Evaluation of Biosolids for Use in Biodegradable Transplant Containers

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#### ABSTRACT

Sustainability practices are leading to the development and use of alternative products in the floriculture and wastewater industries, such as the use of biodegradable containers instead of plastic containers. The objective of this research was to evaluate the efficacy of using digested biosolids from a regional wastewater treatment plant as an ingredient in creating a biodegradable transplant biocontainer. The biosolids were tested for metals limits as specified by the U.S. EPA Part 503 Rule, and met the requirements for Class B. Multiple mixes of biosolids, fibers, starch, polymer, and natural glue were developed to provide overall pot stability and structural strength. Engineering tests, such as tensile strength, pH, and saturated paste tests, were conducted on the different mixes to determine the optimum strength that could be produced.

The top-performing biosolids mixes were used to make 10.2 cm (four-inch) pots that were compared in various ways to the market leaders, Peat Pots and standard plastic pots. A two-part mold was created on a 3D printer, which would allow for positive pressure to be used in forming the BioPots. Mixes were transferred to the lower half of the mold, the upper part was then plunged and fastened into the lower half, and then the mold with its mix was placed in an oven to dry. Laboratory germination bioassays were performed to test for the presence of phytotoxic compounds. Construction of BioPots for the lab-scale studies was tedious. Different methods (e.g., negative pressure systems) need to be investigated for use in producing the BioPots commercially.

Most of the BioPots survived the resiliency study. Leachate quality from the biocontainers was no worse than from the plastic containers. Some discoloration was observed on the biocontainers, but it was not due to algal/fungal growth. Growth of soybeans, marigolds, and romaine in the biocontainers was significantly better (e.g., increased height, leaf sizes, and weight) than in the plastic containers.

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#### ABSTRACT (public)

The Western Virginia Water Authority serves the City of Roanoke, and Counties of Roanoke, Franklin and Botetourt. Approximately 141 million liters per day (37 million gallons per day) of wastewater from the service area is treated at the Roanoke Regional Water Pollution Control Plant (RRWPCP). Solids are anaerobically digested and lagooned prior to agricultural land application; biogas is stored and used to generate electricity. After about nine months in the lagoon, 9.07 million dry kilograms (10,000 dry tons) of biosolids are land applied locally each year. Solids management costs are a significant part of the RRWPCP's operating budget. In an effort to decrease costs and increase sustainability, there has been growing interest in resource recovery by producing a high-quality nutrient product that can be beneficially used. In January 2014, research to develop a high-quality biosolids product for beneficial use was initiated by Virginia Tech, in collaboration with the RRWPCP. The drivers, research goals, methodology, and results from that research will be presented.

The general public is familiar with several commercially available biocontainer products, such as Peat Pots and CowPots<sup>TM</sup>. They are used in nurseries, greenhouses, and households, and minimize plastic waste while also contributing organic material for healthy plant growth. The WPCP was intrigued with developing a biosolids product that could be marketed and used like the Peat Pots.

The objective of this research was to evaluate the efficacy of using digested biosolids from Roanoke WPCP as an ingredient in creating a biodegradable transplant pot. The biosolids were tested for and met the metals and contaminants limits as required by the U.S. EPA Part 503 Biosolids Rule. In addition to the biosolids, other fibrous materials, such as used cardboard or cellulose, were used to stabilize and add structural strength. Multiple blends, or mixes, were developed, each varying in biosolids and fiber content on a dry weight basis, as well as different additives such as starch, polymer, or a natural glue. Tensile and puncture tests were conducted on the different mixes to determine the optimum strength that could be produced.

The top performing mixes were used to create four-inch pots, for comparison to market leader, Peat Pots, and standard plastic pots. Greenhouse studies were conducted in two phases:

- Phase 1 analysis of leachate and assessment of pot stability through watering cycles.
- Phase 2 growth studies for soybeans, marigolds, and romaine. These plants were selected based on growth ability and/or sensitivity.

The RRWPCP does not currently produce Class A biosolids, but by producing biodegradable transplant pots, they hope to produce a high-value, sustainable product that meets Class A requirements and diversifies their current biosolids management program.

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## Chapter 1: Introduction

The Western Virginia Water Authority serves the City of Roanoke, and Counties of Roanoke, Franklin and Botetourt. Approximately 140 million liters per day (37 million gallons per day) of wastewater from the service area is treated at the Roanoke Regional Water Pollution Control Plant (RRWPCP). Solids are anaerobically digested and lagooned prior to agricultural land application; biogas is stored and used to generate electricity. Solids management costs are a significant part of the RRWPCP's operating budget. In an effort to decrease costs and increase sustainability, there has been growing interest in resource recovery by producing a high-quality nutrient product that can be beneficially used.

The general public is familiar with several commercially available biocontainer products, such as Peat Pots and CowPots<sup>TM</sup>. Plants potted in these biocontainers do not need to be transplanted, because they biodegrade over time. They are used in nurseries, greenhouses, and households, and minimize plastic waste while also contributing organic material for healthy plant growth. The RRWPCP was intrigued with developing a biosolids product that could be marketed and used like the Peat Pots, and decided to support research to further evaluate the development of biocontainers using biosolids.

The objective of the research was to evaluate the efficacy of using digested biosolids from RRWPCP as an ingredient in creating a biodegradable transplant pot. The biosolids were tested for and met the metals and contaminants limits as required by the U.S. EPA Part 503 Biosolids Rule. In addition to the biosolids, other fibrous materials, such as used cardboard or cellulose, were used to stabilize and add structural strength. Multiple blends, or mixes, were developed, each varying in biosolids and fiber content on a dry weight basis, as well as different additives such as starch, polymer, or a natural glue. Various engineering tests, such as tensile, pH and saturated paste tests, and puncture tests, were conducted on the different mixes to determine the optimum strength that could be produced.

The top-performing mixes were used to create four-inch pots, for comparison to market leader, Peat Pots, and standard plastic pots. Using these optimal blends, greenhouse studies were conducted in two phases:

- Phase 1 analyzed leachate and determined the longevity of the pot through watering cycles.
- Phase 2 included growth studies for soybeans, marigolds, and romaine lettuce. These plants were selected based on growth ability and sensitivity to plant growth variables.

This research is described and organized as follows:

Background and literature review pertaining to the management and regulation of biosolids in the U.S., demographics and the treatment processes employed at the

RRWPCP, and market drivers associated with the demand and use of biodegradable planters (Chapter 2).

- Background, materials and methods, and results of engineering and producing biodegradable planters from biosolids (Chapter 3).
- Background, materials and methods, and evaluation of greenhouse studies using the biosolids-derived biodegradable planters (Chapter 4).
- Conclusions and recommendations for further research (Chapter 5).

## **Chapter 2: Literature Review**

#### **Overview**

Since passage of the Clean Water Act in 1972, progress has been made in the United States toward achieving higher levels of wastewater treatment. Water utilities are striving to become "water resource recovery facilities" (WRRFs) in an effort to recover numerous resources, such as water, energy, and nutrients, in a sustainable manner. Sustainability is driving the water sector to take on the challenge of transforming wastewater treatment from an energyconsuming and waste-producing activity to one with positive, net-energy production and beneficial use of biosolids. The development, distribution and marketing of high-quality biosolids products are of particular interest to provide a renewable resource that is environmentally safe and reduces the financial burden on water utilities and their customers.

Consumers are willing to pay a premium price for products that utilize waste or recycled materials in substitute of conventional petroleum based plastics (Yue et al., 2010). A particular area of interest in becoming more environmental sustainable is the greenhouse and nursery industry. Growing plants is perceived as a green and eco-friendly process, but there are several ways that growers can become even more sustainable. Many greenhouses and nurseries have shown that embracing sustainable practices is an increasing trend and market driver in their industry (Dennis et al., 2010). There are ways that a grower can be more ecofriendly, but consumers are most interested in plants grown in biodegradable, compostable, and recycled containers (Yue et al., 2011). Public interest has spurred companies to create green and sustainable products that have the ability to replace their plastic counterparts. Biocontainers are often derived from waste or recycled products, or renewable raw materials. The alternatives are usually classified as biodegradable or compostable. By minimizing waste and utilizing other waste materials, these containers can be carbon negative (Yue et al., 2010).

According to the U.S. Environmental Protection Agency's (US EPA) 2012 Clean Watershed Needs Survey, there are 14,691 facilities that provide secondary or more advanced treatment, and the number is projected to increase to 15,242 facilities by 2032. Approximately 129 billion liters (34 billion gallons) of wastewater are treated each day in the U.S. Increasing treatment requirements to meet stringent effluent limits results in increasing amounts of residuals, or biosolids. Biosolids are the nutrient-rich organic materials resulting from the treatment of sewage sludge; the regulatory term for the solid, semisolid or liquid untreated residue generated during the treatment of domestic sewage in a treatment facility. When properly treated and processed, sewage sludge becomes biosolids which can be safely recycled and applied as a fertilizer and organic soil amendment to sustainably improve and maintain productive soils and stimulate plant growth. Only biosolids that meet the most stringent standards specified in federal and state rules can be approved for use as a fertilizer or soil amendment product.

Many utilities are looking for ways to beneficially use biosolids; some utilities treat their biosolids to high enough standards that they can land apply their solids under specified loading rate, public access and use restrictions. Some utilities are interested in producing higher quality biosolids materials that can offset their processing costs and produce potential revenue from the sale of the biosolids-derived products. The RRWPCP commissioned Virginia Tech to evaluate the viability of using anaerobically digested, stabilized biosolids to create biodegradable flower pots. This literature review outlines the requirements for biosolids to be used, explores the issues with conventional agricultural plastics, and analyzes biocontainers that are already on the market.

## **Biosolids Management and Regulations**

Biosolids and biosolids-derived products are governed under federal and parallel state regulations. This section addresses these regulations and the potential impacts on the development and marketing of bio-planters. Emphasis is given to the regulations applicable to biosolids products that can be distributed and marketed to the public.

Federal regulations governing the use and disposal of municipal wastewater solids include: 40 CFR 503 Standards for the Use or Disposal of Sewage Sludge, 40 CFR 257 Criteria for Classification of Solid Waste Disposal Facilities and Practices, and 40 CFR 258.2 Criteria for Municipal Solid Waste Landfills. Solids that are placed in a Municipal Solid Waste (MSW) landfill are not subject to the Part 503 regulations, but must still meet the requirements of both 40 CFR 257 and 40 CFR 258.2.

The Part 503 regulations were promulgated in 1993 and set forth standards for the following use and disposal options: beneficial use through land application, distribution and marketing; disposal at dedicated sites or in sludge-only landfills; and incineration in sludge-only incinerators. The Part 503 regulations were based on a comprehensive risk assessment of pollutant pathways, which resulted in limits for heavy metal concentration related to all biosolids practices. Biosolids management practices are also stipulated to limit exposure and ensure biosolids are utilized in a way that is protective of human health and the environment.

The Part 503 regulations specify requirements in the following three categories for solids applied to land: pollutant limits, pathogen reduction requirements, and Vector Attraction Reduction (VAR) requirements.

