $10^{-3}$, $10^{-5}$, and $10^{-7}$ (left, middle, and right respectively, in duplicate, in each picture) for Bovey field soil (A), stony soil MAC (B) and silt-loam soil Elora (C) indicating multiple microbial colonies after incubation at 25°C for 3 days.


Figure 6.4 SEM image of cocci bacteria found in the Bovey Field Trial (BFT) soil sample. The sample was taken from a plot treated with a subsurface zone of 100g of GRW-AWW. No other microbial colonies were found in the sample. (Image by J. Garnett, 2017)

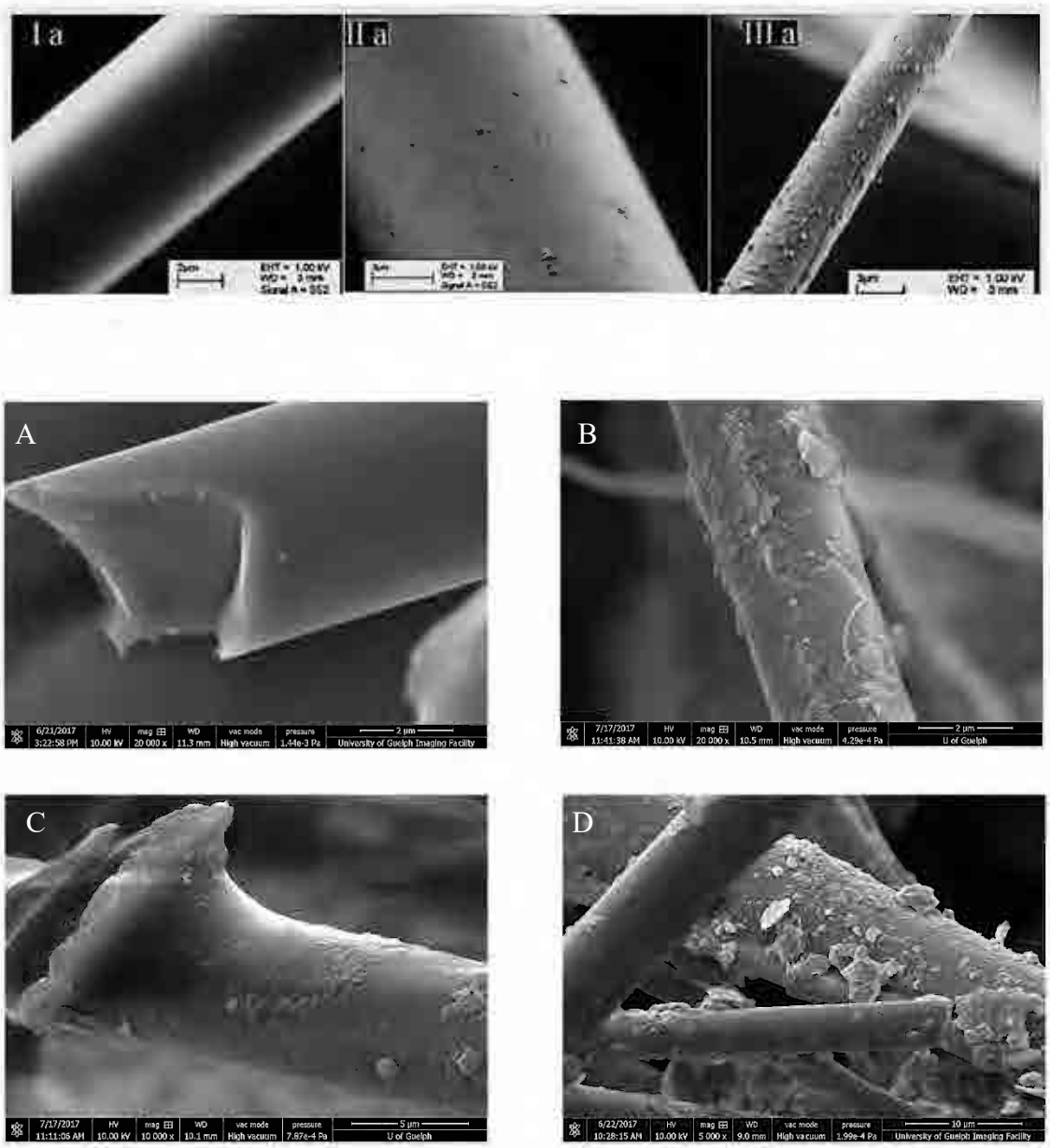



Figure 6.5 SEM images comparing morphological changes in the surface of Grodan rockwool fibres, before and after exposure to moisture, as well as the soil environment. Modified SEM images of Lund and Yue (2008) (top) compare the surface of a fresh rockwool fibre (Ia), to a fibre humidity-exposed 4 weeks (IIa), and a fibre water-exposed 4 weeks ( IIIa). SEM images from this 2017 study compare the surface of a fresh GRW fibre (A), to a GRW-0 fibre after 5 weeks in MAC soil (B), a GRW-0 fibre after 5 weeks in Elora soil (C), and GRW-AWW fibres after 7 months in Bovey field soil (D). (Images A-D by J. Garnett, 2017)

Some of the surface damage to the GRW-0 fibres was likely as a result of exposure to water (Schott and Oelkers,1995; Lund and Yue, 2008) during the saturation process and when the potted soil was watered with AR (pH 5.6). Some of the damage to the GRW-AWW fibres in the Bovey field trial (Fig. 6.5D) was also likely due to the wastewater pre-soak (pH 7.2) as well as the soil moisture. It is not clear how different conditions (i.e., exposure to extreme temperatures, drying, or exposure to plant roots in the natural soil environment), contributed to changes in the surface of the rockwool fibres. The surface damage does seem to indicate that the GRW fibres will degrade in the soil environment, especially if moisture is present at some point. However, more research is needed to determine how other factors, such as temperature, contribute to the surface damage in the soil environment and to establish a GRW degradation timeline.

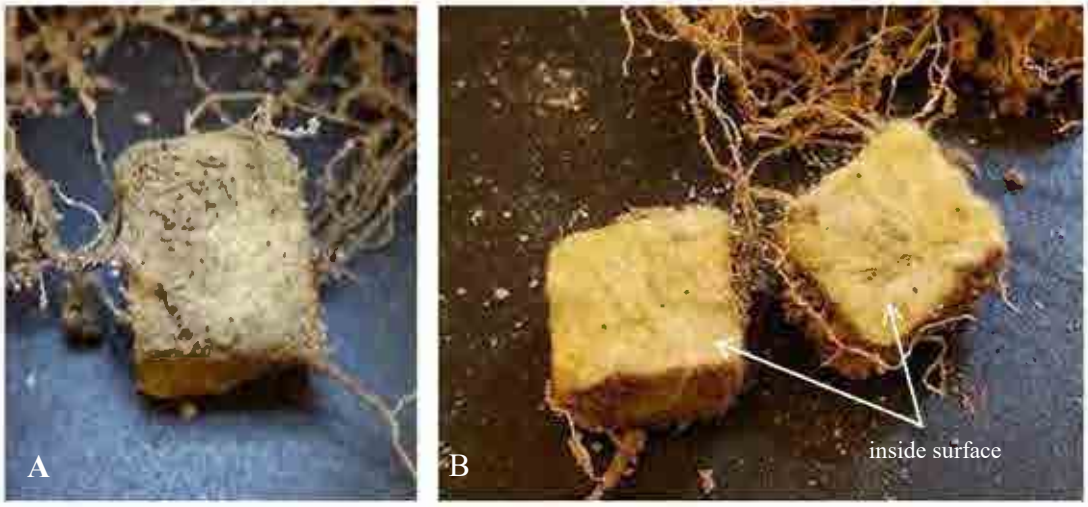



Figure 6.6 Roots of perennial ryegrass (*Lolium perenne* L.) associated with GRW-AWW cubes from the Bovey Field Trial after 7 months. The roots appear to be engaged with the exterior of a GRW-AWW cube (A) but no roots were found to have penetrated into the cube when sectioned (B). (Photo: J. Garnett, 2017)

### *6.4.6 Roots and Grodan Rockwool Cubes*

It was observed that the roots of the perennial ryegrass did not penetrate into the individual rockwool cubes in either in the greenhouse pot study or the Bovey field trial (Fig. 6.6) This was also observed in a preliminary pot study using 5g of shredded rockwool. Some perennial ryegrass roots or root hairs, however, did attach themselves firmly to the loose, outer fibres of the rockwool cubes in both the greenhouse and field trial. One reason for this could be the moisture and wastewater nutrients were easily accessible on the outer surfaces of the GRW cubes by the roots. Another reason could be the GRW cubes, in particular, are too dense and therefore difficult for the roots to penetrate (personal communication with soil scientist, Dr. Irina Solntseva). In a living wall study, Jorgensen et al.(2014) found that plants grown in Grodan rockwool media had reduced root growth and found horizontal root growth appeared hindered in rockwool. They suggested the GRW fibre orientation (previously mentioned in Chapter 2) promotes uneven water distribution in the block and poor water retention, which may have contributed to the root problem. This issue warrants further research to determine the interaction of plant roots with rockwool fibres.

## 6.5 Conclusions

The perennial ryegrass tissue and soil analyses for both the greenhouse and field studies indicated the GRW-AWW treatment was not effective at providing N and P to the perennial ryegrass. However, the mean perennial ryegrass blade length was approximately 1cm longer when the stony soil MAC was treated with 5g GRW-AWW. This is promising but more research is needed to understand the potential for wastewater-treated Grodan rockwool to supply plant-available N and P in nutrient-poor soils. Future studies may need to consider tissue

analysis of the whole plant, given that the root system of perennial ryegrass in particular has been shown to take up N and P but not translocate all of it to the shoot tissue. The observation that perennial ryegrass roots do not penetrate the GRW cubes also needs further investigation to determine if the plant growth or microbial growth is compromised as a result.

Assessing respiration rates using the Solvita $CO_2$-C Burst Test system was fast, reliable, and cost effective. The Digital Colour Reader (DCR) of the Solvita system quantified the colour of the Solvita gel paddle after soil $CO_2$ exposure consistently even when the levels were low. More research may be needed to determine appropriate time intervals for re-assessing $CO_2$-C for sandy soils with particularly low $CO_2$-C concentrations. The increased mean $CO_2$-C from 21.11 mg/kg to 23.95 mg/kg in the Elora soil treated with 5g GRW-AWW treatment, was a promising indication that microbial activities increased with the GRW-AWW treatment. Future experiments may need to be done to investigate the optimum ratio of soil to GRW-AWW.

The SEM did not reveal microbial biofilms, before or after treatments, in the MAC or Elora soils or among the treated rockwool fibres. Dilution plating did however confirm the existence of microbial biomass in all three soils. Part of the challenge was recognizing the microbial biomass among the soil particles. It was expected they would be abundant and self-evident. However, the $CO_2$-C concentrations may have been an indication that finding large amounts of biofilm would be challenging.

More research is needed to be better informed regarding the SEM capabilities and detection of soil microbial biomass in order to determine if a difference in populations has occurred between treatments. Other methods, such as quantitative dilution cultures, should be used in tandem to verify SEM findings.

# Chapter 7 - Overall Conclusions and Future Research Recommendations

## 7.1 Review of Issues and Study Proposal

The disturbed soils of former aggregate mining sites in Ontario can vary in texture, depth, moisture retention, and fertility complicating efforts for re-vegetation. The Management of Abandoned Aggregate Properties (MAAP) program looks to research for the development of new and innovative ideas to deal with the challenges of legacy pit and quarry restoration whereby minimal intervention causes a site to naturalize faster than it would have on its own. Rockwool is an inert, product with characteristics conducive to plant growth. It is widely available either as fresh or waste rockwool from greenhouse production. Wastewater biosolids (liquid) are inexpensive, renewable, and a locally produced fertilizer source. The idea proposed by this study, was to utilize rockwool, treated with wastewater, as a soil amendment for promoting the re-vegetation of disturbed pit and quarry soils.