#### **Pollutant Limits**

The Part 503 regulations have established "Pollutant Limits" for nine (9) metals as shown in Table 2-1.



#### **Table 2-1. 40 CFR §503.13 Pollutant Limits**

 $mg =$  milligrams

(1) Dry Weight basis (3) For sludge applied to a lawn or home garden  $kg =$  kilograms

(2) For sludge applied to land (4) For sludge sold or given away in a bag

#### Pathogen Reduction

The Part 503 regulations clearly specify two classifications, depending upon the quality of biosolids and the level of pathogen reduction achieved, Class A or Class B. Class A pathogen reduction requirements reflect a Process to Further Reduce Pathogens (PFRP) standard, while Class B requirements reflect a Process to Significantly Reduce Pathogens (PSRP) standard. Class A pathogen treatment reduces biosolids pathogen levels to minimal detection, while Class B biosolids may have higher pathogen levels and must be managed in accordance with specific practices that ultimately provide the same level of protection as Class A treatment. Typically, Class A treated biosolids can be distributed in a bag or container and used on lawns and home gardens similar to commercial fertilizers; whereas, Class B biosolids may only be applied to agricultural or forest sites with buffer zones, limited public access, and harvesting restrictions.

A summary of the Class A and Class B pathogen treatment options and treatment technologies are described in Section 503.32 of the federal rule. They range from demonstrating pathogen and VAR through various monitoring and analytical techniques to a list of accepted PRFP and PSRP treatment options. For example, conventional anaerobic digestion is a PSRP resulting in Class B biosolids; composting and heat drying are considered PFRPs that result in Class A biosolids. 

In 1985, the EPA created the Pathogen Equivalency Committee (PEC) which is a federally sponsored technical group that provides technical assistance and recommendations on process equivalencies for pathogen reduction in sewage sludge to government and industry.

The PEC reviews and makes recommendations to relevant federal and/or state permitting authorities on the merits of applications proposing new innovative or alternative sewage sludge pathogen reduction processes are equivalent to the processes currently listed in the 40 CFR Part 503, Subpart D, §503.32. The PEC process may be necessary for some enhanced digestion processes and/or newer pathogen reduction technologies. The EPA website (http://www.epa.gov/biosolids/pathogen-equivalency-committee-documents) provides guidance from the PEC for demonstrating the effectiveness of innovative and alternative sewage sludge pathogen disinfection processes for the purposes of receiving a recommendation of PSRP or PFRP equivalency.

## Vector Attraction Reduction

All biosolids products must meet one of the ten VAR options when used for land application or options 1 through 8 when applied to a lawn or home garden. The VAR options are listed in Table  $2-2$ .





The term "Exceptional Quality" (EQ) has been defined for biosolids that meet the most stringent requirements for all three parameters (pathogen reduction, VAR and pollutant limits). EQ biosolids are those that meet a Class A - Process to Further Reduce Pathogens (PFRP) pathogen reduction process, options 1-8 of the VAR requirements, and metal limits under EPA §503.13 Table 3 Pollutant Concentrations. EQ biosolids are exempt from additional

management practice requirements and may be used freely as soil amendments and/or fertilizers in allowed by the local regulatory agency.

Past research has tended to focus on Class B biosolids land application for agricultural use with very little done to help identify and develop industry standards/specifications for high quality biosolids (Class A or even better). "High standard" biosolids have been relatively well-received in a niche marketplace; however, there are currently no established standards or guidance available to the generator (WRRF) or to the end-user in such circumstances.

As utilities prepare for the future, better guidance on manufacturing high quality biosolids that are suitable for specific purposes and markets is needed. The Water Environment & Reuse Foundation (WE&RF) is currently funding research to develop guidelines for the manufacture and marketing of high-quality biosolids products (NTRY7R15, 2016). The use of biosolids to produce bio-planters for commercial use is a novel, sustainable way to create an environmentally conscious brand for local wastewater treatment facilities and to potentially reduce their biosolids management costs.

## **Roanoke Regional Water Pollution Control Plant**

The RRWPCP receives wastewater from the City of Roanoke, Roanoke County, Franklin County, and Botetourt County. It has a maximum operating capacity of 140 million liters per day (37 million gallons per day). The RRWPCP anaerobically digests their solids and then lagoons them for about nine months. The biosolids are currently rated for Class B land application. The plant land applies about 9.07 million dry kilograms (10,000 dry tons) per year locally. The rest of the solids that are landfilled, which is a significant cost to the utility and is not considered a sustainable practice. The RRWPCP would like to beneficially use more its biosolids and minimize landfill disposal and costs. Figure 2-1, shown below, is a plan view of the treatment plant.



**Figure 2-1. Roanoke Regional Water Pollution Control Plant** 

## **Plastic Containers**

For decades, plastic containers have been the main vessel of choice for greenhouse and nursery growers. Plastic containers were widely adopted by the agriculture industry during the 1950s to phase out more the expensive and fragile terracotta containers (Yue et al., 2010). Plastic containers are favorable for several reasons, including durability, weight, and price. The ability to find plastic containers in virtually any size and shape has allowed for their use in all phases of plant production. However, the fate and disposal of horticultural plastic has proven to be a serious and substantial issue. Botts (2007) reported that the production of nursery pots, flats, and cell packs used approximately 145.2 million kilograms (320 million pounds) of plastic a year. Because of the convenience of plastic containers and trays, plants can be grown year-round and transported easily.

Across all industries, plastic waste has proven to be a significant issue. In 2012, of the 227.8 billion kilograms (251 million tons) of municipal solid waste produced, plastic products comprised 13% of the waste stream (EPA, 2012). Of the 29.9 billion kilograms (33 million tons) of plastic waste generated in 2013, only 9% was recycled. Recycling can be very beneficial to reuse materials such as glass, paper, plastics, and metals to create new products. However, recycling can be challenging for agricultural plastics.

Many recycling facilities require that plastics are presorted by the different types. The Society of the Plastic Industry (SPI) has created a code to streamline identification and sorting of plastics. Even with a helpful code, manually presorting containers can be a long and arduous process. An SPI code is not a guarantee that a recycler will accept the product. Recycling facilities may also deny a product because of contamination. Contamination can occur from excess growing media or moisture still adhered to the plastic container, UV light degradation, or pesticide residue. The container loses its flexibility and the quality decreases from prolonged exposure to heat and UV light. Though the potential effects of pesticide residue on recycled plastics have not been identified, negative public perception is the biggest concern for recyclers. Some nursery growers are concerned about the potential spread of disease from reusing pots and that sanitation practices are not adequate (Yue et al., 2010). Because virgin plastic is often cheap, recyclers are less likely to need recycled plastics. With little to gain from recycling, it is no wonder than many producers resort to just burning their plastic on the farm or production facility. Garthe (2016) reported in a survey conducted by a Penn State Cooperative Extension that up to 60% of farmers resort to burning their used plastic themselves to save on recycling or landfill costs. (Garthe, 2016)

Biocontainers can be good alternatives to conventional plastic, but it is important to understand the needs unique to each situation in choosing the most suitable type (White, 2009). 

## **Biocontainers**

Biocontainers can be found in an almost endless variety of waste, recycled, or renewable raw materials. Manufacturers and growers are noting that consumers are beginning to prefer 'green' products and recognize the need for more sustainable products. Though biocontainers can be found in a variety of materials, such as rice hull, peat, paper, coconut fiber, and more, they can all be basically sorted by being either plantable or compostable.

#### Plantable Biocontainers

Plantable containers, also referred to as biodegradable, allow the user to plant together both the container and the plant. This can be advantageous for greenhouses and nurseries to begin growing their plants in a controlled environment, and then move the plant with container to the ground. This practice reduces transplant shock, saves transplant time and cost, and avoids disposal of the container (Nambuthiri et al., 2013). In order to be plantable, the container, once in the ground, must break down quickly to allow the root to grow into the surrounding soil. There are many factors that affect the rate of biodegradation, such as material type, nitrogen, moisture, temperature, pH, and microbial community (Nambuthiri et al., 2013).

Currently, a number of products on the market are advertises as being plantable. CowPots <sup>™</sup> (CowPots Co., Brodheadsville, PA), utilize composted and compressed cow manure with an added binding agent. Peat Pots (Jiffy Products; Kristaiansand, Norway) are widely regarded as the standard and market leader in the biodegradable category and are made with a

combination of peat and waste wood pulp or paper. Paper containers (Western Pump Products; Corvallis, OR) are made of recycled and post-consumer paper and have various lifespans to suit the need of the grower. Rice Straw containers (Ivy Acres Inc.; Baiting Hollow, NY) contain 80% rice straw, 20% coconut fiber, and proprietary natural adhesive as a binding agent. Wood fiber containers (Fertil International; Boulogne, France) are comprised of 80% cedar fiber, 20% peat, and lime to promote healthy plant growth. Coconut fiber containers (ITML Horticultural Products; Brantford, Ontario, Canada) are made with varying size coconut fibers and a binding agent. These are only a few of the numerous plantable containers on the market (Beeks and Evans, 2013). 

#### Compostable Biocontainers

Contrary to plantable containers, compostable containers are not meant to be planted with the plant. Compostable containers should not be planted in the soil with the plant because the containers degrade too slowly and are too strong to allow the plants roots system to penetrate the container walls (Beeks and Evans, 2013). Compostable containers should be separated before final planting, broken apart, and composted for proper disposal (Evans et al., 2010). Like plantable containers, they do break down under the influence of naturally occurring microorganisms; however, the difference is that compostable containers must eventually break down into carbon dioxide, water, and non-toxic biomass. Another caveat about being labeled compostable is that the container must degrade at the same rate as paper (White, 2009).

Many of the compostable containers on the market are derived from renewable raw materials. Ricehull containers (Summit Plastic Co.; Tallmadge, OH) are produced from ground rice hulls with an added binding agent. However, the vast majority of containers in this category are some form of bioplastic. An example of a bioplastic container is the OP47 (Summit Plastic Co.; Tallmadge, OH). These containers use a bioplastic that has been derived from polylactic acid or wheat starch (Beeks and Evans, 2013). Because of the wide array of container materials, some may need to be industrially composted to increase degradation rate. They may be too tough to break down under normal composting conditions because of inconsistent temperature, moisture, or pH (Nambuthiri et al., 2013). ASTM has set standards for materials testing for industrially composted plastics in the United States. In order to meet that certification, the containers must degrade by 60% in 90 days at a temperature of at least 60 °C (ASTM D6400).

#### Testing

In order for biodegradable pots to be marketable and widely accepted, they must be capable of growing a plant competitive to one grown in a plastic container. There are several different tests and metrics to grade alternatives to determine if the biocontainers will perform at the same level, or nearly the same level, as plastic containers. Most research on biocontainers focuses on strength of containers, plant growth, water usage, and algal coverage on the walls (Beeks and Evans, 2013).

## *Durability*

Possibly the biggest disadvantage of biocontainers is that they may not be as strong as their plastic counterparts, especially when wet. However, this is not necessarily a disadvantage because they are not meant to be used multiple times and need to be weak enough to either degrade in the soil with the plant or be composted. At a minimum, biocontainers need to be strong enough for handling, packing, shipping, and to support the plant and associated growing media (Beeks and Evans, 2013).

#### Compatibility with Greenhouse Production

Research has shown that getting biocontainers wet significantly inhibits their structural integrity and can thus make them predisposed to tearing or breaking during greenhouse production, packaging, shipping, or even hinder their marketability in retail (Nambuthiri et al., 2013). For its price, plastic is strong, versatile, and lightweight, which enables plastic containers to be compatible with mechanized production and ideal for shipping (Evans and Hensley, 2004). Because plastic has been so reliable, growers have been hesitant to experiment with new alternatives, though many agree that consumers will want sustainable containers. About a quarter of growers surveyed have either used biocontainers or would like to implement them (Koeser et al., 2013). This statistic is fairly low because of the perceived limitations of biocontainers that could sacrifice profitability for the grower.

Biocontainers would be more widely accepted by the nursery and greenhouse industry if they were demonstrated to be durable enough and compatible with a mechanized production process. The current biocontainers on the market have not been thoroughly researched to determine their level of compatibility with a mechanized process for a high output of plants for greenhouse production (Koeser et al., 2013).

Koeser et al. (2013) set out to evaluate the impacts on system efficiency if a biocontainer is used in a mechanized production process. The researchers conducted two successive experiments:  $(1)$  Putting the biocontainers through a mechanical production process, and  $(2)$ evaluating the effects of shipping on the biocontainers. In the first experiment, a plastic pot (control) and seven biocontainers were put through mechanical filling and spacing experiments at a wholesale commercial greenhouse facility. Using a randomized block design, all the containers were sent through a gravity-fed, pot filling machine. Koeser et al. (2013) recorded the number of pots damaged by the machinery, the number of unfilled pots, and the total elapsed time for filling. For the shipping experiment, Koeser et al. (2013) filled the pots with soilless media and watered the pots prior to being loaded into a box truck that was transported 200 km. Upon arrival, the pots were inspected for fraying, tears, gashes, creasing, crushed areas, and various other damages.

Koeser et al. (2013) found the proportion of unfilled containers did not vary by type or trial, but the proportion of damaged containers did vary among the containers tested. When compared to the plastic control pot, coir, pressed manure, paper, and peat pots were more likely to be damaged by the filling machine. However, despite the difference among containers, none of

the containers experienced more than 1.5% damage. Koeser et al. (2013) speculate that this could be attributed to the workers' lack of familiarity with using biocontainers.

The results from the shipping experiment showed that the proportion of pots damaged varied by container type. The differences among container types was attributed to the damage experienced by the pressed manure (27% of pots damaged), and peat pots (35% of pots damaged). Biocontainers require extra care when being handled and transported, especially after being in production. Koeser et al. concluded that biocontainers were generally compatible with a mechanical filling process, but require extra care during transport to ensure a sellable product. 

## **Strength Simulations**

Several investigators have attempted to quantify the strength of biocontainers for a comparison of various brands and materials (Koeser et al., 2013; Evans and Karcher, 2004; Beeks and Evan, 2013; Evans et al., 2010). Strength is often tested under both dry and wet conditions, vertically and laterally. Puncture resistance is often evaluated, as well.

Evans et al. (2010), Evans and Karcher (2004), and Koeser et al. (2013) performed experiments that involved measuring the force needed to crush biocontainers. Koeser et al. (2013) crushed the containers as they were standing up. Evans et al. and Evans and Karcher crushed the pots vertically, just as Koeser et al. did, and with the container laying on its side with the weight placed on the bottom edge of the container.

Koeser et al. (2013) compared seven different biocontainers to a plastic pot control. The researchers sought to understand the effects that different irrigation techniques have on the biocontainers. In their experiment, they watered plants in different biocontainer by hand, drip tubing, or ebb-and-flood irrigation. A random selection of pots representing each container type and irrigation method was chosen for crush and puncture strength tests. Koeser et al. found that container type and irrigation method were both significant in crush tests. The plastic and bioplastic container strengths did not vary significantly between the different irrigation methods. For the other biocontainers, differences did occur across irrigation methods. The other biocontainers had higher crush loads when they were hand watered or tube irrigated than ebb-and-flood irrigation. Koeser et al. also tested the puncture resistance of the biocontainers, but did not elaborate on the methods used to evaluate puncture strength. They found that puncture strength did vary significantly across biocontainers and with irrigation method. As with crush loads, ebb-and-flood irrigation resulted in lower puncture resistance than hand watering and drip irrigation. Overall, Koeser et al. found that biocontainers performed better and were stronger than the bioplastic and plastic controls. It should be noted that the researchers used a thermoformed plastic container, and speculated that a direct injected plastic container would have been much stronger. (Koeser et al., 2013)

Evans and Karcher (2004) also conducted crush strength and puncture resistance tests. In this experiment, pots standing vertically and also on their sides were crushed with a texture analyzer. To test puncture resistance, a 0.5 cm ball probe was used. The different pots were

tested dry and wet, after being saturated for 7 days. Evans and Karcher found that peat pots and a feather container, developed in cooperation with Tyson Foods, were able to withstand greater crush strengths than the plastic control. However, the plastic container had a much higher puncture strength. The plastic container's strength was little changed after being saturated for 7 days. The peat and feather pots experienced a significant decrease in strength when wet, and the feather containers supported a slightly greater load than peat containers. (Evans and Karcher, 2004)

In another study conducted by Evans et al. (2010), crush strength and puncture resistance were tested again, but with additional biocontainers. New, unused containers were again used for dry strength testing, but for wet strength, containers were filled with substrate and watered daily in a greenhouse for four weeks. The crush strength was conducted in the same way, by measuring the pressure need to crush the pots vertically and on their sides. In the puncture test, a 0.5 cm ball probe at a speed of 10 mm/s was used. Rice hull containers exhibited the highest crush strength of all the containers, followed by paper, plastic, peat, cowpot, Fertil, coconut fiber, and OP47 bioplastic pots in decreasing order. Plastic and paper containers kept their strength better when wet, but rice hull was again of the stronger biodegradable containers. Wet strength decreased for all containers that were able to absorb water. The plastic containers had the greatest dry and wet punch strengths. The researchers speculated that biocontainers should have a minimum of 2 kg wet punch and crush strength in order to withstand normal handling in production. In this study, Fertil, peat, and Cowpot did not meet this recommended minimum strength and could therefore result in problems during greenhouse production. (Evans et al., 2010)

Crush strength is an important characteristic to know about a biocontainer, but the majority of the forces that a pot will undergo in a greenhouse is perhaps quite the opposite. Handling during greenhouse production often involves the need to pick up a pot on the lip with one hand, resulting is a pulling or tensile force. Crushing forces are more likely to be experienced during shipping when pots are stacked on top of each other. In order to develop a more representative test to quantify strength, Beeks and Evans created a device that suspended the container 12 cm above a catch basin. The pots are then gradually loaded with 4.5 mm diameter steel balls, each weighing 354 mg. By suspending the pot, the researchers were attempting to simulate the tensile forces that come into effect when the pot is picked up. The maximum weight used was 10 kg. Beeks and Evans found that all new, dry containers were able to hold the test limit of 10 kg. Used peat, dairy manure, rice straw, and wood fiber containers all produced significantly lower tensile strengths than the plastic control containers. (Beeks and Evans, 2013)

These experiments showed that biocontainers can be just as strong as their plastic counterparts. The disadvantage comes when these biocontainers become saturated and lose strength. Extra care when handling wet biocontainers should be taken to ensure a marketable product.

## *Plant Growth*

Another basic requirement of any container is that they must be able to produce a plant on par with, or better than, the industry's standard plastic pot. In order to be economically viable, the more expensive biocontainers need to produce a healthy plant that can sell for a premium. The majority of studies on biocontainers have been focused on plant growth. These studies on biocontainer compatibility have been evaluated with short term crops, long-term crops, and different irrigation techniques.

#### Short-Term Crops

Most of the research conducted on plant growth in biocontainers has been focused on shortterm crops or annual bedding plants (Beeks and Evans, 2013). The focus on biocontainer compatibility is justified because of the huge market of floriculture crops. In the United States, floriculture generated about \$6.5 billion in revenue. Of that \$6.5 billion in revenue, bedding plants made up about 58% of total gross sales (USDA, 2009).

Evans and Hensley (2004), in cooperation with Tyson Foods, created a biodegradable container made from processed waste poultry feathers. They evaluated growth of 'Better Boy' tomato (*Lycopersicum esculentum* L.), 'Janie Bright Yellow' marigold (*Tagetes patula* L.), 'Dazzler Rose Star' impatiens (*Impatiens walleriana* Hook.f.), 'Cooler Blush' vinca [*Catharanthus roseus* (L.) G. Don.], and 'Orbit Cardinal' geranium (*Pelargonium xhortorum* L.H.Baily) in peat, plastic, and the feather containers.

In the first experiment, uniform fertilization and irrigation were used for all containers and plants. The plants were all irrigated when the substrate of about 25% of the containers was visually determined to be dry (Evans and Hensley, 2004). Fertilizer was added along with irrigation so all plants received the same amount and frequency of water and fertilizer. Due to inherent differences in each plant's needs, some received excess water, while others began to wilt before watering. The dry weight of the plants was measured after three weeks for tomatoes, five weeks for marigolds, and eight weeks for impatiens, geranium, and vinca.

The dry weights were significantly higher for marigold, vinca, and geranium grown in the plastic containers than in the plants grown in the feather or peat containers. However, the dry weights of those plants grown in feather containers were greater than the ones grown in peat containers. Dry weight of tomato plants was significantly greater in plastic containers than feather and peat, with no differences occurring between feather and peat containers. No differences occurred between containers for dry weight of impatiens. Evans and Hensley observed that the substrate in peat containers dried much faster than in plastic containers and slightly faster than the substrate in feather containers.

In their second experiment, Evans and Hensley performed the same experiment but used only 'Dazzler Rose Star' impatiens and 'Cooler Blush' vinca with non-uniform irrigation and fertilizer application. Each container was now irrigated individually when each plant and substrate was visually determined to be drying. Because of non-uniform watering, plants received different amounts of water, but none of the plants reached wilting before receiving water.

In experiment two, dry weights of impatiens and vinca plants were significantly higher when grown in feather containers than peat or plastic, with no significant difference occurring between peat or plastic. By watering the plants as needed, the potential effect of water stress was removed. This experiment also confirmed that the advantage of plastic containers is that they do not dry as quickly. Evans and Hensley attribute the increased growth in feather containers to the nitrogen in the containers from the feathers.