## 7.2 Thesis Goal and Objectives Achieved and Future Research Recommendations

The goal of this thesis research was to provide scientific insight into the use of wastewater-treated Grodan rockwool as a soil amendment and fertilizer for promoting the re-vegetation of disturbed pit and quarry soil. This study set out to achieve this through a series of experiments and objectives starting with determining the persistence of Grodan rockwool in the environment through silica and pH analysis. In Chapter 3, two experiments investigating the Si dissolution of GRW in pH adjusted rainwater were unsuccessful at determining a rate of GRW deterioration representative of its degradability in the soil environment. The development of four equations representing GRW Si dissolution in pH 5.5, 7, 8 and 8.5 rainwater were weak

according to the Pseudo Efron $R^2$ values of <36. More research is needed to improve the equations; ideally with data obtained from a long-term field trial focused on GRW deterioration and Si dissolution in the soil environment.

The second objective was to determine if a subsurface zone of GRW can prolong moisture retention in the top 5 cm of stony and agricultural soil. In Chapter 4, three out of the four sensor experiments consistently demonstrated 10 g (dry weight) GRW-0 in stony soil (MAC) maintained a mean higher VWC% than the control in the top 5 cm as the soil dried. This response was demonstrated by the silt-loam (Elora) soil but only during the rehydration experiment. This may indicate that GRW is a good candidate as a soil amendment to maintain moisture in the top 5 cm of stony soils which are typically found in disturbed aggregate mining sites. Waste-rockwool, recovered from greenhouse production, may be an option for this application as well. Either way, more research is needed to characterize the interaction of GRW-0 with the properties of different soil-types.

The third objective was to determine if a subsurface zone of GRW, treated with wastewater, can provide plant-accessible P and N and diminish P and N leaching. In Chapter 5, the leaching experiment demonstrated the most TP leached with the AR came from the column with 82 mg MAP fertilizer incorporated into the stony soil MAC. This verified the need to find an alternative method of applying P fertilizer particularly in stony soil. The large pores and high hydraulic conductivity of stony soil promotes P as well as N leaching. None of the treatments utilizing GRW appeared to enhance or reduce P leaching. On the other hand, some leaching of $NH_4^+$ appears to have been reduced when a zone of 1g of GRW-0 was placed below Elora soil containing 82 mg of MAP fertilizer. The $NH_4^+$ leached was significantly lower as compared to the control without GRW-0. This finding warrants further research that should include another

leaching experiment with MAP fertilizer incorporated into MAC soil above a subsurface-zone of 1g GRW-0 to verify the ability of GRW-0 to reduce N and P leaching in stony soil

In chapter 6, the Greenhouse Pot experiment and Bovey Field Trial were designed to determine if a subsurface zone of GRW, treated with wastewater, can provide plant-accessible P and N. The nutrient poor silt-loam and stony soils chosen represented the disturbed soils of pit and quarry soils. Perennial ryegrass was chosen for this study because of its adaptability and potential to establish quickly in poor soil. The MAC soil treated with the GRW-AWW did have a positive outcome in that the mean length of the perennial ryegrass blade was significantly longer by approximately 1cm than the control. However, the shoot tissue and soil analysis for both the greenhouse and field study did not indicate a significant uptake of N and P from the GRW-AWW as compared to the control. Future studies should consider increasing the nutrient content of the GRW-AWW so that nutrient amounts are more detectable. More importantly, future studies should consider tissue analysis that includes the roots of the plant because previous studies have found N and P uptake by p. ryegrass shoots did not account for N and P remaining in the root system.

The last objective was to determine the effect of wastewater-treated GRW on soil microbial growth as evidenced by $CO_2$ respiration and SEM-detected biofilm. The Solvita $CO_2$-C Burst Test system, including the Digital Colour Reader (DCR), was a reliable, fast, and cost effective way to measure soil respiration. The response of the soil microbial $CO_2$-C to soil treated with GRW-0 (Chapter 4) appeared to decline from day 1 to the 40th day after two soil saturation and drying events. This response warrants more research to investigate the effect of GRW-0 soil treatments on the microbial communities. In Chapter 6, it was expected that the GRW-AWW and the root exudates of the perennial ryegrass would boost the $CO_2$-C. Instead the

$CO_2$-C values did not significantly increase from the control for the MAC soil and significantly decreased by almost 50% from day one to day 212 for the Bovey field trial. Research would be needed to ascertain why this happened. For the field trial, part of the reason may be the microbes did not respond favourably to the initial habitat disturbance caused by the installation of the field plots. There was however a significant increase in $CO_2$-C concentration in the greenhouse experiment for the Elora soil treated with 5g GRW-AWW as compared to the control. This is promising however, more research is needed to develop methodology that allows for the installation of the GRW-AWW with minimal soil disturbance.

Finally, it was expected that soil microbes would be easily visualized with the SEM. However, only one community of cocci bacteria, in the Bovey field soil, was found after repeated sample preparations and SEM observations. Hagen et al. (1968) found the minimal concentration of microorganisms required in soil for SEM detection is critical. The Solvita manual equated the low, initial $CO_2$-C concentrations of the MAC soil particularly to a very low microbial content which may have been an indication that finding an abundant biofilm would be remote. More research is needed to better understand the SEM capabilities in the detection of soil microbial biomass and to develop methods that quantify differences in microbial populations as a result of treatments. Other methods, such as quantitative dilution cultures and $CO_2$-C values should be used in tandem to verify SEM findings.

The SEM was able to illuminate changes in the GRW fibres possibly resulting from pre-treatments and weathering in the soil environment. This finding suggests that GRW is degradable however more studies are needed to determine GRW's persistence in the soil environment .

This thesis research was able to provide some insight into the use of wastewater-treated Grodan rockwool as a soil amendment and fertilizer for promoting the re-vegetation of disturbed pit and quarry soil. However, a long-term field study is recommended to investigate the interaction of GRW with soil properties and to determine the length of time required for GRW to degenerate in the soil environment.

**References:**


APHA. 1999. Standard methods for the examination of water and wastewater. 1060 C, Sample storage and preservation, page 60 and 315. Available from https://www.mwa.co.th/download/file_upload/SMWW_1000-3000.pdf

Acuna, R., Bonachela, S., Magan, J., Marfa, O., Hernandez, J. and Caceres, R. 2013. Reuse of rockwool slabs and perlite grow-bags ina low-cost greenhouse: substrates' physical properties and crop production. Sci. Hort. 160: 139-147.

Agriculture and Agri-Food Canada, 2012. Canadian farm fuel and fertilizer: prices and expenses. Market Outlook Report 4:1 AAC No. 11727F.

Agriculture and Agri-Food Canada. 2016a. 2015 Statistical overview of the Canadian greenhouse vegetable industry. AAFC No. 12507E Cat. No. A71-39/2015E-PDF.

Agriculture and Agri-Food Canada. 2016b. Canadian farm fuel and fertilizer: prices and expenses. Market Outlook Report 7:1 AAC No. 12607E.

Agriculture and Agri-Food Canada. 2016c. Crop profile for greenhouse tomato in Canada, 2014. AAFC No. 12575E. Cat. No., A118-10/24-2014E-PDF.

Allaire, S.E., Caron, J., Menard, C., and Dorais, M. 2004. Potential replacements for rockwool as growing substrate for greenhouse tomato. Can. J. Soil Sci. 85: 67-74.

Anderson, P., Davidson, C.M., Duncan, A.L., Littlejohn, D., Ure, A.M., and Garden, L.M. 2000. Column leaching and sorption experiments to assess the mobility of potentially toxic elements in industrially contaminated land. J. Environ. Monit. 2: 234-239.

Bailey, J.S. 1991. A re-examination of phosphorus-lime interactions in perennial ryegrass. Plant and Soil 135: 185-196.

Bailey, L.D., and Grant, C.A. 1990. Fertilizer placement studies on calcareous and non-calcareous chernozemic soils: growth, P-uptake, oil content and yield of Canadian rape. Com. in Soil Sci. Plant Anal. 21(17&18): 2089-2104.

Bougoul, S., Ruy, S., de Groot, F., and Boulard, T. 2005. Hydraulic and physical properties of stonewool substrates in horticulture. Sci. Hort. 104: 391-405.

Bowley, S. 2015. A hitchhiker's guide to statistics in biology. Generalized linear mixed model edition. Plants et al., Inc. Ont. Canada.

Bowman, D.C., and Paul, J.L. 1988. Uptake and assimilation of $NO_3^-$ and $NH_4^+$ by nitrogen-deficient perennial ryegrass turf. Plant Physiol. 88: 1303-1309.

Brady, N., Weil, R. 2008. The nature and properties of soils, 14th ed. Pearson Prentice Hall, New Jersey, USA.

Bravo-Martin-Consuegra, S, Garcia-Navarro, F., Amoros-Ortiz-Villajos, J., Perez-de-los-Reyes, C., and Higueras, P. 2016. Effect of the addition of sewage sludge as a fertilizer on a sandy vineyard soil. J. Soils Sed. 16: 1360-1365.

Burkitt, L.L., Turner, L.R., Donaghy, D.J., Fulkerson, W.J., Smethurst, P.J. and Roche, J.R. 2009. Characterization of phosphorus uptake by perennial ryegrass (*Lolium perenne* L.) during regrowth. N.Z. J. Agri. Research. 52: 195-202.

Bussell, W.T., and Mckennie, S. 2004. Rockwool in horticulture and its importance and sustainable use in New Zealand. NZ J. Crop and Hort. Sci. 32(1): 29-37.

C.C.M.E. 2012. Guidance document for the beneficial use of municipal biosolids, municipal sludge and treated septage. Available from http://www.ccme.ca/files/Resources/waste/biosolids/pn_1473_biosolids_guidance_eng_1.0.pdf

Campopiano, A., Cannizzaro, A., Angelosanto, F., Astolfi, M., Ramires, D., Olori, A., Canepari, S., and Iavicoli, S. 2014. Dissolution of glass wool, rock wool and alkaline earth silicate wool: morphological and chemical changes in fibers. Reg. Tox. Pharm. 70:393-406.

Canadian Environmental Protection Act 1993. Mineral fibres (Man-made vitreous fibres) Can. Cat. Publ. Data., Can. Com. Gr. Publ. Ottawa, Canada.

Carter M.R. and Gregorich, E.G (Eds). 2008. Soil sampling and methods of analysis, 2nd Ed. Can. Soc. Soil Sci. CRC Press, Boca Raton, USA.

Chenu, C., Hassink, J., and Bloem, J. 2001. Short-term changes in the spatial distribution of microorganisms in soil aggregates as affected by glucose addition. Biol. Fertil. Soils 34: 349-356.

Chowdhury, N., Burns, R.G., and Marschner, P. 2011. Recovery of soil respiration after drying. Plant Soil, 348: 269-279.

Christensen, V.R., Jensen, S.L., Guldberg, M., and Kamstrup, O. 1994. Effect of chemical composition of man-made vitreous fibres on the rate of dissolution in vitro at different pHs. Environ. Health Perspect. 102(Suppl. 5):83-86.

Cousin, I., Nicoullaud, B., and Coutadeur, C. 2003. Influence of rock fragments on the water retention and water percolation in a calcareous soil. Catena 53: 97-114.

Corry, R.C., Lafortezza, R., Brown, R.D., Kenny N., and Roberrtson, P.J. 2008. Using landscape context to guide ecological restoration: An approach for pits and quarries in Ontario. 26(2): 120-126.

da Silva, F., Wallach, R., and Chen, Y. 1995. Hydraulic properties of rockwool slabs used as substrates in horticulture. Growing media and plant nutrition. Acta Hort. 401: 71-75.