Koeser et al. (2013a) also tested the effects of container type on a short-term crop with a wider variety of biocontainers and bioplastic containers, against a plastic control. In total, one plastic, seven biocontainers, and two bioplastic containers were used to grow 'Yellow Madness' petunia (*Pentunia xhybrida*) for a period of five weeks. The experiment used a sample set of twenty replications per container for a total of 200 petunias. Each plant was watered by hand individually when the substrate surface moisture was visually determined to be below 40%. Irrigation frequency for each container type was recorded because the containers dried at different rates. At the conclusion of the growth period, plants were harvested and dry weight was measured.

Koeser et al. (2013a) found that both water usage and dry weight across the containers were significantly different. The plastic, bioplastic, and rice hull containers required the least amount of water. The wood fiber container required the most amount of water and also had one of the lowest dry plant weights. The bioplastic sleeve and slotted rice hull containers produced the greatest dry weights and were in the middle for water usage. In conclusion, the experiment showed that more frequent irrigation was required for peat, manure, and wood pulp containers to produce the same level of growth as the other containers and would thus negate some of the environmental benefits gained.

In another experiment, Koeser et al. (2013b) tested the short-term crop, 'Florida Sun Jade' (*Solenostemon scutellarioides*), with uniform irrigation. The containers used were a plastic control, a bioplastic, and six different biocontainers. Plants were watered when it was visually determined that 25% of the containers were dry on the surface of the substrate. After the plants reached marketability at seven weeks, they were harvested and the dry weights were measured. Koeser et al. found no significant difference of dry weight between the different containers. They found that biocontainer choice made no significant difference on plant growth, and that biocontainers can be a suitable alternative to plastic containers.

#### Long-Term Crops

Growing long-term crops in biocontainers could create issues with container integrity because of the prolonged exposure to elements and conditions that plants require. As with bedding plants and annuals, the container must be able to withstand growing and greenhouse conditions to produce a sellable and profitable product. As with crop duration, the biocontainers were also tested under different watering conditions because the biocontainers have been shown to absorb and hold water at different rates. In an experiment comparing

hand, drip, and ebb-and-flood irrigation, Koeser et al. (2013) found that the different irrigation methods produced significantly different dry weights of plants.

#### Various Watering Methods

#### *Subirrigation*

Many greenhouse crops that require longer production times are grown using subirrigation systems such as ebb-and-flood (Beeks and Evans, 2013). Beeks and Evans (2013) recognized a lack of research using biocontainers for long-term crops, specifically in a subirrigation system, and sought to determine if biocontainers could produce a viable plant under those conditions. In their experiment, a plastic control, three compostable containers (bioplastic, solid ricehull, and slotted ricehull), and six plantable containers (paper, peat, dairy manure, wood fiber, rice straw, and coconut fiber) were used. The containers, because of differences in brands, ranged in size from 12.5 cm to 15.8 cm in top diameter. 'Rainier Purple' cyclamen (*Cylcamen persicum*) was transplanted into the containers being tested because it is a long term production crop. Nine containers of a single type were place into a bench container. The benches were flooded to a depth of 2 cm for 10 minutes when the moisture level of three of the containers decreased below 40%, as read from a moisture reader. The benches were flooded on an individual basis. Beeks and Evans (2013) only compared biocontainers to the plastic control, and not to one another, because of large variations in size of containers.

Since the plants were grown until flowering, the study duration varied. The plants in the dairy manure were the first to flower at 70 days, and the plants in the solid rice hull took the longest at 79 days. The plants in the plastic control took 76 days to flower. Statistically, there was no significant difference between plants grown in biocontainers compared to the plastic container. Dry shoot weights were greater than the plastic control for all containers, with the exception of the wood fiber container. In order for the substrate to absorb the fertilizer solution, it must first be absorbed by the wall of the biocontainer, especially for the wood fiber containers, because they did not have a hole on the bottom. As a result, the wood fiber containers dried out faster than the other containers and sometimes created mild water stress on the cyclamen in these containers. 

#### *Overhead Irrigation*

In addition to testing biocontainers compatibility with a semi-mechanized production process, Koeser et al. (2013) also evaluated differences in dry shoot weight and total leaf area of 'Florida Sun Jade' coleus (Solenostemon scutellarioides) between seven different biocontainers and a plastic control. The containers were watered uniformly when about 25% of plants showed visible drying on the surface of the substrate. Drip irrigation was used as the irrigation method. After the plants reached market size at about 7 weeks, they were harvested to measure dry shoot weight and leaf area. Koeser et al. (2013) found that neither final leaf area nor dry shoot weight varied significantly by container type.

Poinsettias are one of the most popular flowering crops with a wholesale value of \$140 million in 2014 for the 15 largest producing states (USDA, 2016). Poinsettias popularity as a holiday

indoor flower require a presentable container. Lopez and Camberato (2011) recognized the popularity and growth time of poinsettias could be potential incompatibility issues when grown in a biocontainer. Lopez and Camberato (2011) used a plastic container as control and seven different biocontainers, with diameters ranging from 12.5 cm to 15.3 cm, to grow 'Eckespoint Classic Red' poinsettias (*Euphorbia pulcherrima*). Plants were irrigated as necessary and grown for 14 weeks. They were then harvested and dry weights were recorded. The containers were also visually inspected and rated on a scale of 1-5, with a score of 5 meaning containers were intact and had no visible changes.

The plastic, rice hull, wheat starch-derived bioresin, and molded fiber containers all received scores of 5. Straw and coconut coir received scores of 2.9 and 2.8, respectively, because of significant changes to appearances, but container integrity was unchanged. Canadian sphagnum moss, wood pulp, and cow manure received ratings of 1.6, 1.6, and 1.4, respectively, because of not only significant changes in appearance, but it also severely compromised container integrity.

All the plants and containers produced plants that met the minimum marketable height of 35.6 cm (Lopez and Camberato, 2011). The molded fiber and straw containers produced plants significantly taller than the other containers, but this growth difference was not translated into greater dry shoot weight. The dry shoot weights of all containers were not statistically significant. Though there was no difference in dry shoot weights, plants grown in molded fiber containers had significantly greater dry root weight, as compared to plants grown in plastic and wheat starch-derived containers. Lopez and Camberato (2011) concluded that biocontainers can be used to produce an acceptable quality poinsettia. The biocontainer's limitations are compromises in appearance and integrity from the growth process. Because of changes in appearance and integrity, biocontainers will not be equally marketable.

The majority of studies have shown that biocontainers do not have negative effects on plant growth when compared to plastic containers. However, some biocontainers may perform better with certain irrigation techniques than others. Koeser et al. (2013) sought to determine if biocontainers performed differently under different irrigation techniques. Their first experiment used drip irrigation to grow 'Florida Sun Jade' coleus in a variety of biocontainers and a plastic control. Once the plants were grown to market ready size, their dry shoot weights were recorded. Koeser et al. (2013) observed no significant differences between container types. This result confirmed what many of the other researchers also concluded, that biocontainers can produce a viable plant.

The question the researchers then addressed was whether a particular irrigation type, hand, drip, and ebb-and-flood, would be more beneficial than the others. Koeser et al. planted the same plant, 'Florida Sun Jade' coleus, in the same biocontainers and plastic control, but set up different irrigation stations. Plants were irrigated with either ebb-and-flood, drip tubing, or hand watering. All plants were irrigated uniformly when 25% of plants showed visible drying on the media surface. The plants were again grown to market size and dry weights were recorded. Koeser et al. (2013) found that container type was not significant, but irrigation method was.

Ebb-and-flood outperformed the other irrigation approaches and produced bigger plants. However, the researchers noted that the rate of fertilization was likely greater in the ebb-andflood method and attributed the increased dry weight to this. They found no significant difference between hand watering and drip irrigation, and concluded that biocontainers are a suitable alternative to conventional plastic containers.

## *Water Usage*

The majority of studies have not shown significant negative impacts on growth from biocontainer use. Because no significant effects on growth have been shown, when choosing to use a biocontainer, water consumption and cost should take precedence over plant performance (Koeser et al., 2013). Although biocontainers have been shown to produce equivalent plants, other variables may be different. Biocontainers have been shown to dry out at faster rates and therefore require more attention and water. Due to their semi-porous and often times hydrophilic properties, water may be lost through the side of the container at an increased rate, and lost to evaporation before the plant has the opportunity to use that water (Nambuthiri et al., 2013).

Evans and Karcher (2004) recognized that water usage was a critical difference between biocontainers and plastic containers. In their first experiment they measured water loss through container walls using plastic, peat, and feather containers. They did this by filling the three types of containers with the same volume of substrate. The substrate was then watered until saturated, while being careful not to pour directly onto the container walls. The containers were allowed to drain freely. Once all containers ceased draining, the top and bottom were covered using paraffin wax. The containers were weighed and placed in a greenhouse where they were weighed daily for a week. Because of differences in container sizes, Evans and Karcher (2004) expressed water loss on a per cm<sup>2</sup> basis. After one week, Evans and Karcher (2004) observed significant differences in weight across the three types of containers tested. The water loss of the peat container was about 2.5 times greater than the feather container, while no appreciable water loss occurred in the plastic container. Evans and Karcher concluded that the peat containers were hydrophilic and drew water from the soil, where the water is then able to evaporate into the atmosphere.

Evans and Karcher (2004) then wanted to see what the effect of water loss through container walls has on plants. They tested 'Dazzler Rose Star' impatiens and 'Cooler Bush' vinca plugs in the feather, peat, and plastic containers. The plants were irrigated individually when the substrate appeared to be dry. Trays were also placed under each container to collect any excess water not absorbed but the soil or taken up by the plant. Throughout the five-week experiment, the researchers recorded the number of irrigations, average volume retained, total water required, and the irrigation interval.

Evans and Karcher (2004) found that, for both plants, the total volume of water used and total number of irrigations, was significantly more for peat and feather containers than plastic containers. When they compared only the biocontainers to each other, for both plants, they found that the total volume of water used and total number of irrigations were significantly less for feather containers than peat containers. Not surprisingly, Evans and Karcher (2004) also observed longer irrigation intervals for the plants in the plastic containers. Their results in this experiment confirmed the results of the first experiment when no plants were used. Water is not absorbed by the plastic container walls, so evaporation can only occur through the substrate's surface, dramatically limiting water loss to evaporation. (Evans and Karcher, 2004)

In another study, Evans et al. (2010) performed similar experiments, but with more biocontainers and longer growing period. In this experiment, Evans et al. (2010) expanded beyond the peat and feather containers to include OP47 bioplastic, Fertil pot, coconut fiber, Cowpot, peat, rice hull, paper, and rice straw containers. A 4-in plastic container was used as the control for all biocontainers, except the OP47 bioplastic, where a 12.7 cm (5-in) plastic was used as the control because of the larger size.

To determine the effect of the biocontainer on plant growth, Evans et al. used 'Orbit Cardinal' geranium (*Pelargonium xhortorum*). The plants were irrigated individually when the substrate surface showed visible signs of drying. The drainage was again collected and recorded in order to quantify total water used and the irrigation interval. The experiment was concluded after 8 weeks, once the plants had reached market size. Evans et al. concluded that the peat, coconut fiber, paper, rice straw, and Cowpot containers required more water and more frequent watering than the plastic container of comparable size. The rice hull and OP47 containers, however, were similar to plastic in both total water volumes used and average irrigation interval. 