Debicka, M., Kocowicz, A. Weber, J., Jamroz, E. 2015. Organic matter effects on phosphorus sorption in sandy soils. Archives of Agronomy and Soil Science.

de Meringo, A., Morscheidt, C., Thelohan, S., and Tiesler, H. 1994. In vitro assessment of biodurability acellular systems. Environ. Health Perspect. 102(5): 47-53.

Dias, G.M., Ayer, N.W., Khosla, S., Van Acker, R., Young, S.B., Whitney, S., and Hendricks, P. 2017. Life cycle perspectives on the sustainability of Ontario greenhouse tomato production: Benchmarking and improvement opportunities. J. Cleaner Prod. 140: 831-839.

Doran, J., Kettler, T., Tsivou, M. 1997. Field and laboratory solvita soil test evaluation. University of Nebraska, USDA-ARS.

Drury, C.F., Reynolds, W.D., Parkin, G.W., Lauzon, J.D., Saso, J.K., Zhang, T.Q., Yang, X.M., Tan, K, Liu, T.K., Calder, W., Oloya, T.O., Welacky, T.W., and Reid, D.K.. 2016. Solute dynamics and the Ontario nitrogen index: II Nitrate leaching. Can. J. Soil Sci. 96(2):122-135.

Drever, J.I. 1994. The effect of land plants on weathering rates of silicate minerals. Geo. Cosm. Acta, 58(10) 2325-2332.

Dupuis, E.M., and Whalen, J.K. 2007. Soil properties related to the spatial pattern of microbial biomass and respiration in agroecosystems. Can. J. Soil Sci. 87: 479-484.

Eghball, B., Binford, G., and Baltensperger, D. 1996. Phosphorus movement and adsorption in a soil receiving long-term manure and fertilizer application. J. Environ. Qual. 25: 1339-1343.

Environment Canada. 2004. Canadian guidance framework for the management of phosphorus in freshwater systems. Ecosystem Health: Science-based solutions report no. 1–8. Cat. No. En1–34/8–2004E. National Guidelines and Standards Office, Water Policy and Coordination Directorate, Environment Canada, Ottawa, Ontario, Canada.

Fetter, C.W. 2001. Applied Hydrogeology, 4th Ed. Prentice-Hall, Inc. New Jersey, USA.

Fonteno, W.C., and Nelson, P.V. 1990. Physical properties of and plant responses to rockwool amended media. J. Amer. Soc. Hort. Sci. 115(3): 375-381.

Franzluebbers, A.J., Haney, R.L., Honeycutt, C.W., Schomberg, H.H., and Hons, F.M. 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Sci. Soc. Am. J. 64: 613-623.

Fulkerson, B. 2007. Future Dairy Tech Note, Perennial Ryegrass. Available from http://futuredairy.com.au/wp-content/uploads/2016/02/TechNoteRyegrassPerennial.pdf

Funke, B.R., and Harris, J.O. 1968. Early respiratory responses of soil treated by heat or drying. Plant and Soil, 28 (1): 38-48.

Gilewska, M. 2006. Utilization of wastes in reclamation of post mining soils and ash dump sites. Roczniki Gleboznawcze, Tom LVII NR 1/2 Warszawa 75-81 (trans. polish).

Goupil, K., and Nkongolo, K. 2014. Assessing soil respiration as an indicator of soil microbial activity in reclaimed metal contaminated lands. Am. J. Environ. Sci. 10(4): 403-411.

Grodan 2005. Material safety data sheet. Available from http://grodan101.com/sites/default/files/leaflets/MSDS%20USEU%20version%20actual%20April%2002%20-%20updated%20180803%20and%20271005%20FINAL.pdf

Grodan 2014. Material safety data sheet. Available from http://www.grodan101.com/sites/default/files/leaflets/MSDS-US-09012014.pdf

Guan, W., Ji, F., Chen, Q., Yan P., and Zhang, Q. 2013. Preparation and phosphorus recovery performance of porous calcium-silicate-hydrate. Ceramics International 39: 1385-1391.

Gudbrandsson, S., Wolff-Boenisch, D., Gislason, S., and Oelkers, E. 2011. An experimental study of crystalline basalt dissolution from $2 \leq pH \leq 11$ and temperatures from 5 to 75$^{o}$C. Geo. Cosm. Acta 75: 5496-5509.

Hagen, C.A., Hawrylewicz, E.J., Anderson, B.T., Tolkacz V.K., and Cephus, M. L. 1968. Use of the scanning electron microscope for viewing bacteria in soil. Appl. Micro. 16(6): 932-934.

Haney, R, Brinton, W.. and Evans, E. 2008. Soil CO2 respiration: comparison of chemical titration, CO2 IRGA analysis and the Solvita gel system. Renewable Agriculture and Food Systems 23(2): 171-176.

Haney, R., Franzluebbers, A., Jin, V., Johnson,M., Haney,E., White,M., Harmel, R. 2012. Soil organic C:N vs. water-extractable organic C:N. Open J. Soil Sci. 2: 269-274.

Haney, R.L., Jin, V., Johnson, M.V., Haney, E., Harmel, R.D., Arnold, J., and White, M. 2015. Analysis methods for the determination of anthropogenic additions of P to agricultural soils. Open J. Soil Sci. 5: 59-68.

Hannaway, D., Fransen, S., Cropper, J., Teel, M., Chaney, M., Griggs, T., Halse, R., Hart, J., Cheeke, P., Hansen, D., Klinger, R., and Lane, W. 1999. Perennial ryegrass (*Lolium perenne* L.), Oregon State Univ., Pacific Northwest Extension Publications.

Health Canada 2006. The safety of man-made vitreous fibres. Available from http://www.hc-sc.gc.ca/hl-vs/iyh-vsv/prod/vitre-eng.php

Hillel, D. 1982. Introduction to soil physics. Academic Press Inc. USA.

IARC. 2002. IARC monographs on the evaluation of carcinogenic risks to humans: Man-made vitreous fibres. World Health Organization.. Available from https://monographs.iarc.fr/ENG/Monographs/vol81/mono81.pdf

Iler, R.K. 1979. The chemistry of silica. Solubility, polymerization, colloid and surface properties, and biochemistry. J. Wiley and sons. New York, USA.

Jaroszuk-Sierocinska, M., Slowinska-Jurkiewicz A., Bryk, M, and Kolodziej, M. 2014. Estimation of structure and water and air properties of Grodan rockwool waste after production cycle. Pol. J. Environ. Stud. 23(6): 2039-2045.

Jin, C., and Parker, W. 2017. Needs assessment for sludge processing technologies in Ontario. University of Waterloo. Available from https://sowc.ca/wp-content/uploads/2017/10/SOWC-report_-Executive-summary_17.09.15.pdf

Jin, L., Williams, E., Szramek, K., Walter, L., and Hamilton, S. 2008. Silicate and carbonate mineral weathering in soil profiles developed on Pleistocene glacial drift (Michigan, USA): Mass balances based on soil water geochemistry. Geo. et Cosm. Acta, 72: 1027-1042.

Jorgensen, L., Dresboll, D.B., and Thorup-Kristensen, K. 2014. Spatial root distribution of plants growing in vertical media for use in living walls. Plant Soil. 380: 231-248.

Kreyling J., Schuerings, J., Malyshev, A., Vogt, L., Werner, C., and Jentsch, A., 2015. Nitrogen leaching is enhanced after a winter warm spell but mainly controlled by vegetation composition in temperate zone mesocosms. Plant Soil. 396: 85-96.

Kroetsch, D. and Wang, C. 2008. Particle size distribution. In: Soil Sampling Methods of Analysis, Eds. M.R. Carter and E.G. Gregorich. CRC Press, Boca Raton, Fl. 713-726.

Loganathan, P., Vigneswaran, S., Kandasamy, J., and Bolan, N. 2014. Removal and recovery of phosphate from water using sorption. Critical Rev. Environ. Sci. Tech. 44:847-907.

Lund, M.D., and Yue, Y. 2008. Influences of chemical aging on the surface morphology and crystallization behavior of basaltic glass fibres. J. Non-Crystalline Solids. 354: 1151:1154.

Lutgens, F.Kl, and Tarbuck, E.J. 2015. Essentials of geology, 12th Ed. Pearson Education Inc. New Jersey, USA.

Lyons, E.M., Synder, R.H., and Lynch, J.P. Regulation of root distribution and depth by phosphorus localization in *Agrostis stolonifera.* Hort. Sci. 43(7): 2203-2209.

Mackintosh, E.E., and Mozuraitis, E.J. 1982. Agriculture and the aggregate industry. Rehabilitation of extracted sand and gravel lands to an agricultural after-use. University of Guelph. Available from http://www.toarc.com/pdfs/Agri_Aggre.pdf

Mastalerz, J.W. 1977. The greenhouse environment. John Wiley and Sons. New York, USA.

McClellan, T.A., Shearman, R.C., Gaussoin, R.E., Mamo, M., Wortmann, C.S., Horst, G.L., and Marx, S.B. 2007. Nutrient and chemical characterization of aging golf course putting greens: Establishment and rootzone mixture treatment effects. Crop Sci. 47: 193-199.

OMAFRA. 2006. Publication 611 Soil fertility handbook. Available from: http://www.omafra.gov.on.ca/english/crops/pub611/p611order.htm

OMAFRA 2008. Lawn establishment. Available from http://www.omafra.gov.on.ca/english/crops/facts/08-025w.htm

OMAFRA 2012. Cover crops: Ryegrass Available from http://www.omafra.gov.on.ca/english/crops/facts/cover_crops01/ryegrass.htm

OMNR. 2010. The state of the aggregate resource in Ontario study, consolidated report. No. 52624. Queen's Printer for Ontario.

OSSGA. 2010. Study of aggregate site rehabilitation in Ontario Part 1. Ontario Stone, Sand & Gravel Assoc. Available from ossga.com/multimedia/51/ossga_rehabilitation_study_part_1_-_1971-2009.pdf

Papadopoulos T., and Gosselin, A. 2007. Greenhouse vegetable production in Canada. Chronica Hort. 47(3):23-28.

Paul, E.A., and Clark, F.E.,(eds.) 1989. Soil microbiology and biochemistry. Ch. 12 Phosphorus Transformations in Soil pgs. 222-231. Elsevier Inc. USA.

Pell, M., and Nyberg, F. 1989. Infiltration of wastewater in a newly started pilot sand-filter system: 1. Reduction of organic matter and phosphorus. J. Environ. Qual. 18: 451-457.

Poesen, J., Ingelmo-Sanchez, F., and Mucher, H. 1990. The hydrological response of soil surfaces to rainfall as affected by cover and position of rock fragments in the top layer. Earth Surf. Proc. and Land. 15: 653-671.

Raviv, M. and Lieth, J.H. 2008. Soilless culture: theory and practice. Ch. 12 Inorganic and synthetic organic components of soilless culture and potting mixes. Elsevier, London, UK.

Resh, H.M. 2004. Hydroponic food production. A definitive guidebook of soilless food-growing methods, 6th Ed. Newconcept Press. New Jersey, USA.

Richardson, A.E., Barea J.M., McNeill, A.M., and Prigent-Combaret, C. 2009. Acquisition of phosphorus and nitrogen in the rhizospere and plant growth promotion by microorganisms. Plant Soil 321: 305-339.

Rochette, P., Ellert, B., Gregorich, E.G., Desjardins, R.L., Pattey, E., Lessard, R., and Johnson, B.G. 1997. Description of a dynamic closed chamber for measuring soil respiration and its comparison with other techniques. Can. J. Soil Sci. 77: 195-203.

Rockwool 2015. Rockwool international annual report 2015. Available from http://static.rockwool.com/globalassets/rockwool-com/investor-relations/financial-reports/annual-reports/Annual-Report-2015_ENrockwool-international.pdf

SAROS. 2009. State of aggregate resource study in Ontario. Rehabilitation paper 6. MNR Number 52659. Queen's Printer for Ontario.