Evans et al. recognized that differences in individual plant needs and substrate surface area can affect the water required for each plant. In order to positively attribute water demand to container type, they performed the same experiment as Evans and Karcher (2004), where they filled each biocontainer with substrate and irrigated until saturation. Once drainage ceased, they plugged all exposed holes with paraffin wax and placed the containers in a greenhouse where they were weighed daily for one week. As expected, based on the results from the first experiment, rice straw, coconut fiber, Fertil, and peat containers had the highest rate of water loss. Rice hull and OP47 containers performed similarly to plastic containers. Cowpots and paper containers were in the middle of the two groups. Evans et al., concluded that dramatic differences in water demand can occur based on the type of biocontainer used. Therefore, increased water usage and demand should be weighed against the benefits of reducing plastic waste. The results of this experiment also confirm the results obtained by Evans and Karcher (2004), that biocontainers lose water through the container walls at a faster rate than plastic containers. (Evans et al., 2010)

Beeks and Evans (2012) did not specifically set out to determine the water requirement of different biocontainers, but through their testing of different biocontainers to grow long term crops in an ebb-and-flood irrigation system, were able to determine the total volume of water used per container and the average irrigation interval. Beeks and Evans (2012) determined that peat, dairy manure, wood fiber, and rice straw containers all had irrigation intervals lower than the plastic control, therefore requiring more frequent irrigation. The bioplastic, solid ricehull,

slotted ricehull, paper, and coconut fiber containers all had irrigation intervals not significantly different from the plastic containers. These results confirm most of the results obtained by Evans et al. (2010), that bioplastic and ricehull containers perform similarly to plastic containers in regards to the water requirements of plants. However, in this experiment the coconut and paper containers seemed to have retained water better than the results obtained by Evans et al. (2004), possibly because of the different irrigation methods. Beeks and Evans (2012) still concluded that containers with water-permeable walls, like the majority of biocontainers, might require a greater volume of water and shorter irrigation intervals.

#### *Algal Coverage*

Biocontainers, because of the higher percentage of organics and the tendency to absorb water, have a tendency to produce algae or fungi on their surface. This is usually neither harmful to the plant nor decreases the strength of the container, but it could possibly decrease the marketability of the container.

Evans and Karcher (2004) also evaluated algal and fungal growth when they grew impatiens in plastic, peat, and the feather containers. When the plants and soil were removed after eight weeks, the containers were allowed to dry. The area of algal or fungal growth was measured using an area meter, and the area covered was expressed as a percentage or total container surface area. Evans and Karcher (2004) found that, as expected, no algal or fungal growth was found on plastic containers. They also found that the area covered by algal or fungal growth was significantly higher on peat containers than on feather containers. It was noted that the discoloration that occurred on peat containers was largely due to algal growth, whereas the discoloration on feather containers was due to fungal growth. Evans and Karcher theorized that the difference in container chemistry determined which organism grew on the walls. The peat container stayed wet longer, which could have been more favorable to algae, whereas the feather containers had higher nitrogen content that could have been favorable to fungus. (Evan et al., 2004)

In another experiment, Evans et al. (2010), expanded on the results from his experiment in 2004. This experiment followed the same procedure, but with a broader array of containers. In this experiment plastic, OP47 (bioplastic), Fertil, Cowpot, coconut fiber, peat, rice hull, paper, and rice straw containers were used. Evans et al. (2010) found that after six weeks, no algal or fungal growth was seen on the coconut fiber, rice hull, OP47, or plastic containers. Cowpot, paper, and rice hull containers showed a slight decrease of wall coverage, around 2 to 4%. Fertil and peat pots had the greatest and most significant amount of coverage at 26% and 47%, respectively. This time, Evans et al. (2010) attributed the difference in algal/fungal coverage, to the absorption capacity of the materials. The containers with no or minimal coverage, dried quickly, whereas Fertil and peat pots, retained moisture. (Evans et al., 2010)

#### Sustainability

A customer's willingness to pay a premium for a biodegradable container often comes from the desire to be more sustainable and environmentally friendly. Biocontainers are more sustainable because they are non-petroleum based and can be planted in the ground, but they often require additional inputs and care throughout the growing cycle. Koeser et al. (2014) quantified the material and energy inputs required to produce a petunia, from plug production to delivery at a retail garden center, in a variety of biocontainers. Koeser et al. (2014) only measured secondary impacts, such as water usage, or additional energy use from a longer growing cycle, because these impacts could be directly measured.

Global Warming Potential (GWP) was used as the primary environmental impact unit to allow for comparison between the containers. Koeser et al. (2014) found that plug production in the controlled greenhouse accounted for almost half of the plants carbon footprint. The majority of GWP for plug production was due to the electricity needed to provide lighting for the plants. Koeser et al. (2014) determined that any differences in GWP between biocontainers was due to the differences in volume. The additional volume resulted in a greater amount of soil, which increased shipping weight and additional water demand. Additional water demand between biocontainers and plastic container did not produce significant differences in GWP. Koeser et al. (2014) concluded that petunias grown in similarly sized biocontainers and conventional plastic containers had nearly the same GWP. (Koeser et al., 2014)

#### Demand

One of the largest advantages that plastic containers have over biocontainer alternatives is a lower price point. Growers must be able to charge a premium for containers grown in biocontainers because of the increased cost. Growers will be very resistant to increasing their overhead costs if they cannot recoup that cost down the line when sold to the costumer.

Yue et al. (2010) studied what consumers valued based on how much more or less they were willing to spend. Yue et al. (2010) conducted an online survey of 834 people. The survey first began with several baseline questions to categorize the type of costumers and then presented the costumers questions on willingness to pay (WTP). WTP questions are important because cost is often the major factor in decision making and is a good measure of attributes the consumer values. The WTP questions included pictures of eight different kinds of pots, with the materials clearly presented. The containers pictured were recycled plastic, wheat-starch, rice hulls, straw, coconut coir, resin from poultry feathers, cow manure, and peat. The participants were then asked how much more or less they would pay, from  $-51.50$  to \$1.50, as compared to a plastic container.

The survey concluded that participants were willing to pay a premium for containers containing wheat starch, rice hulls, straw, coir, and peat. On average, consumers were willing to pay about a quarter more for these containers. However, participants were not willing to pay more for containers made from poultry feathers or cow manure.

In a complementary journal article that elaborated on the same survey, Hall et al. (2010) concluded that biocontainers could be sold for a premium to particular groups of people, such as the "Environmentally Conscious," or "Carbon-Sensitive." However, as a whole, consumers hesitated to purchase low-quality products, regardless of how environmentally friendly they

were. The 'green attributes' of biocontainers are not enough to demand a higher price tag; they must perform as well, or better, than similar products.

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## Chapter 3: Engineering Biodegradable Planters from Lagooned Biosolids

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## Abstract

Sustainability practices are leading to the development and use of alternative products in the floriculture and wastewater industries, such as the use of biodegradable containers instead of plastic containers. The objective of this research was to evaluate the efficacy of using digested biosolids from a regional wastewater treatment plant as an ingredient in creating a biodegradable transplant pot. The biosolids were tested for metals and contaminants limits as required by the U.S. EPA Part 503 Rule, and met the pathogen reduction requirements for Class B biosolids. Multiple blends, or mixes, were developed, each varying in biosolids and fiber content on a dry weight basis, as well as different additives such as starch, polymer, or a natural glue to provide overall pot stability and structural strength. Various engineering tests, such as tensile, pH and saturated paste tests, and puncture tests, were conducted on the different mixes to determine the optimum strength that could be produced.

The top-performing biosolids mixes were used to make 10.2 cm (four-inch) pots that were compared in various ways to the market leaders, Peat Pots and standard plastic pots. A twopart mold was created on a 3D printer, which would allow for positive pressure to be used in forming the BioPots. Mixes were transferred to the lower half of the mold, the upper part was then plunged and fastened into the lower half, and then the mold with its mix was placed in an oven to dry. Laboratory germination bioassays were performed to test for the presence of phytotoxic compounds. Construction of BioPots for the lab-scale studies was tedious. Different methods (e.g., negative pressure systems) need to be investigated for use in producing the BioPots commercially. Standard agronomic soil tests showed that BioPots should not have an adverse effect on plant growth.

#### **Introduction**

The floriculture industry, in a 15-state program in the U.S., is estimated to be valued at a \$4.37 billion in 2015 (USDA, 2016). The floriculture market is a subset of a much bigger agriculture and greenhouse industry. The industry heavily relies on the use of plastic to keep production up and costs down. Plastics are widely used due to their durability, light weight, and low price. Plastic containers also come in a wide variety of shapes and sizes, allowing growers to have a near infinite selection of containers that will best suit their products. With such large-scale use of plastic, the fate and disposal of horticulture plastics has been increasingly problematic. Botts (2007) reported that production of nursery pots, flats, and cell packs used approximately 320 million pounds of plastic annually. Recycling plastic can be difficult due to: presorting requirements depending on the container material type; possible washing of the containers due to contamination from growing media adhered to the container walls; or diminishing quality due to UV light degradation. Some growers may be hesitant to reuse containers because of the fear of a possible spread of disease.

Growers have expressed a desire to incorporate more sustainable practices and one of those practices is the use of biocontainers (Dennis et al., 2010). Biocontainers can both be plantable or compostable and come in a variety of waste, recycled, or renewable raw materials. Plantable containers alleviate the need to dispose of the container after planting the plant because the plant and container are both planted together. The container is broken down naturally in the soil and can decrease the transplant shock of removing the plant from the container. Compostable containers still require the plant and container to be separated before planting but allow the grower to compost the container. Consumers have also expressed a willingness to pay more for a sustainable product.

Since passage of the Clean Water Act in 1972, progress has been made in the United States toward achieving higher levels of wastewater treatment. Water utilities are striving to become "water resource recovery facilities" (WRRFs) in an effort to recover numerous resources, such as water, energy, and nutrients, in a sustainable manner. The development, distribution and marketing of high-quality biosolids products are of particular interest to provide a renewable resource that is environmentally safe and reduces the financial burden on water utilities and their customers. Biosolids are the nutrient-rich organic materials resulting from the treatment of sewage sludge; the regulatory term for the solid, semisolid or liquid untreated residue generated during the treatment of domestic sewage in a treatment facility. When treated and processed, sewage sludge becomes biosolids which can be safely recycled and applied as fertilizer to sustainably improve and maintain productive soils and stimulate plant growth. Only biosolids that meet the most stringent standards specified in the federal and state rules can be approved for use as a fertilizer or nutrient product.

Federal regulations governing the use and disposal of municipal wastewater solids include: 40 CFR 503 Standards for the Use or Disposal of Sewage Sludge, 40 CFR 257 Criteria for Classification of Solid Waste Disposal Facilities and Practices, and 40 CFR 258.2 Criteria for Municipal Solid Waste Landfills. Solids that are placed in a Municipal Solid Waste (MSW) landfill are not subject to the Part 503 regulations, but must still meet the requirements of both 40 CFR 257 and 40 CFR 258.2.

The Part 503 regulations were promulgated in 1993 and set forth standards for the following use and disposal options: beneficial use through land application, distribution and marketing; disposal at dedicated sites or in sludge-only landfills; and incineration in sludge-only incinerators. The Part 503 regulations specify requirements in the three categories for solids applied to land: pollutant limits, pathogen reduction requirements (Class A and Class B), and Vector Attraction Reduction (VAR) requirements.

Many utilities are exploring ways to beneficially use biosolids; some utilities treat their biosolids to regulatory standards so that they can land apply their solids. Some utilities are interested in producing higher quality biosolids materials that can offset their processing cost and produce potential revenue from the sale of the biosolids-derived products. The Western Virginia Water Authority awarded a contract to Virginia Tech to evaluate the viability of using biosolids to create a biodegradable flower pot. The Roanoke Regional Water Pollution Control Plant

(RRWPCP), where the biosolids used in this study were obtained, anaerobically digests its solids and then lagoons them for about nine months. The biosolids are currently treated to allow for Class B land application restrictions. The plant land applies about 9.07 million dry kilograms (10,000 dry tons) per year locally. The rest of the solids are landfilled, which is a significant cost to the utility and is not considered a sustainable practice.

The objective of this research was to evaluate the efficacy of using digested biosolids from RRWPCP as an ingredient in creating a biodegradable transplant pot. In addition to the biosolids, other fibrous materials, such as used cardboard or cellulose, were used to stabilize and add structural strength. Multiple blends, or mixes, were developed, each varying in biosolids and fiber content on a dry weight basis, as well as different additives such as starch, polymer, or a natural glue. Various engineering and soil media standard tests, such as tensile, pH and saturated paste tests, and puncture tests, were conducted on the different mixes to determine the optimum strength that could be produced.

## **Materials and Methods**

#### Selecting the Mixes

The lagooned biosolids received from the RRWPCP were about 5% solids by weight. The biosolids were collected in five-gallon buckets and stored in a refrigerated room until use. The total solids concentration of the plant's biosolids were not high enough to produce a solid container without the addition of materials for structural support and integrity. For the sake of sustainability, different biodegradable and recyclable fiber sources were tested for compatibility. Ultimately, cardboard and cellulose fiber (Terra-Mulch Cellulose fiber) were used as fiber sources. The cardboard was made into a pulp by soaking in water and blending until a semi-homogenous consistency was made. The cellulose fiber was blended to a fine powder. The two fiber materials were then mixed with biosolids at ratios of 1:1, 2:1, and 5:1 on a fiber to biosolids dry weight basis. The intent and goal of this study was to utilize as much biosolids as possible for maximum reuse of the biosolids to offset solids management costs incurred by the treatment authority.

In addition to different fiber materials, other additives were also considered to enhance structural strength. Starch was chosen because it is commonly found as an ingredient in other biocontainers and a natural binding agent. Starch was added at a rate of 2% on a wet weight basis per container. This percentage was chosen because it was found that at greater amounts of starch, the mixture would not blend well. The polymer, FLOPAM 4550 (SNF Inc.), was selected to enhance dewaterability. With the different fiber sources and additives, numerous combinations were considered. The different combinations are shown in the hierarchical tree shown in Figure 3-1.



**Figure 3-1. Hierarchy of Biosolids Container Mixes on a Fiber to Biosolids Dry Weight Basis (no add=no additive)**

## Tensile Strength Tests

A strength test was performed on the 18 different combinations, to eliminate the weaker mixtures from further testing. A common way to test biocontainer strength involves use of a texture analyzer to crush the container standing vertically and on its side. Crush strength is an important metric to know, but the forces exerted on the container during the growing process are more likely tensile forces. These forces occur when the container is held by the lip and transported by hand. A tensile test, commonly used to test metallic and polymeric materials, was used to evaluate the tensile properties of the different mixes. When testing the tensile properties of metallic materials, a coupon (strip of material) is used. The coupon is widest on each end with a slight taper to the middle. The taper is created to concentrate the stress in the middle of the sample to create a smooth stress strain curve.

A stencil type mold was fabricated, with dimensions similar to the dimensions of a metallic coupon sample. Each coupon was about 20 cm by 3.2 cm. The mold is shown in Figure 3-2.



**Figure 3-2. Fabricated Stencil Mold Used to Create Coupons for Tensile Testing** 

The mold was loaded with the volume of wet mixture required to create the necessary dry volume to fully fill each coupon. The sides were closed and the water was squeezed out. The entire mold was allowed to cure in an oven at  $60^{\circ}$ C for two days to dry the mixes. Four replicates of each mix were prepared, and the samples were tested in the Engineering and Science Mechanics Lab at Virginia Tech. An Instron Texture Analyzer, similar to the machine used by others to crush containers (Koeser et al., 2013; Evans and Karcher, 2004; Beeks and Evan, 2013; Evans et al., 2010), was used. However, instead of being programmed for compression, the machine was programmed to gradually pull on each end of the coupons. With metallic samples, the Instron machine is able to draw a stress-strain curve, but because of the variability and the inelastic nature of the biocontainer coupons, only data for load at failure (kN) could be recorded. Four replicates of each mixture were tested.

## Saturated Paste Test

As a preliminary measure to ensure that the biocontainer mixtures would be conducive for plant growth, the five top performing mixtures from the tensile tests were ground using a commercial coffee grinder. A saturated paste extract was made with the grindings, as described in Rhoades (1996). The pH of the extract was measured using a Thermo Scientific Orion 370 PerpHecT LogR pH meter, and the extract's specific conductance was determined with a VWR Model 2052 conductance meter calibrated to a standard 1,000 ( $\mu$ S/cm) KCl solution.

#### Germination

To test for the presence of any potentially phytotoxic compounds, laboratory germination bioassays were performed with soybean seeds [Glycine max (L.) Merr]. Filter paper was placed within petri dishes initially saturated with the saturated paste extract from the ground BioPot materials, or with distilled water as a control. Eight soybean seeds were placed within each petri dish. Distilled water as added as needed to prevent seeds from drying. Three replicates were set up for each BioPot treatment. The dishes were placed in a sunny window and checked
daily for the number of seeds that had germinated. Final germination counts were taken 14 days after the study was initiated.

## Mold Construction

Many commercially available biocontainers are created through use of a suctioning process. This type of equipment was not available for this effort. Instead, a compression mold, modeled after 4-inch Peat Pots, was drafted using AutoCAD Inventor and then created using a 3D printer. Shop drawings are shown in Figure 3-3 and 3-4. The mold consisted of two pieces, a bottom piece used to hold the mixes and a top price to force water out and form the pots. The mold contained six cups and was made of ABS (Acrylonitrile butadiene styrene) M30 Plastic.

The interior of each cup was lined with two different permeable layers to hold the solids, but allow the water to filter out. The first layer was an aluminum mesh that added support to the second layer, a mesh fabric, which was in direct contact with the biosolids mixture.



**Figure 3-3. Top Piece of Pot Mold** 



**Figure 3-4. Bottom Piece of Pot Mold** 

## BioPot Construction Process

After significant trial and error, calculations to determine the required volume of mixture needed to completely form a biocontainer were found to consistently under-estimate the actual needed volume. Because of variability and non-homogeneity of the mixtures, the first containers produced had gaps and holes in the container walls. To overcome this, additional mixture was added to each well in the mold. If the BioPot formed above the container lip, amounts of it could be trimmed back later, once dry.

Another difficulty encountered was the tendency for pots to stick to the top of the mold. Several non-stick sprays were tested, but did not alleviate the sticking issue. This was eventually solved by covering each cone with saran wrap that could be easily removed from the containers after drying.

The cardboard based mixture required that 400 mL be used to adequately fill all voids and form a complete pot in the well, whereas only 300 mL of the cellulose-based mixture was needed because it did not contain as much water. Once the required volume was poured into each cup, saran wrap was laid over each cone on the top piece of the mold. The top piece of the mold was positioned so that each cone fit into each cup evenly, and then the top was forced down. Long screws were inserted through the bottom of each cup and tightened evenly to ensure a uniform thickness of material in the container walls.

The entire mold was then placed in an oven and heated at a temperature of 60 °C for two days. After two days, the top piece of the mold was removed, and the bottom piece with the containers was placed in the oven to dry for another day. After three days in the oven, the containers were removed, and the filter layers were peeled off the BioPots.

All data collected was analyzed using an Analysis of Variance (ANOVA) with mean separation using Fisher's Least Significant Difference (LSD). An ANOVA is used to determine if the data are derived from more than one sample population. If significant differences between groups do occur, the mean separation procedure is used to determine where significant differences occur.

### **Results and Discussion**

#### Tensile Tests

The objective of this test was to identify the feasible combinations of materials. To fulfill that purpose, a differentiating factor needed to be determined. To determine if significant differences occurred between coupons based on additive, fiber base, or biosolids volume, an ANOVA with mean separation was used. The results of this testing are given in Table 3-1. The 2:1 cellulose +starch mixture was the strongest of all the mixes and one of two, significantly different from all other mixes. The 2:1 cardboard  $+$  polymer mixture was also significantly different from all the others; however, it was the weakest of all the mixes. In several cases, with the exceptions of 1:1 cardboard, 1:1 cellulose, and 5:1 cardboard, the additives in the mixtures were significantly different from each other. In all cases, the mixes with polymer were weaker than the mixes with starch. In all mixes, mixes without additives were not significantly different from the mixes that contained starch.



## **Table 3-1. Tensile Test Results**

\*Means followed by the same letter are not significantly different (P≤0.05; Fisher's LSD).

Because significant differences were noted between starch and polymer in some of the mixes and polymer was associated consistently with the lower strength readings, polymer was eliminated as a potential ingredient. The mixes that were strongest and exhibited the most potential were then selected for further testing, as shown in Table 3-2. The ratios provided in the table represent fiber to biosolids on a dry weight basis.



## **Table 3-2. Mixes Considered for Further Testing**

#### Saturated Paste Test

All samples had pH and specific conductance values within a range that is unlikely to have adverse effects on plant growth. Table 3-3 shows the average pH and specific conductivity readings for each mixture. Each replicate produced consistent pH and SC measurements within each mixture. 5:1 Cardboard, was the only mixture with a significantly different pH and SC values than every other mixture. 5:1 Cardboard has a significantly lower SC values than the

other mixtures, most likely because of the lack of ions in the fiber. The 2:1 Cardboard + starch and  $2:1$  Cellulose + starch did not have significantly different  $pH$  values, with a possible conclusion that the pH is dictated by biosolids volume and starch. All SC values were significantly different from each other with 2:1 Cellulose measuring the averaging highest at 2.61 dS/m and 5:1 Cardboard with the averaging lowest at 0.88 dS/m.