Schachtman, D.P., Reid, R.J., and Ayling, S.M. 1998. Phosphorus Uptake by plants: from soil to cell. Plant Physiol. 116: 447-453.

Scholze, H. and Conradt, R. 1987. An *in vitro* study of the chemical durability of siliceous fibres. Ann. Occup. Hyg. 31(4B): 683-692.

Schott, J., and Oelkers, E.H. 1995. Dissolution and crystallization rates of silicate minerals as a function of chemical affinity. Pure and Appl. Chem. 67(6): 903-910.

Sebastien, P. 1994. Biopersistence of man-made vitreous silicate fibers in the human lung. Environ. Health Perspect 102 (suppl. 5): 225-228.

Smil,V. 2000. Phosphorus in the environment: natural flows and human interferences. Annu. Rev. Energy Environ. 25: 53-88.

Soldat, D.J., and Petrovic, A.M. 2008. The fate and transport of phosphorus in turfgrass exosystems. Crop Sci. 48: 2051-2065.

Tantiado, R.G., Tajolosa, S.E., and Estrella, K.J. 2016. Antibacterial screening of soil bacteria isolates from Guimaras Island, Philippines against escherichia coli and staphylococcus aureus. Int. J. Bio. Sci. Bio. Tech. 8(2): 419-432.

TOARC 2015. Aggregate resources statistics in Ontario. Production statistics 2015. Available from http://www.toarc.com/pdfs/Stats_2015.pdf

TOARC 2016. The Ontario aggregate resources corporation. Available from http://www.toarc.com/research/annual-reports.html and http://www.toarc.com/maap-1/about-maap.html

Tunesi, S., Poggi, V., and Gessa C. 1999. Phosphate adsorption and precipitation in calcareous soils: the role of calcium ions in solution and carbonate minerals. Nutrient Cycling in Agroecosystems 53: 219-227.

Urrestarazu, M., Guillen, C., Mazuela, P., and Carrasco, G. 2007. Wetting agent effect on physical properties of new and reused rockwool and coconut coir waste. Sci. Hort. 116: 104-108.

Vantsi, O., and Karki, T. 2014. Mineral wool waste in Europe: a review of mineral wool waste quantity, quality, and current recycling methods. J. Mater Cycles Waste Manag. 16: 62-72.

Wang, F.L., and Alva, A.K. 1996. Leaching of nitrogen from slow-release urea sources in sandy soils. Soil Sci. Soc. Am. J. 60: 1454-1458.

Wang, Y.T., Zhang, T.Q., O'Halloran, I.P., Tan, C.S., Hu, Q.C., and Reid, D.K. 2012. Soil tests as risk indicators for leaching of dissolved phosphorus from agricultural soils in Ontario. SSSAJ, 76(1): 220-228.

Weaver, D.M., Ritchie, G.S., and Anderson, G.C. 1988. Phosphorus leaching in sandy soils II. Laboratory studies of the long-term effects of the phosphorus source.

Wever, G., and Kipp, J. 1998. Characterization of the hydrophysical behaviour of stonewool. In Proc. 16th World Congress of Soil Science. Sci. Reg. no. 2580.

White, A. F., and Brantley, S. L. 2003. The effect of time on the weathering of silicate minerals: why do weathering rates differ in the laboratory and field? Chem. Geology, 202: 479-506.

Yeates, J.S., and Clarke, M.F. 1993. Developing alternatives to phosphate fertilizers of high water solubility. Fert. Research 36: 141-150.

Yiqi, L., and Zhou, X. 2006. Soil respiration and the environment. Academic Press, San Diego California, USA.

# Appendices

## Appendix 1

Table A1.1 Mean (n=18) deionized water absorption by Grodan 1cm Grow Cubes® (GRW) prior to use in the Sensor 1, 2, and 3 experiments. The dry GRW was weighed then placed in a self-sealing bag with 500 mL of deionized water (pH 5.5) for a minimum of 24 hours. The GRW sample was drained for two minutes then weighed to determine the saturated weight. The mean absorbed $H_2O$ was determined by subtracting the mean initial weight from the mean saturated weight and converting to milliliters.

| **GRW Sample ID** | Mean GRW Initial Wt (g) (n=18) | Mean GRW Saturated Wt (g) (n=18) | Mean $H_2O$ Absorbed (ml) (n=18) |
|---|---|---|---|
| 1g | 1.02 | 13.86 | 12.84 |
| 5g | 5.02 | 66.72 | 61.70 |
| 10g | 10.02 | 129.13 | 119.11 |

Equation A1.1

Calculations for monoammonium phosphate (MAP) fertilizer in the leaching column.

10,000 $m^2$ in a hectare
10,000 $cm^2$ in a meter

Area of leaching tube: $A = \pi r^2 = 3.14\ (2.6\ cm)^2 = 21.2\ cm^2$

200 kg per ha x 1 ha /10,000 $m^2$ = 0.02 kg/ $m^2$
0.02 kg/ $m^2$ x 1$m^2$/10,000 $cm^2$ = 0.000002 kg/$cm^2$

0.000002 kg/ $cm^2$ x 21.2 $cm^2$ x 1000 g/kg = 0.0424 g
0.0424g /.52 ($P_2O_5$) = 0.0815 g = 82 mg

MAP nutrient ratio by weight: 11% N, 52% $P_2O_5$, 0 $K_2O$ %
Ortho P= (31 g/mol x 2) / ((31 g/mol x 2) + (16 g/mol x 5) = 0.43 x .52 = 0.2236 or 22.36 %

82 mg of MAP contains:
P = 0.2236 x 0.082 g = 0.018 g = 18 mg
N = 0.11 x 0.082 g = 0.009 g = 9 mg

Table A1.2 Silicon Mesh-Lid Experiment mean Al (A), Fe (B), and Ca (C) recovered from a 50 mL artificial rainwater solution adjusted to either pH 5.5, 7, 8, or 8.5, containing 1g Grodan® rockwool. The solution was replaced daily for the first 35days and then once a week up to day 63. The means represent a product of 3 replicates per pH treatment for four randomly chosen days (day 2, 7, 21, 43, and 63).

**A**

| pH | Element | Mean mg/l | Standard Error | Significant Grouping* |
|---|---|---|---|---|
| 5.5 | Al | 0.16 | 0.016 | ab |
| 7 | Al | 0.11 | 0.015 | b |
| 8 | Al | 0.18 | 0.016 | a |
| 8.5 | Al | 0.19 | 0.015 | a |

**B**

| pH | Element | Mean mg/l | Standard Error | Significant Grouping* |
|---|---|---|---|---|
| 5.5 | Fe | 0.25 | 0.011 | ab |
| 7 | Fe | 0.24 | 0.005 | b |
| 8 | Fe | 0.26 | 0.005 | ab |
| 8.5 | Fe | 0.27 | 0.008 | a |

**C**

| pH | Element | Mean mg/l | Standard Error | Significant Grouping* |
|---|---|---|---|---|
| 5.5 | Ca | 1.01 | 0.031 | a |
| 7 | Ca | 0.86 | 0.013 | b |
| 8 | Ca | 0.95 | 0.020 | a |
| 8.5 | Ca | 0.95 | 0.026 | a |

*pH treatments with the same letter are not significantly different at $p < 0.05$

Table A1.3 Leaching Experiment mean values (3 replicates, 4 days) for K (A), $NH_4^+$ (B), $NO_3^-$ (C), Al (D), Fe (E), Mg (F), P (G), and S (H). Treatments were leached at a rate of 1mL $min^{-1}$ of artificial rainwater once a day for 14 consecutive days.

**A**

| Treatment | Element | Mean mg/L | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| GRW-0 | K | 9.36 | 0.401 | a |
| GRW-AWW | K | 9.02 | 0.401 | a |
| MAPMac | K | 4.35 | 0.401 | b |
| M5g GRW-AWW | K | 3.72 | 0.401 | bc |
| MAC | K | 3.08 | 0.401 | bc |
| MAPElora | K | 3.04 | 0.491 | bc |
| EMAPGRW-01g | K | 2.88 | 0.401 | bc |
| M1g GRW-AWW | K | 2.81 | 0.401 | bc |
| E5g GRW-AWW | K | 2.72 | 0.401 | bc |
| Elora | K | 2.65 | 0.401 | bc |
| E1g GRW-AWW | K | 2.35 | 0.401 | c |

**B**

| Treatment | Element | Mean mg/L | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| MAPElora | $NH_4$ | 4.20 | 0.154 | a |
| MAPMac | $NH_4$ | 3.40 | 0.128 | b |
| Elora | $NH_4$ | 2.68 | 0.130 | c |
| E5g GRW-AWW | $NH_4$ | 2.43 | 0.130 | cd |
| E1g GRW-AWW | $NH_4$ | 2.00 | 0.130 | de |
| EMAPGRW-01g | $NH_4$ | 1.95 | 0.128 | de |
| GRW-AWW | $NH_4$ | 1.70 | 0.130 | e |
| M5g GRW-AWW | $NH_4$ | 0.60 | 0.130 | f |
| GRW-0 | $NH_4$ | 0.47 | 0.130 | f |
| M1g GRW-AWW | $NH_4$ | 0.44 | 0.130 | f |
| MAC | $NH_4$ | 0.39 | 0.130 | f |

**C**

| Treatment | Element | Mean mg/L | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| EMAPGRW-01g | $NO_3$ | 130.81 | 10.000 | a |
| E5g GRW-AWW | $NO_3$ | 115.87 | 10.000 | a |
| E1g GRW-AWW | $NO_3$ | 99.72 | 10.000 | a |
| Elora | $NO_3$ | 38.62 | 10.000 | b |
| MAPElora | $NO_3$ | 16.63 | 12.250 | b |
| GRW-AWW | $NO_3$ | 11.15 | 10.000 | b |
| M5g GRW-AWW | $NO_3$ | 1.83 | 10.000 | b |
| MAPMac | $NO_3$ | 1.76 | 10.000 | b |
| M1g GRW-AWW | $NO_3$ | 1.11 | 10.000 | b |
| MAC | $NO_3$ | 0.45 | 10.000 | b |
| GRW-0 | $NO_3$ | 0.13 | 10.000 | b |

**D**

| Treatment | Element | Mean mg/L | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| GRW-AWW | Al | 0.18 | 0.004 | a |
| MAPMac | Al | 0.01 | 0.004 | b |
| M1g GRW-AWW | Al | 0.01 | 0.004 | b |
| Elora | Al | 0.0 | 0.004 | b |
| M5g GRW-AWW | Al | 0.0 | 0.004 | b |
| EMAPGRW-01g | Al | 0.0 | 0.004 | b |
| GRW-0 | Al | 0.0 | 0.004 | b |
| E1g GRW-AWW | Al | 0.0 | 0.004 | b |
| E5g GRW-AWW | Al | 0.0 | 0.004 | b |
| MAPElora | Al | 0.0 | 0.005 | b |
| MAC | Al | 0.0 | 0.004 | b |

**E**

| Treatment | Element | Mean mg/L | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| E1g GRW-AWW | Fe | 0.11 | 0.014 | a |
| E5g GRW-AWW | Fe | 0.11 | 0.014 | a |
| Elora | Fe | 0.10 | 0.014 | a |
| MAPElora | Fe | 0.10 | 0.016 | ab |
| EMAPGRW-01g | Fe | 0.09 | 0.014 | abc |
| MAPMac | Fe | 0.09 | 0.014 | abc |
| GRW-AWW | Fe | 0.06 | 0.014 | abc |
| MAC | Fe | 0.05 | 0.014 | abc |
| M1g GRW-AWW | Fe | 0.05 | 0.014 | abc |
| GRW-0 | Fe | 0.03 | 0.014 | bc |
| M5g GRW-AWW | Fe | 0.03 | 0.014 | c |