## Table 3-3. pH and Electrical Conductivity Measurements

\*Means followed by the same letter within columns are not significantly different (P≤0.05; Fisher's LSD).

### **Germination**

As shown in Table 3-4 below, soybeans germinated adequately in all samples. In most mixtures, greater than 90% of the seeds germinated.  $2:1$  cellulose + starch and  $5:1$  cardboard were similar in they had the lowest percentage germinate but were still able to germinate 88% of the seeds. None of the mixtures or blank were significantly different from one another.



#### **Table 3-4. Germination Results**

\*Means followed by the same letter are not significantly different (P≤0.05; Fisher's LSD).

### Mold Construction

Using the method previously described, four containers of each of the five mixes were created to undergo additional testing in a greenhouse. Example photos of the BioPots are shown in Figure 3-5.



 **Figure 3-5. Photo of 2:1 Cellulose Container**

## **Conclusions**

With many utilities seeking alternative biosolids management methods to become more sustainable, biocontainer production can be a viable solution. Based on this research, it appears that lagooned biosolids with the correct mix of additives can yield solid biocontainers that are marketable. The fiber sources provided rigidity, and help to increase marketability as a legitimate commercial product. This research was performed with biosolids from a single source and solids handling process. A dewatered cake product, at a higher total solids concentration, would be a preferred feedstock for producing potentially marketable biocontainers, and would require less additional fibers. Biosolids from different sources might also contain different constituents, such as metals, organics, and added polymers that would need to be considered if constructing biocontainers on a larger scale from multiple sources. For distribution and marketing purposes, the biosolids feedstock would need to meet the Class A standards as stipulated in the federal Part 503 rule.

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# Chapter 4: Evaluation of Biosolids Derived Biocontainers in Greenhouse Production

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## Abstract

Sustainability practices are leading to the development and use of alternative products in the floriculture and wastewater industries, such as the use of biodegradable containers instead of plastic containers. The objective of this research was to evaluate the efficacy of using digested biosolids from a regional wastewater treatment plant as an ingredient in creating a biodegradable transplant pot. The biosolids were tested for metals and contaminant limits as required by the U.S. EPA Part 503 Rule, and met the pathogen reduction requirements for Class B biosolids. Multiple blends, or mixes, were developed, each varying in biosolids and fiber content on a dry weight basis, as well as different additives such as starch, polymer, or a natural glue to provide overall pot stability and structural strength.

Top-performing BioPots were constructed and then evaluated in a greenhouse setting, including their ability to yield healthy plants. Media capacity, resiliency, leachability, algal/fungal growth were tested. Greenhouse studies were conducted in two phases:  $(1)$ analysis of leachate and assessment of pot stability through watering cycles; and (2) growth studies for soybeans, marigolds, and romaine lettuce. These plants were selected based on growth ability and sensitivity.

Most of the BioPots survived the resiliency study. Leachate quality from the biocontainers was not different than plastic containers. Some discoloration was observed on the biocontainers, but it was not due to algal/fungal growth. Growth of soybeans, marigolds, and romaine in BioPots was significantly better (e.g., increased height, leaf sizes, and weight) than in plastic containers.

### Introduction

The greenhouse floricultural industry produces small perennials, flowering potted plants, and annual bedding plants. In a 15-state study, the floriculture industry is estimated to be valued at \$4.37 billion in 2015 (Floriculture Crops 2015 Summary). The industry relies heavily on the use of plastic to keep production up and costs down. Plastics are widely used due to their durability, light weight, and low price. Plastic containers also come in a wide variety of shapes and sizes, allowing growers to have a near infinite selection of containers that will best suit their products. With such large-scale use of plastic, the fate and disposal of horticulture plastics has been increasingly problematic. Botts (2007) reported that production of nursery pots, flats, and cell packs used approximately 145 million kilograms (320 million pounds) of plastic annually. Recycling plastic can be difficult due to: presorting requirements depending on the container material type; possible washing of the containers due to contamination from growing media adhered to the container walls; or diminishing quality due to UV light degradation. Some growers may be hesitant to reuse containers because of the fear of a possible spread of disease. Because of the prevalence and large amount of plastic used, the greenhouse industry not as sustainable as it could be.

Growers have expressed desire to implement more sustainable practices in effort to attract the environmentally-minded consumer (Dennis et al., 2010). One sustainable practice that growers and buyers have found enticing is the use of biocontainers. Biocontainers are often produced from a variety of waste, recycled, or renewable raw materials and allow the user to either plant the container in the ground with the plant or to dispose of the container through composting. The development and use of biocontainers would significantly decrease the amount of waste a greenhouse would produce.

In addition to decreasing plastic use, biocontainers offer growers other advantages such as reducing transplant shock by planting the container in the ground with the plant and the possibility of producing a bigger, healthier plant. However, biocontainers have some special considerations for use. Many growers are hesitant to incorporate biocontainers because of their limited durability and perceived incompatibilities with production practices. Biocontainers are also far more expensive than their plastic counterparts, though consumers have been shown to be willing to pay a premium for a more sustainable product (Yue et al., 2010).

Several studies have researched the effects of biocontainers on plant growth. Keoser et al. (2013) compared growth of a 'Florida Sun Jade' (Solenostemon scutellarioides) in a plastic, bioplastic, and six different biocontainers and found that the type of container made no significant in the dry weight of the plants. Lopez and Camberato (2011) grew poinsettias (*Euphorbia pulcherrima*) in plastic and seven different types of biocontainers for a 14-week period before harvesting. They found no significant differences in the dry shoot weight of the plants and that the biocontainers produced a plant of acceptable quality.

To be competitive, biocontainers not only have to produce a plant on par with plastic, but also be aesthetically attractive. Biocontainers have a tendency to produce fungal or algal growth on container walls because of the hydrophobic and organic properties of the container materials. Evans et al. (2010), in a study using plastic and eight different biocontainers, found that while most biocontainers had minimal or no algal coverage, Fertil and peat pots had 26% and 47% coverage, respectively. Algal growth is attributed to the absorptive capacity of the container material. It is neither harmful to the plant no decreases the container strength, but it could decrease the marketability of the container.

Since passage of the Clean Water Act in 1972, progress has been made in the United States toward achieving higher levels of wastewater treatment. Water utilities are striving to become "water resource recovery facilities" (WRRFs) in an effort to recover numerous resources, such as water, energy, and nutrients, in a sustainable manner. The development, distribution and marketing of high-quality biosolids products are of particular interest to provide a renewable resource that is environmentally safe and reduces the financial burden on water utilities and their customers. Biosolids are the nutrient-rich organic materials resulting from the treatment of sewage sludge; the regulatory term for the solid, semisolid or liquid untreated residue generated during the treatment of domestic sewage in a treatment facility. When treated and processed, sewage sludge becomes biosolids which can be safely recycled and applied as fertilizer to sustainably improve and maintain productive soils and stimulate plant growth. Only biosolids that meet the most stringent standards specified in the federal and state rules can be approved for use as a fertilizer or nutrient product.

Federal regulations governing the use and disposal of municipal wastewater solids include: 40 CFR 503 Standards for the Use or Disposal of Sewage Sludge, 40 CFR 257 Criteria for Classification of Solid Waste Disposal Facilities and Practices, and 40 CFR 258.2 Criteria for Municipal Solid Waste Landfills. Solids that are placed in a Municipal Solid Waste (MSW) landfill are not subject to the Part 503 regulations, but must still meet the requirements of both 40 CFR 257 and 40 CFR 258.2.

The Part 503 regulations were promulgated in 1993 and set forth standards for the following use and disposal options: beneficial use through land application, distribution and marketing; disposal at dedicated sites or in sludge-only landfills; and incineration in sludge-only incinerators. The Part 503 regulations specify requirements in the three categories for solids applied to land: pollutant limits, pathogen reduction requirements (Class A and Class B), and Vector Attraction Reduction (VAR) requirements.

Many utilities are exploring ways to beneficially use biosolids; some utilities treat their biosolids to regulatory standards so that they can land apply their solids. Some utilities are interested in producing higher quality biosolids materials that can offset their processing cost and produce potential revenue from the sale of the biosolids-derived products. This study was performed for the Western Virginia Water Authority. The Roanoke Regional Water Pollution Control Plant (RRWPCP), where the biosolids used in this study were obtained, anaerobically digests their solids and then lagoons them for about nine months. The biosolids are currently rated as a Class B and can be land applied. About 9.07 million dry kilograms (10,000 dry tons) of solids from the plant are land applied per year. The rest of the solids that are landfilled at a significant cost to the utility and is not considered a sustainable practice.

The objective of this study was to evaluate the performance of biosolids-derived biocontainers (BioPots) in a greenhouse setting and their ability to produce healthy plants.

### Materials and Methods

#### Media Capacity

Media capacity is the volume of water that the soil and container is able to absorb before water begins to leach out. Determination of container capacity was necessary in order to perform resiliency and leachability studies on the containers. Five different container mixes were tested and coded by their ratio of fiber to biosolids on a dry weight basis:  $2:1$  Cardboard;  $2:1$ Cardboard + Starch; 2:1 Cellulose; 2:1 Cellulose +Starch; and 5:1 Cardboard. Two types of control pots were used: 10.2 cm (4-inch) plastic and 10.2 cm (4-inch) Peat Pots. Four replicates of each container type were tested, making the total number of pots tested 28. Each container was filled with 40 grams of Sunshine #2 potting soil. Containers were watered up to approximate pot capacity, before leakage occurred. Containers were then covered loosely with

plastic wrap and allowed to sit overnight. The next day, containers were watered until leaking began through the bottom of containers. Each container was then weighed and compared to its respective initial weight to determine the water capacity of the media.

## Resiliency

Biocontainers must be strong enough to withstand daily watering, but weak enough to be plantable in the soil and not inhibit plant growth. In order to determine how the BioPots performed from daily watering and typical greenhouse conditions for an extended period of time, containers were watered daily to capacity without significant leaching for six weeks. This watering schedule simulated normal greenhouse operations at the average time that plants are housed in a greenhouse.

## **Leachability**

Concurrent with the resiliency evaluation, leachability tests were conducted after 1-week and 6-weeks. Containers were first watered to capacity and allowed to sit undisturbed for 30 minutes before an additional 150 mL of water was added. Containers were placed on top of plastic cups that collected the leachate. Containers were given an opportunity to drain until each produced about 110-140 mL of leachate. The leachate samples were decanted into wide mouth bottles and transported to lab for analysis.

Each 10 mL sample was decanted into a test tube for EC and pH measurements. The pH was measured using a Thermos Scientific Orion pH meter (370 PerpHecT LoR) and EC was measured using VWR conductance meter (Model 2052) calibrated to a standard 1,000 µS/cm KCl solution.

The remaining volume of each sample was filtered through a 0.45  $\mu$  filter and split into two parts. One part was acidified for priority pollutant metals list (PPL) metals (As, Cd, Cu, Pb, Hg, Mo, Ni, Se, and Zn) analysis via ICP-MS. The other part was frozen for nutrient analysis (nitrate, ammonium, and ortho-P) via Lachat.