**F**

| Treatment | Element | Mean mg/L | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| Elora | Mg | 10.16 | 0.774 | a |
| MAPElora | Mg | 10.16 | 0.948 | ab |
| E5g GRW-AWW | Mg | 8.85 | 0.774 | ab |
| MAPMac | Mg | 8.48 | 0.774 | ab |
| MAC | Mg | 7.71 | 0.774 | ab |
| EMAPGRW-01g | Mg | 7.53 | 0.774 | ab |
| E1g GRW-AWW | Mg | 7.39 | 0.774 | ab |
| M1g GRW-AWW | Mg | 6.4 | 0.774 | ab |
| M5g GRW-AWW | Mg | 6.2 | 0.774 | ab |
| GRW-AWW | Mg | 1.44 | 0.774 | c |
| GRW-0 | Mg | 1.23 | 0.774 | c |

**G**

| Treatment | Element | Mean mg/L | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| MAPMac | P | 3.34 | 0.242 | a |
| GRW-AWW | P | 1.05 | 0.242 | b |
| E1g GRW-AWW | P | 0.93 | 0.242 | b |
| MAPElora | P | 0.9 | 0.297 | b |
| E5g GRW-AWW | P | 0.89 | 0.242 | b |
| EMAPGRW-01g | P | 0.82 | 0.242 | b |
| Elora | P | 0.69 | 0.242 | b |
| M5g GRW-AWW | P | 0.08 | 0.242 | b |
| MAC | P | 0 | 0.242 | b |
| M1g GRW-AWW | P | 0 | 0.242 | b |
| GRW-0 | P | 0 | 0.242 | b |

**H**

| Treatment | Element | Mean mg/L | Standard Error | Significant Grouping * |
|---|---|---|---|---|
| E5g GRW-AWW | S | 7.38 | 0.729 | a |
| MAPMac | S | 6.71 | 0.729 | ab |
| M5g GRW-AWW | S | 6.27 | 0.729 | ab |
| E1g GRW-AWW | S | 5.75 | 0.729 | ab |
| MAPElora | S | 5.36 | 0.894 | ab |
| Elora | S | 5.21 | 0.729 | ab |
| MAC | S | 5.1 | 0.729 | ab |
| GRW-0 | S | 5.09 | 0.729 | ab |
| GRW-AWW | S | 4.29 | 0.729 | ab |
| EMAPGRW-01g | S | 3.86 | 0.729 | b |
| M1g GRW-AWW | S | 3.83 | 0.729 | b |

* Treatment with the same letter are not significantly different at $p<0.05$.

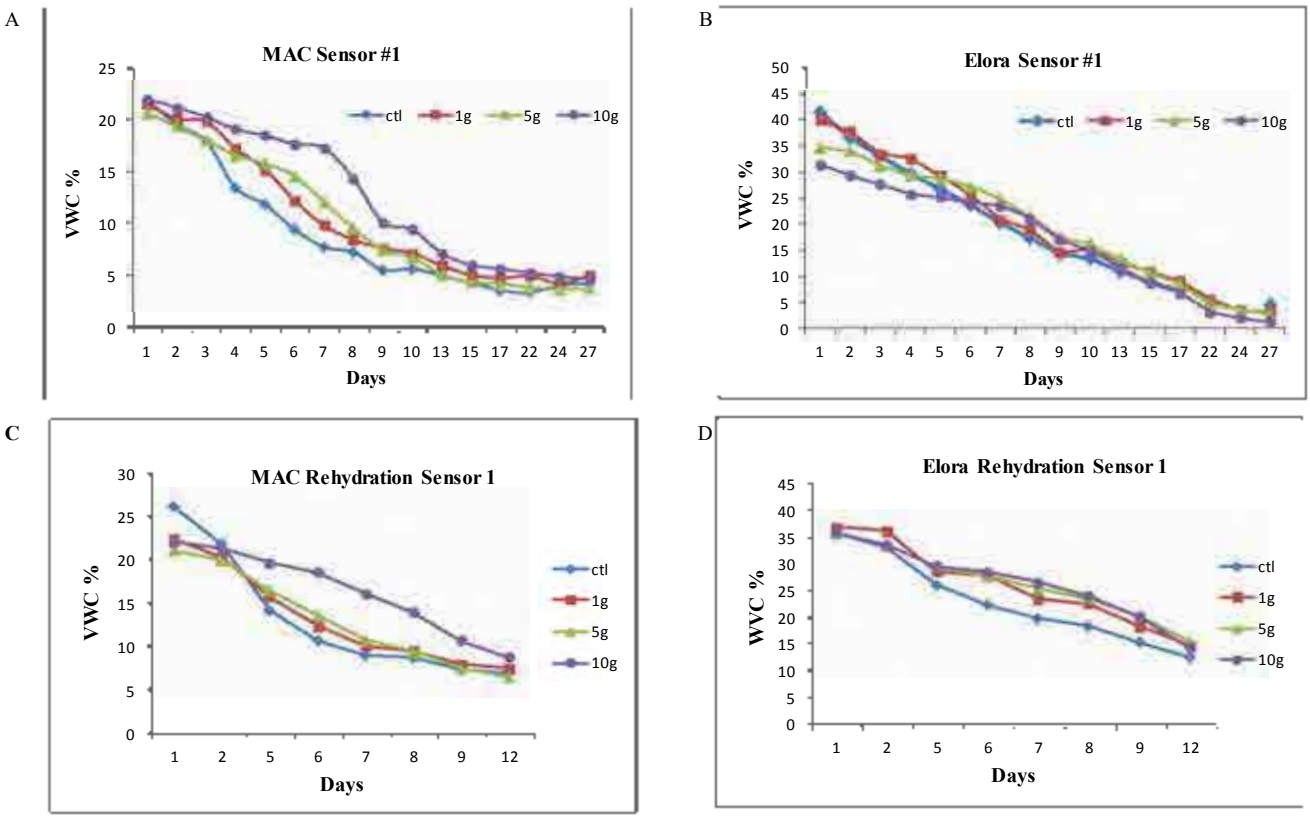



Figure A1.1 Sensor #1 and Rehydration Sensor #1 Experiment results. Data points represent the daily mean (two replicates for 5g and 10g only) volumetric water content percent (VWC%) in the top 5 cm of stony MAC soil (A) and silt-loam Elora soil (B) after saturation and drying 27 days. The treatments were a subsurface zone of 1g, 5g, and 10g saturated Grodan rockwool (GRW-0) and a control without GRW-0. For the Rehydration of Sensor #1 Experiment, MAC (C) and Elora (D) pots from Sensor #1 were re-saturated and allowed to dry for 12 days.

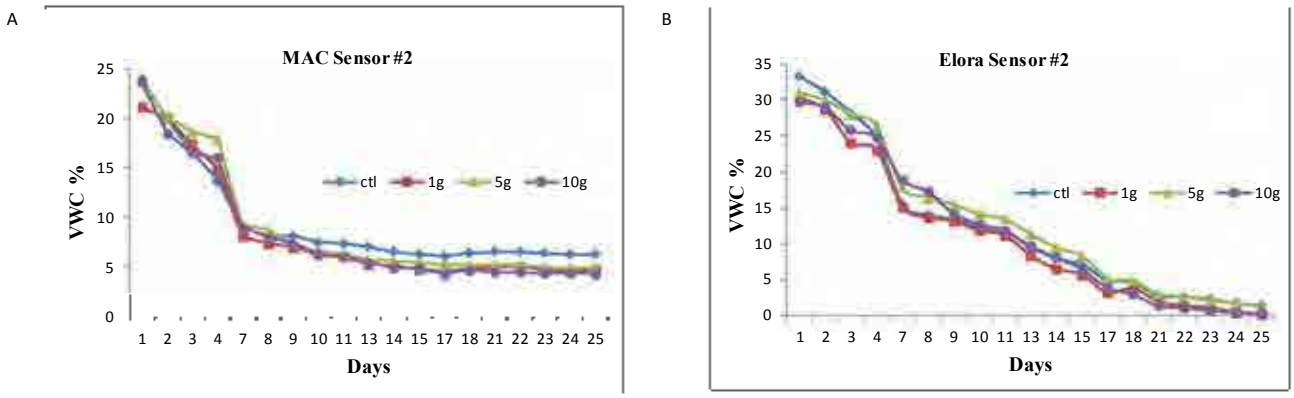



Figure A1.2 Sensor #2 Experiment results. Data points represent the daily mean (two replicates) volumetric water content percent (VWC%) in the top 5 cm of stony MAC soil (A) and silt-loam Elora soil (B) after saturation and drying 25 days. The treatments were a subsurface zone of 1g, 5g, and 10g saturated Grodan rockwool (GRW-0) and a control without GRW-0.
*Note: The relative humidity (Rh%) was not found to have an effect on the results but it was <32% for several days during this experiment.

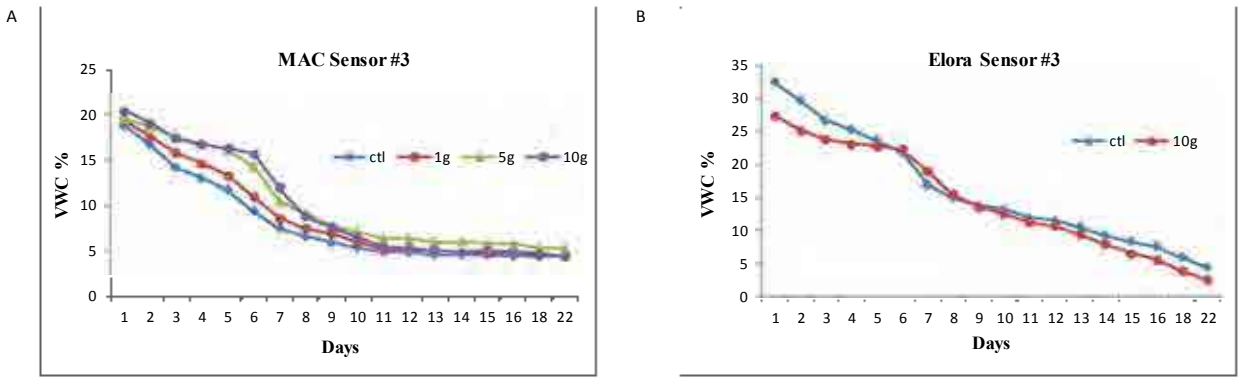



Figure A1.3 Sensor #3 Experiment results. Data points represent the daily mean (three replicates for MAC, two replicates for Elora) volumetric water content percent (VWC%) in the top 5 cm of stony MAC soil (A) and silt-loam Elora soil (B) after saturation and drying 22 days. The treatments were a subsurface zone of 1g, 5g, and 10g saturated Grodan rockwool (GRW-0) and a control without GRW-0. For the Elora soil, the treatment consisted of two controls and two, 10g GRW-0 treatments only.

Table A1. 4 Leaching Experiment mean results per day 2, 5, 10, and 14 for nutrients Al, Fe, K, Mg, P, S, $NH_4^+$ and $NO_3^-$. The column contents included stony soil MAC (Mac) or silt-loam Elora soil with either a 1g or 5g zone of rockwool treated with artificial wastewater (GRW-AWW). The Elora/MAP-GRW0-1g treatment included 82 mg monoammonium phosphate (MAP) fertilizer mixed into the soil above a 1g (saturated, untreated) GRW-0 zone. Peristaltic pumps flowed the artificial rainwater leaching solution at a rate of 1mL $min^{-1}$ for one hour a day for 14 consecutive days.

| | | Mean (3 replicates) mg/l | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trmt | Day | Al | Fe | K | Mg | P | S | NH4 | NO3 | Vol.(L) |
| MAC | 2 | 0.00 | 0.00 | 4.39 | 12.85 | 0.00 | 14.59 | 0.34 | 1.18 | 0.0450 |
| MAC | 5 | 0.00 | 0.07 | 3.36 | 6.66 | 0.00 | 2.67 | 0.38 | 0.63 | 0.0410 |
| MAC | 10 | 0.00 | 0.11 | 2.54 | 6.41 | 0.00 | 1.87 | 0.44 | 0.00 | 0.0430 |
| MAC | 14 | 0.00 | 0.03 | 2.03 | 4.91 | 0.00 | 1.29 | 0.37 | 0.00 | 0.0440 |
| Elora | 2 | 0.00 | 0.17 | 3.97 | 0.00 | 1.75 | 0.00 | 4.08 | 145.57 | 0.0470 |
| Elora | 5 | 0.00 | 0.02 | 3.09 | 22.92 | 0.61 | 14.67 | 2.69 | 8.21 | 0.0420 |
| Elora | 10 | 0.00 | 0.02 | 2.56 | 13.42 | 0.27 | 4.73 | 2.22 | 0.65 | 0.0432 |
| Elora | 14 | 0.00 | 0.00 | 1.00 | 4.32 | 0.12 | 1.46 | 1.73 | 0.07 | 0.0430 |
| GRW-0 | 2 | 0.00 | 0.13 | 32.33 | 1.91 | 0.00 | 17.23 | 0.59 | 0.48 | 0.0480 |
| GRW-0 | 5 | 0.00 | 0.00 | 2.97 | 1.29 | 0.00 | 1.53 | 0.42 | 0.00 | 0.0420 |
| GRW-0 | 10 | 0.00 | 0.00 | 1.34 | 1.00 | 0.00 | 1.06 | 0.42 | 0.00 | 0.0430 |
| GRW-0 | 14 | 0.00 | 0.00 | 0.81 | 0.71 | 0.00 | 0.53 | 0.45 | 0.04 | 0.0470 |
| GRW-AWW | 2 | 0.72 | 0.23 | 28.80 | 2.04 | 3.69 | 13.24 | 4.38 | 4.02 | 0.0496 |
| GRW-AWW | 5 | 0.00 | 0.00 | 5.47 | 1.94 | 0.52 | 2.46 | 1.26 | 40.59 | 0.0458 |
| GRW-AWW | 10 | 0.00 | 0.00 | 1.39 | 1.52 | 0.00 | 1.07 | 0.57 | 0.00 | 0.0466 |
| GRW-AWW | 14 | 0.00 | 0.00 | 0.41 | 0.27 | 0.00 | 0.40 | 0.61 | 0.00 | 0.0470 |
| MAPMac | 2 | 0.00 | 0.04 | 7.19 | 10.89 | 4.61 | 16.14 | 5.36 | 5.49 | 0.0453 |
| MAPMac | 5 | 0.04 | 0.13 | 5.24 | 6.83 | 4.18 | 3.06 | 4.64 | 0.43 | 0.0415 |
| MAPMac | 10 | 0.00 | 0.11 | 2.52 | 4.80 | 2.87 | 1.42 | 2.35 | 0.15 | 0.0443 |
| MAPMac | 14 | 0.00 | 0.07 | 2.44 | 11.40 | 1.73 | 6.23 | 1.26 | 0.97 | 0.0460 |
| MAPElora | 2 | 0.00 | 0.17 | 4.50 | 0.00 | 2.16 | 0.00 | 7.15 | 60.80 | 0.0470 |
| MAPElora | 5 | 0.00 | 0.13 | 3.42 | 22.87 | 0.72 | 14.87 | 3.12 | 4.78 | 0.0415 |
| MAPElora | 10 | 0.00 | 0.11 | 2.86 | 13.13 | 0.49 | 4.98 | 3.35 | 0.63 | 0.0435 |
| MAPElora | 14 | 0.00 | 0.00 | 1.38 | 4.63 | 0.26 | 1.58 | 3.19 | 0.31 | 0.0438 |
| Mac1g GRW-AWW | 2 | 0.00 | 0.00 | 2.63 | 7.34 | 0.00 | 9.26 | 0.42 | 3.40 | 0.0460 |
| Mac1g GRW-AWW | 5 | 0.00 | 0.04 | 3.07 | 6.93 | 0.00 | 2.87 | 0.42 | 0.92 | 0.0380 |
| Mac1g GRW-AWW | 10 | 0.04 | 0.13 | 3.16 | 6.53 | 0.00 | 1.88 | 0.44 | 0.14 | 0.0465 |
| Mac1g GRW-AWW | 14 | 0.00 | 0.03 | 2.39 | 4.87 | 0.00 | 1.33 | 0.48 | 0.01 | 0.0475 |
| Mac5g GRW-AWW | 2 | 0.00 | 0.00 | 5.14 | 7.98 | 0.08 | 16.12 | 0.72 | 7.10 | 0.0440 |
| Mac5g GRW-AWW | 5 | 0.00 | 0.04 | 3.99 | 8.32 | 0.24 | 6.34 | 0.59 | 0.17 | 0.0362 |
| Mac5g GRW-AWW | 10 | 0.00 | 0.07 | 2.72 | 3.96 | 0.00 | 1.23 | 0.65 | 0.07 | 0.0455 |
| Mac5g GRW-AWW | 14 | 0.00 | 0.00 | 3.03 | 4.55 | 0.00 | 1.40 | 0.46 | 0.00 | 0.0463 |
| Elora1g GRW-AWW | 2 | 0.00 | 0.03 | 3.92 | 0.00 | 2.51 | 0.00 | 2.16 | 388.67 | 0.0488 |
| Elora1g GRW-AWW | 5 | 0.00 | 0.02 | 2.49 | 17.93 | 0.83 | 17.55 | 1.97 | 10.13 | 0.0418 |
| Elora1g GRW-AWW | 10 | 0.00 | 0.02 | 1.77 | 7.23 | 0.27 | 3.59 | 2.26 | 0.09 | 0.0471 |
| Elora1g GRW-AWW | 14 | 0.00 | 0.02 | 1.24 | 4.39 | 0.13 | 1.87 | 1.64 | 0.00 | 0.0486 |
| Elora5g GRW-AWW | 2 | 0.00 | 0.03 | 4.32 | 0.00 | 2.15 | 0.00 | 3.30 | 386.67 | 0.0466 |
| Elora5g GRW-AWW | 5 | 0.00 | 0.02 | 2.77 | 21.08 | 0.94 | 21.69 | 2.53 | 73.93 | 0.0370 |
| Elora5g GRW-AWW | 10 | 0.00 | 0.02 | 2.10 | 8.45 | 0.29 | 4.99 | 2.14 | 2.29 | 0.0448 |
| Elora5g GRW-AWW | 14 | 0.00 | 0.02 | 1.75 | 5.87 | 0.18 | 2.85 | 1.76 | 0.59 | 0.0476 |
| EloraMAPGRW-01g | 2 | 0.00 | 0.03 | 3.54 | 0.00 | 2.21 | 0.00 | 1.98 | 476.33 | 0.0463 |
| EloraMAPGRW-01g | 5 | 0.00 | 0.02 | 2.66 | 13.85 | 0.41 | 9.71 | 2.09 | 8.47 | 0.0403 |
| EloraMAPGRW-01g | 10 | 0.00 | 0.02 | 3.69 | 10.95 | 0.21 | 4.01 | 1.47 | 4.03 | 0.0443 |
| EloraMAPGRW-01g | 14 | 0.00 | 0.02 | 1.63 | 5.31 | 0.44 | 1.72 | 2.27 | 34.42 | 0.0463 |

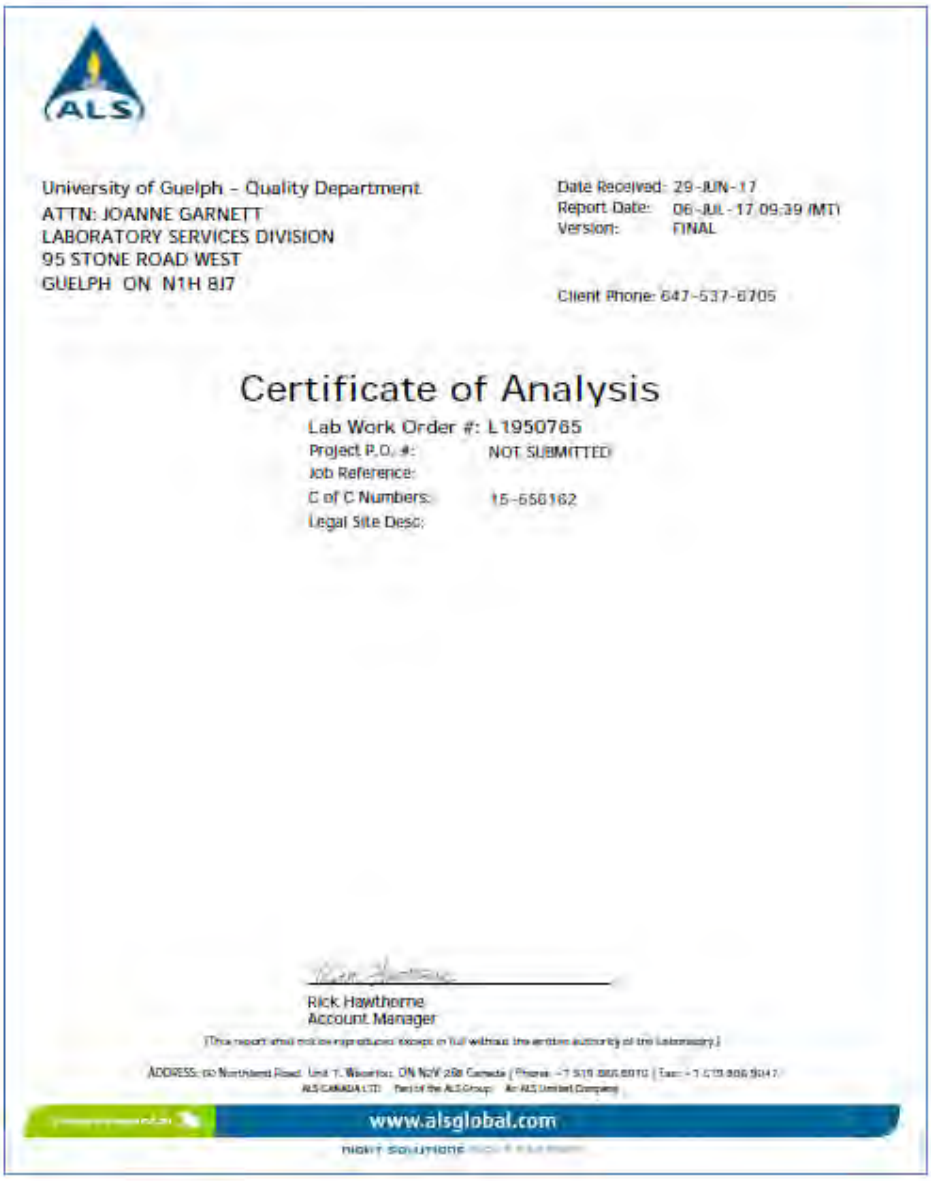
## Appendix 2

Table A2.1 Precipitation totals and temperatures for the Bovey Field Trial from Nov. 1, 2016 to June 15, 2017. The information was obtained from the Argus monitoring system, that includes the Argus Session Manager and the Argus Control Systems software 2014 1.0.0.14, installed at the University of Guelph, Guelph, Ontario. The Argus system develops means from data taken at multiple intervals each day.

(Argus Control System) Precipitation and Temperatures for the 2016 - 2017 Bovey Field Trial

| Date | Precip. (mm) | High - Low Temp. ($^{o}$C) | Mean Temp (86 - 96 intervals) |
|---|---|---|---|
| Nov. 1 - Nov. 29 | 53.76 | 17.5 - -4.4 | 5.6 |
| Nov. 30 - Dec. 30 | 43.58 | 9.7 - -12.6 | -2.5 |
| Dec. 31 - Jan. 30 | 63.4 | 6.2 - -15.9 | -2.8 |
| Jan. 31 - Feb. 28 | 59.14 | 13.0 - -10.3 | -1.5 |
| Mar. 1 - Mar. 30 | 39.33 | 10.3 - -13.0 | -1.6 |
| Mar. 31 - Apr. 29 | 129.88 | 23.0 - -9.0 | 8.3 |
| Apr. 30 - May 30 | 86.03 | 26.6 - 0.9 | 11.2 |
| May 31 - June 15 | 9.61 | 29.1 - 8.7 | 18.6 |

## Appendix 3



University of Guelph - Quality Department
ATTN: JOANNE GARNETT
LABORATORY SERVICES DIVISION
95 STONE ROAD WEST
GUELPH ON N1H 8J7

Date Received: 29-JUN-17
Report Date: 06-JUL-17 09:39 (MT)
Version: FINAL

Client Phone: 647-537-6705

Certificate of Analysis

Lab Work Order #: L1950765
Project P.O. #: NOT SUBMITTED
Job Reference:
C of C Numbers: 15-656162
Legal Site Desc:

Rick Hawthorne
Account Manager

[This report shall not be reproduced except in full without the written authority of the Laboratory.]

ADDRESS: 60 Northland Road, Unit 1, Waterloo, ON N2V 2B8 Canada | Phone: +1 519 886 6910 | Fax: +1 519 886 9047
ALS CANADA LTD Part of the ALS Group An ALS Limited Company

www.alsglobal.com

Figure A3.1 The Certificate of analysis (page 1 of 5) from ALS Global Laboratory in Waterloo, Ontario. Analysis for formaldehyde, phenols, and volatile organic compounds (VOC) was conducted on the Bovey field trial control soil (Bovey #1C) and the soil exposed to Grodan rockwool (Bovey #2G) for seven months. The Bovey soil samples were collected at 15 cm depth from multiple plots, composited, and transported in a cooler with ice packs. Soil samples for the VOC analysis were firmly packed in 250 mL jars with no headspace as directed by ALS. The following pages are the results and the reference information.

| Sample Details/Parameters | Result | Qualifier* | D.L. | Units | Extracted | Analyzed | Batch |
|---|---|---|---|---|---|---|---|
| L1950765-1 BOVEY #1C<br>Sampled By: CLIENT on 22-JUN-17 @ 16:00<br>Matrix: SOIL | | | | | | | |
| **Physical Tests** | | | | | | | |
| % Moisture | 6.59 | | 0.10 | % | 29-JUN-17 | 29-JUN-17 | R3758895 |
| **Aggregate Organics** | | | | | | | |
| Phenols (4AAP) | <0.10 | | 0.10 | mg/kg | 05-JUL-17 | 05-JUL-17 | R3766018 |
| **Volatile Organic Compounds** | | | | | | | |
| Acetone | <0.50 | VOCJ | 0.50 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Benzene | <0.0068 | VOCJ | 0.0068 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Bromodichloromethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Bromoform | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Bromomethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Carbon Disulfide | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Carbon tetrachloride | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Chlorobenzene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Dibromochloromethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Chloroethane | <0.020 | VOCJ | 0.020 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Chloroform | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Chloromethane | <0.020 | VOCJ | 0.020 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,2-Dibromoethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Dibromomethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,2-Dichlorobenzene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,3-Dichlorobenzene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,4-Dichlorobenzene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Dichlorodifluoromethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1-Dichloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,2-Dichloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1-Dichloroethylene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| cis-1,2-Dichloroethylene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| trans-1,2-Dichloroethylene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Dichloromethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,2-Dichloropropane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| cis-1,3-Dichloropropene | <0.030 | VOCJ | 0.030 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| trans-1,3-Dichloropropene | <0.030 | VOCJ | 0.030 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Ethylbenzene | <0.018 | VOCJ | 0.018 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| n-Hexane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 2-Hexanone | <0.50 | VOCJ | 0.50 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Methyl Ethyl Ketone | <0.50 | VOCJ | 0.50 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Methyl Isobutyl Ketone | <0.50 | VOCJ | 0.50 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| MTBE | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Styrene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1,1,2-Tetrachloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1,2,2-Tetrachloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |

Figure A3.2 ALS Global Laboratory analysis (page 2 of 5) of the Bovey field trial control soil (Bovey #1C), and the soil exposed to Grodan rockwool (Bovey #2G) for seven months.

| Sample Details/Parameters | Result | Qualifier* | D.L. | Units | Extracted | Analyzed | Batch |
|---|---|---|---|---|---|---|---|
| L1950765-1 BOVEY #1C<br>Sampled By: CLIENT on 22-JUN-17 @ 16:00<br>Matrix: SOIL | | | | | | | |
| **Volatile Organic Compounds** | | | | | | | |
| Toluene | <0.080 | VOCJ | 0.080 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1,1-Trichloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1,2-Trichloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Trichloroethylene | <0.010 | VOCJ | 0.010 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Trichlorofluoromethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Vinyl chloride | <0.020 | VOCJ | 0.020 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| o-Xylene | <0.020 | VOCJ | 0.020 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| m+p-Xylenes | <0.030 | VOCJ | 0.030 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Xylenes (Total) | <0.050 | | 0.050 | ug/g | | 05-JUL-17 | |
| Surrogate: 4-Bromofluorobenzene | 92.9 | | 70-130 | % | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Surrogate: 1,4-Difluorobenzene | 92.1 | | 70-130 | % | 30-JUN-17 | 05-JUL-17 | R3765517 |
| **Trihalomethanes** | | | | | | | |
| Total THMs | <0.10 | | 0.10 | ug/g | | 05-JUL-17 | |
| **Aldehydes** | | | | | | | |
| Formaldehyde | 0.050 | | 0.040 | ug/g | 30-JUN-17 | 04-JUL-17 | R3764987 |
| L1950765-2 BOVEY #2G<br>Sampled By: CLIENT on 22-JUN-17 @ 16:00<br>Matrix: SOIL | | | | | | | |
| **Physical Tests** | | | | | | | |
| % Moisture | 6.30 | | 0.10 | % | 29-JUN-17 | 29-JUN-17 | R3758895 |
| **Aggregate Organics** | | | | | | | |
| Phenols (4AAP) | <0.10 | | 0.10 | mg/kg | 05-JUL-17 | 05-JUL-17 | R3766018 |
| **Volatile Organic Compounds** | | | | | | | |
| Acetone | <0.50 | VOCJ | 0.50 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Benzene | <0.0068 | VOCJ | 0.0068 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Bromodichloromethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Bromoform | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Bromomethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Carbon Disulfide | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Carbon tetrachloride | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Chlorobenzene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Dibromochloromethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Chloroethane | <0.020 | VOCJ | 0.020 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Chloroform | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Chloromethane | <0.020 | VOCJ | 0.020 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,2-Dibromoethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Dibromomethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,2-Dichlorobenzene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,3-Dichlorobenzene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,4-Dichlorobenzene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |

Figure A3.3 ALS Global Laboratory analysis (page 3 of 5) of the Bovey field trial control soil (Bovey #1C), and the soil exposed to Grodan rockwool (Bovey #2G) for seven months.

| Sample Details/Parameters | Result | Qualifier | D.L. | Units | Extracted | Analyzed | Batch |
|---|---|---|---|---|---|---|---|
| L1950765-2 BOVEY #2G<br>Sampled By: CLIENT on 22-JUN-17 @ 15:00<br>Matrix: SOIL | | | | | | | |
| **Volatile Organic Compounds** | | | | | | | |
| 1,2-Dichloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1-Dichloroethylene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| cis-1,2-Dichloroethylene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| trans-1,2-Dichloroethylene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Dichloromethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,2-Dichloropropane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| cis-1,3-Dichloropropene | <0.030 | VOCJ | 0.030 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| trans-1,3-Dichloropropene | <0.030 | VOCJ | 0.030 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Ethylbenzene | <0.018 | VOCJ | 0.018 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| n-Hexane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 2-Hexanone | <0.50 | VOCJ | 0.50 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Methyl Ethyl Ketone | <0.50 | VOCJ | 0.50 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Methyl Isobutyl Ketone | <0.50 | VOCJ | 0.50 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| MTBE | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Styrene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1,1,2-Tetrachloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1,2,2-Tetrachloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Tetrachloroethylene | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Toluene | <0.080 | VOCJ | 0.080 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1,1-Trichloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| 1,1,2-Trichloroethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Trichloroethylene | <0.010 | VOCJ | 0.010 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Trichlorofluoromethane | <0.050 | VOCJ | 0.050 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Vinyl chloride | <0.020 | VOCJ | 0.020 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| o-Xylene | <0.020 | VOCJ | 0.020 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| m+p-Xylenes | <0.030 | VOCJ | 0.030 | ug/g | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Xylenes (Total) | <0.050 | | 0.050 | ug/g | | 05-JUL-17 | |
| Surrogate: 4-Bromofluorobenzene | 103.2 | | 70-130 | % | 30-JUN-17 | 05-JUL-17 | R3765517 |
| Surrogate: 1,4-Difluorobenzene | 101.2 | | 70-130 | % | 30-JUN-17 | 05-JUL-17 | R3765517 |
| **Trihalomethanes** | | | | | | | |
| Total THMs | <0.10 | | 0.10 | ug/g | | 05-JUL-17 | |
| **Aldehydes** | | | | | | | |
| Formaldehyde | 0.051 | | 0.040 | ug/g | 30-JUN-17 | 04-JUL-17 | R3764987 |

Figure A3.4 ALS Global Laboratory analysis (page 4 of 5) of the Bovey field trial control soil (Bovey #1C), and the soil exposed to Grodan rockwool (Bovey #2G) for seven months.

## Reference Information

**Sample Parameter Qualifier key listed:**

| Qualifier | Description |
|---|---|
| VOCJ | Soil jar was submitted as VOC sample container. VOC results may be biased low, and do not meet federal (CCME) or provincial requirements (for BC, AB-Tier1, MB, ON, SK). |

**Test Method References:**

| ALS Test Code | Matrix | Test Description | Method Reference** |
|---|---|---|---|
| FOR-BUF4-L-ECD-WT | Soil | Formaldehyde in soil - pH4 extrn | EPA 556.1 |
| Soil is extracted with water buffered at pH 4.2. The extracted sample is derivatized with PFBHA by heating in a hotblock. The sample is then extracted with hexane & analyzed by GC-ECD. | | | |
| MOISTURE-WT | Soil | % Moisture | Gravimetric: Oven Dried |
| PHENOLS-4AAP-WT | Soil | Phenol (4AAP) | EPA 9066 |
| A manual method is used to distill the sample. The distillate is then buffered to pH 9.4 and reacts with 4AAP and alkaline ferricyanide to form a red complex which is measured colorimetrically. | | | |
| THM-SUM-CALC-WT | Soil | Total Trihalomethanes (THMs) | CALCULATION |
| Total Trihalomethanes (THMs) represents the sum of bromodichloromethane, bromoform, chlorodibromomethane and chloroform. For the purpose of calculation, results less than the detection limit (DL) are treated as zero. | | | |
| VOC-ROU-HS-WT | Soil | Volatile Organic Compounds | SW846 8260 |
| Soil and sediment samples are extracted in methanol and analyzed by headspace-GC/MS. | | | |
| XYLENES-SUM-CALC-WT | Soil | Sum of Xylene Isomer Concentrations | CALCULATION |
| Total xylenes represents the sum of o-xylene and m&p-xylene. | | | |

** ALS test methods may incorporate modifications from specified reference methods to improve performance.

*The last two letters of the above test code(s) indicate the laboratory that performed analytical analysis for that test. Refer to the list below:*

| Laboratory Definition Code | Laboratory Location |
|---|---|
| WT | ALS ENVIRONMENTAL - WATERLOO, ONTARIO, CANADA |

**Chain of Custody Numbers:**

15-556162

**GLOSSARY OF REPORT TERMS**

*Surrogates are compounds that are similar in behaviour to target analyte(s), but that do not normally occur in environmental samples. For applicable tests, surrogates are added to samples prior to analysis as a check on recovery. In reports that display the D.L. column, laboratory objectives for surrogates are listed there.*
*mg/kg - milligrams per kilogram based on dry weight of sample*
*mg/kg wwt - milligrams per kilogram based on wet weight of sample*
*mg/kg lwt - milligrams per kilogram based on lipid weight of sample*
*mg/L - unit of concentration based on volume, parts per million.*
*< - Less than.*
*D.L. - The reporting limit.*
*N/A - Result not available. Refer to qualifier code and definition for explanation.*

Figure A3.5 Reference information regarding the ALS Global Laboratory procedures and analysis of the Bovey field trial soil for formaldehyde, phenols, and volatile organic compounds (VOC's) (page 5 of 5).

## Appendix 4

Table A4.1 Silicon Shaker Experiment
Analysis of variance for elements Si (A), Al (B), Ca (C), and Fe (D).

**A**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | 4 | 219.64 | 54.91 | 248.74 | <.0001 |
| Error | 10 | 2.21 | 0.22 | | |
| Corrected Total | 14 | 221.84 | | | |

**B**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | 4 | 44.84 | 11.21 | 134.54 | <.0001 |
| Error | 10 | 0.83 | 0.08 | | |
| Corrected Total | 14 | 45.68 | | | |

**C**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | 4 | 208.47 | 52.12 | 547.14 | <.0001 |
| Error | 10 | 0.95 | 0.10 | | |
| Corrected Total | 14 | 209.42 | | | |

**D**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | 4 | 20.37 | 5.09 | 464.40 | <.0001 |
| Error | 10 | 0.11 | 0.01 | | |
| Corrected Total | 14 | 20.48 | | | |

Table A4.2 Silicon Mesh-Lid Experiment.
Analysis of variance for elements Si (A), Al (B), Ca (C), and Fe (D).

**A**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | 3 | 0.32 | 0.11 | 3.99 | 0.0141 |
| Day | 4 | 0.61 | 0.15 | 5.67 | 0.0010 |
| pH*Day | 12 | 0.42 | 0.03 | 1.3 | 0.2543 |
| Error | 40 | 1.07 | 0.03 | | |
| Corrected Total | 59 | 2.42 | | | |

**B**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | 3 | 0.09 | 0.03 | 4.23 | 0.0109 |
| Day | 4 | 0.45 | 0.11 | 15.64 | <.0001 |
| pH*Day | 12 | 0.12 | 0.01 | 1.39 | 0.2104 |
| Error | 40 | 0.29 | 0.01 | | |
| Corrected Total | 59 | 0.95 | | | |

**C**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | 3 | 0.19 | 0.06 | 5.43 | 0.0031 |
| Day | 4 | 1.69 | 0.42 | 35.99 | <.0001 |
| pH*Day | 12 | 0.31 | 0.03 | 2.18 | 0.0323 |
| Error | 40 | 0.47 | 0.01 | | |
| Corrected Total | 59 | 2.66 | | | |

**D**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | 3 | 0.01 | 0.00 | 2.34 | 0.0880 |
| Day | 4 | 0.10 | 0.02 | 13.29 | <.0001 |
| pH*Day | 12 | 0.02 | 0.00 | 1.04 | 0.4302 |
| Error | 40 | 0.07 | 0.00 | | |
| Corrected Total | 59 | 0.20 | | | |

Table A4.3 Leaching Experiment. Analysis of variance for P (A), $NH_4^+$ (B), and $NO_3^-$ (C).

**A**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 10 | 108.32 | 10.83 | 36.88 | <.0001 |
| Day | 3 | 42.15 | 14.05 | 47.85 | <.0001 |
| Trmt*Day | 30 | 36.95 | 1.23 | 4.19 | <.0001 |
| Error | 84 | 24.67 | 0.29 | | |
| Corrected Total | 127 | 212.09 | | | |

**B**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 10 | 176.31 | 17.63 | 82.63 | <.0001 |
| Day | 3 | 36.97 | 12.32 | 57.76 | <.0001 |
| Trmt*Day | 30 | 63.87 | 2.13 | 9.98 | <.0001 |
| Error | 84 | 17.92 | 0.21 | | |
| Corrected Total | 127 | 295.07 | | | |

**C**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 10 | 3.16E+05 | 3.16E+04 | 28.94 | <.0001 |
| Day | 3 | 4.14E+05 | 1.38E+05 | 126.39 | <.0001 |
| Trmt*Day | 30 | 7.58E+05 | 2.53E+04 | 23.14 | <.0001 |
| Error | 84 | 9.17E+04 | 1.09E+03 | | |
| Corrected Total | 127 | 1.58E+06 | | | |

Table A4.4 Sensor Experiments. Analysis of variance for the Volumetric Water Content % (VWC%) for Sensor 1 (A), Sensor 2 (B), Sensor 3 (C), and Rehydration Sensor 1 (D).

**A**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Day | 15 | 1.067 | 0.071 | 106.21 | <.0001 |
| Soil | 1 | 0.194 | 0.194 | 289.04 | <.0001 |
| Soil*Day | 15 | 0.063 | 0.004 | 6.3 | <.0001 |
| Error | 158 | 0.106 | 0.001 | | |
| Corrected Total | 189 | 1.429 | | | |

**B**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Day | 18 | 1.71 | 0.09 | 163.30 | <.0001 |
| Soil | 1 | 0.07 | 0.07 | 122.51 | <.0001 |
| Soil*Day | 18 | 0.19 | 0.01 | 18.35 | <.0001 |
| Error | 266 | 0.15 | 0.00 | | |
| Corrected Total | 303 | 2.12 | | | |

**C**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Day | 17 | 0.95 | 0.06 | 161.71 | <.0001 |
| Soil | 1 | 0.19 | 0.19 | 564.39 | <.0001 |
| Soil*Day | 17 | 0.06 | 0.00 | 10.18 | <.0001 |
| Error | 252 | 0.09 | 0.00 | | |
| Corrected Total | 287 | 1.29 | | | |

**D**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Day | 7 | 0.34 | 0.05 | 80.29 | <.0001 |
| Soil | 1 | 0.34 | 0.34 | 562.53 | <.0001 |
| Soil*Day | 7 | 0.01 | 0.00 | 2.16 | 0.0462 |
| Error | 80 | 0.05 | 0.00 | | |
| Corrected Total | 95 | 0.74 | | | |

Table A4.5 Greenhouse Pot Study. Analysis of variance for perennial ryegrass (*Lolium perenne* var. 'Fiesta 4') tissue analysis for P (A), TN (B), leaf length (C) and soil analysis for P (D), and TN (E).

**A**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 1 | 2.20E-08 | 2.20E-08 | 1.54 | 0.2232 |
| Soil | 1 | 2.69E-07 | 2.69E-07 | 18.84 | 0.0001 |
| Trmt*Soil | 1 | 9.92E-08 | 9.92E-08 | 6.96 | 0.0128 |
| Error | 32 | 4.56E-07 | 1.43E-08 | | |
| Corrected Total | 35 | 8.46E-07 | | | |

**B**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 1 | 8.40E-07 | 8.40E-07 | 0.29 | 0.5961 |
| Soil | 1 | 8.47E-03 | 8.47E-03 | 2888.76 | <.0001 |
| Trmt*Soil | 1 | 1.26E-05 | 1.26E-05 | 4.3 | 0.0463 |
| Error | 32 | 9.38E-05 | 2.93E-06 | | |
| Corrected Total | 35 | 8.57E-03 | | | |

**C**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 1 | 3.80 | 3.80 | 5.65 | 0.0209 |
| Soil | 1 | 528.66 | 528.66 | 785.58 | <.0001 |
| Trmt*Soil | 1 | 3.50 | 3.50 | 5.21 | 0.0263 |
| Error | 56 | 37.69 | 0.67 | | |
| Corrected Total | 59 | 573.65 | | | |

**D**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 1 | 0.41 | 0.41 | 0.48 | 0.4956 |
| Soil | 1 | 524.79 | 524.79 | 605.7 | <.0001 |
| Trmt*Soil | 1 | 7.25 | 7.25 | 8.36 | 0.0068 |
| Error | 32 | 27.73 | 0.87 | | |
| Corrected Total | 35 | 560.17 | | | |

**E**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 1 | 2.250E-08 | 2.250E-08 | 0.03 | 0.8696 |
| Soil | 1 | 2.791E-05 | 2.791E-05 | 50244.5 | <.0001 |
| Trmt*Soil | 1 | 2.000E-08 | 2.000E-08 | 40.5 | <.0001 |
| Error | 32 | 2.000E-08 | 0.000E+00 | | |
| Corrected Total | 35 | 2.798E-05 | | | |

Table A4.6 Bovey Field Trial. Analysis of variance for perennial ryegrass (*Lolium perenne* var. 'Karma') tissue analysis for P (A), TN (B), and soil analysis for P (C) and TN (D).

**A**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 2 | 7.45E-08 | 3.72E-08 | 0.93 | 0.4445 |
| Block | 3 | 1.09E-07 | 3.64E-08 | 0.91 | 0.4899 |
| Error | 6 | 2.40E-07 | 4.00E-08 | | |
| Corrected Total | 11 | 4.24E-07 | | | |

B

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 2 | 2.10E-07 | 1.10E-07 | 0.04 | 0.9595 |
| Block | 3 | 1.09E-05 | 3.63E-06 | 1.43 | 0.3247 |
| Error | 6 | 1.53E-05 | 2.54E-06 | | |
| Corrected Total | 11 | 2.64E-05 | | | |

**C**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 2 | 0.905 | 0.4525 | 0.31 | 0.7438 |
| Block | 3 | 1.11 | 0.37 | 0.25 | 0.8563 |
| Error | 6 | 8.73 | 1.45 | | |
| Corrected Total | 11 | 10.74 | | | |

**D**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 2 | 2.10E-07 | 1.10E-07 | 0.04 | 0.9595 |
| Block | 3 | 1.09E-05 | 3.63E-06 | 1.43 | 0.3247 |
| Error | 6 | 1.53E-05 | 2.54E-06 | | |
| Corrected Total | 11 | 2.64E-05 | | | |

Table A4.7 Solvita CO2_C Burst Test. Analysis of variance for Sensor 1and Rehydration (A), Bovey Greenhouse Experiment (B), and Bovey Field Trial (C).

**A**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 3 | 30.71 | 10.24 | 0.57 | 0.6395 |
| Day | 2 | 357.70 | 178.85 | 10.01 | 0.0010 |
| Soil | 1 | 9830.06 | 9830.06 | 549.98 | <.0001 |
| Trmt*Soil | 3 | 33.07 | 11.02 | 0.62 | 0.6122 |
| Trmt*Day | 6 | 0.00 | 0.00 | 0.00 | 1.0000 |
| Error | 20 | 357.47 | 17.87 | | |
| Corrected Total | 35 | 10580.08 | | | |

**B**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 1 | 8.14 | 8.14 | 6.48 | 0.0159 |
| Soil | 1 | 3049.62 | 3049.62 | 2426.74 | <.0001 |
| Trmt*Soil | 1 | 6.95 | 6.95 | 5.53 | 0.0250 |
| Error | 32 | 40.21 | 1.26 | | |
| Corrected Total | 35 | 3104.92 | | | |

**C**

| Source | df | SS | MS | F Value | Pr > F |
|---|---|---|---|---|---|
| Trmt | 2 | 64.59 | 32.30 | 0.3 | 0.7429 |
| Block | 3 | 362.49 | 120.83 | 1.14 | 0.3724 |
| Trmt*Block | 6 | 280.52 | 46.75 | 0.44 | 0.8378 |
| Error | 12 | 1271.92 | 105.99 | | |
| Corrected Total | 23 | 1979.52 | | | |