An Analysis of Variance (ANOVA) with mean separation using Fisher's Least Significant Difference (LSD) was used to analyze the data from the leachability tests to determine if significant differences occurred between the leachate from biosolids containers, peat pots, and plastic containers. An ANOVA is able to determine if the data are derived from more than one sample population. If the overall F-test for the ANOVA indicates that significant differences due to treatment were probable ( $p < 0.05$ ), then a subsequent mean separation analysis reveals where the differences occurred.

## Algal/Fungal Growth

At the conclusion of the 6-week resiliency study, algal coverage on container walls was estimated via visual observation using USDA-NRCS "Percent of Area Covered" graphics (Schoeneberger et al., 2012). This process involves comparing the algal coverage on the BioPots to the graphic shown in Figure 4-1.

#### **EXAMPLES OF PERCENT OF AREA COVERED**

The following graphic can be used for various data elements to convey "Amount" or "Quantity." **NOTE:** Within any given box, each quadrant contains the same total area covered, just different sized objects.



#### **Figure 4-1. USDA-NRCS "Percent of Area Covered" charges commonly used to semi-quantify** color variegations on soil surface (Schoeneberger et al., 2012).

#### Strength Testing

Once the resiliency study was completed, the strength of the used containers was tested to determine how well the containers withstood normal greenhouse conditions. Several out there have performed experiments to test the strength of biocontainers (Koeser et al., 2013; Evans and Karcher, 2004; Beeks and Evan, 2013; Evans et al., 2010). In these research efforts, the strength of the containers was tested by crushing containers using a texture analyzer while the containers were both upright and on their side. These measurements are a good indicator of the container's structural integrity during shipping or production where crushing forces may occur. However, our research team decided to measure tensile force which would be a good indicator of container stability as it typically handled and moved in a greenhouse setting. Beeks and Evans (2013) tested tensile strength by gradually filling suspended containers with ball bearings until failure occurred. A replication of this test was conducted, but the containers

did not fail when fully loaded. Two of the authors, Stone and Boardman, developed a procedure, the Inflation Test, which can be easily replicated for different types of containers.

When subjected to plant growth, containers experience a majority of forces emanating from the inside of the container, pushing the walls outwards by the weight of saturated soil or roots trying to penetrate the container walls. To replicate these forces, an inflation test was developed. A funnel with a top diameter similar to the container's was placed on top of the containers. A balloon was threaded through the bottom of the funnel with the open end of the balloon still exposed. The balloon was gradually inflated with air and began to fill the interior of the biocontainer. A pressure gauge was attached between the air supply and the balloon. Once the balloon built up enough pressure, the walls of the container ruptured, and the pressure at breakage was recorded. Figure 4-2 shows the configuration of the apparatus used to hold the balloon and biocontainer steady as the pressure reading (kPa) was recorded.



**Figure 4-2. Inflation Test Apparatus**

This test was conducted on new and used containers from the resiliency study to determine if container strength was compromised by the daily watering and conditions in the greenhouse.

## Growth and Plant Health

Based on the results of the resiliency study and the inflation test, the top two types of containers were selected for a greenhouse study. The plants selected for the study were Soybeans (Glycine max (L.) Merr cv. Hutcheson), Romaine lettuce (*Lactuca sativa* L. cv. Green Towers), and Sulfur Cosmos (*Cosmos suphureus* av. Cv. Cosmic Orange). Soybeans were

selected because the Virginia Team greenhouse bioassay team was very familiar with their growth and signs of stress in the plant. Romaine Lettuce was chosen because growth problems can be easily detected on the leaves. Sulfur Cosmos were chosen because the BioPots are more likely to be used for flowering plants. Each plant was grown in four replicates of each of two biocontainers and in peat pots (the control), for a total of 36 containers.

Seeds were germinated under mist irrigation with supplemental watering. The soybean and Cosmos seeds began germinating on April 20, 2016, and the lettuce began germinating on April 21, 2016. Germination counts were taken on April 24, 2016. When the seedlings were about a week old (April  $27<sup>th</sup>$ ), daily fertigation was begun with about 100 mL per container of a dilute solution of Peters Professional 20-20-20 fertilizer containing 200 ppm of N. This was supplemented with mist irrigation for 60-90 seconds, 6 times per day. All species were gradually thinned to one plant per container by May 11, 2016. On May 12, 2016, plants were sprayed with a solution containing 0.01% Pyrethrins and 0.10% Piperonyl Butoxide to control thrips (minute black-winged insects). The plants were harvested and their height was measured. Plant tissue was then dried in a  $60^{\circ}$  C oven for 48 hours and then weighed to determine biomass production. 

### **Results and Discussion**

#### Media Capacity

Media capacity was measured to be approximately 100 mL for all the containers. This volume was used for daily watering of the containers to replicate normal greenhouse and plant growth conditions. 

#### **Resiliency**

When the biosolids containers became saturated, they became more fragile. Although special care was taken when handling, several containers did not remain intact during the 6-week study. Several times during the 6-week study, containers had to be moved to make additional room in the greenhouse testing area. In some of the containers, the strength had become significantly weakened and the container wall ruptured. Of the 20 biosolids containers tested, three were broken during the resiliency study  $(2:1$  Cardboard+Starch, 2:1 Cellulose, and 2:1 Cardboard). Figure 4-3 shows a BioPot that was damaged due to watering and handling.



**Figure 4-3. Ruptured Biosolids Container**

Although most of the containers withstood the rigor of the evaluations, the majority did show some signs of wear and tear. Most of the containers were noticeability shorter than their original height due to the frequency of the wetting and drying cycles. The strength of the remaining containers was tested using the inflation test.

## Leachability

Leachate collected from all containers had EC and pH values that would not inhibit plant growth. The average values for EC and pH from the first leachability test on January 12, 2016 are shown in Table 4-1. The pH readings did not vary much between the different container types. Only the leachate from 2:1 Cardboard + Starch and 2:1 Cellulose had pH values significantly different from every other container. The leachate from neither the peat nor the plastic containers had pH values significantly different from the biosolids containers with the exception of 2:1 Cardboard + Starch and 2:1 Cellulose. Electrical conductivity values were significantly higher in the plastic containers than the Peat Pots and BioPots. None of the BioPots were significantly different from one another. The biosolids containers had EC values ranging from 0.156 to 0.215 dS/m. Peat Pots had significantly lower EC values, averaging 0.061 dS/m. A possible explanation is adsorption of the ions by the organic matter in the biosolids and peat containers. 

<b>Container Type</b>	$EC$ (dS/m)	рH
Plastic	$0.842a*$	7.21ab
Peat	0.061c	6.98 bc
2:1 Cellulose	$0.156$ bc	6.94 c
2:1 Cellulose + Starch	0.215 b	7.05 abc
2:1 Cardboard	$0.132$ bc	$7.10$ abc
2:1 Cardboard + Starch	$0.122$ bc	7.25a
5:1 Cardboard	$0.156$ bc	7.10 abc

**Table 4-1. EC and pH Results of Initial Leachability Test**

\*Means followed by the same letter within columns are not significantly different (P $\leq$ 0.05; Fisher's LSD).

Average leachate measurements for the different biocontainers and control plastic container are shown in Table 4-2. Significant differences were noted for the majority of the nutrients and metals between the BioPots, peat pots, and plastic containers. BioPots, with the exception of 2:1 Cardboard + Starch and 2:1 Cardboard, yielded significantly greater levels of Ortho-P than plastic and Peat Pots. Leachates from the 2:1 Cellulose + Starch and 2:1 Cellulose mixes contained the greatest amount of Ortho-P, 0.13 and 0.11 mg/L, respectively. Similar results were seen with  $NH_3-N$  measurements. All BioPots, with the exception of the 2:1 Cardboard + Starch and 2:1 Cardboard, produced leachate that had significantly greater levels of NH<sub>3</sub>-N than plastic and peat pots. The leachate from plastic containers contained significantly more  $NO<sub>3</sub>-N$ than that of all other biocontainers, whose concentrations were not significantly different from each other. 

Regarding metals in the leachate, Ni concentrations were not significantly different from one another, with the exception of 2:1 Cellulose + Starch, whose Ni concentration was significantly greater than all others at 8.00 ppb. The 2:1 Cellulose and 5:1 Cardboard mixes contained significantly greater concentrations of Cu, averaging 19.23 and 18.08 ppb, respectively. The only significant differences noted for Zn was between the plastic and peat containers. Peat Pots had a significantly greater concentration of Zn at 126.48 ppb, as compared to 14.80 pbb in leachate from the plastic containers. Leachate from the plastic control contained a significantly higher concentration of As than leachate from all other biocontainers. The Pb concentration in leachate from the plastic container was significantly higher than that from all the BioPots. Se, Cd, and Hg leachate concentrations were not significantly different in any of the containers.

<b>Container</b> <b>Type</b>	Ortho-P (mg/L)	$NO3-N;$ (mg/L)	$NH3-N$ $mg/L$ )	Ni (ppb)	Cu (ppb)	Zn (ppb)	As (ppb)	Se (ppb)	Mo (ppb)	Cd (ppb)	<b>Pb</b> (ppb)	Hg (ppb)
<b>Plastic</b>	$0.01 d*$	0.9a	0.35c	2.68 <sub>b</sub>	2.90 <sub>b</sub>	14.80 b	0.88a	0.43a	0.35c	0.10a	0.38a	0.05a
Peat	0.02 <sub>d</sub>	0.05 <sub>b</sub>	0.37c	1.55 <sub>b</sub>	3.68 <sub>b</sub>	126.48 a	0.25 <sub>b</sub>	0.25a	0.30c	$BD**$	0.25ab	0.05a
2:1 <b>Cellulose</b>	$0.11$ ab	0.06 <sub>b</sub>	1.42a	6.83ab	19.23a	32.20 ab	0.45 <sub>b</sub>	0.55a	12.50a	0.03a	0.03c	0.06a
2:1 <b>Cellulose</b> + Starch	0.13a	0.04 <sub>b</sub>	1.22a	8.00a	11.40 ab	96.28 ab	0.45 <sub>b</sub>	0.53a	9.95ab	0.03a	$0.13$ bc	0.07a
2:1 <b>Cardboard</b>	$0.05$ cd	0.03 <sub>b</sub>	0.15c	4.25ab	$10.48$ ab	24.13 ab	0.25 <sub>b</sub>	0.23a	5.90 abc	0.10a	0.08c	0.05a
2:1 Cardboard + Starch	0.02 <sub>d</sub>	0.03 b	$0.41$ bc	4.80 ab	9.63ab	50.18 ab	0.13 <sub>b</sub>	0.23a	3.78 bc	0.05a	$0.10$ bc	0.07a
5:1 <b>Cardboard</b>	0.08 <sub>bc</sub>	0.06 <sub>b</sub>	1.16ab	4.15ab	18.08a	73.75 ab	0.30 <sub>b</sub>	0.28a	5.40 abc	$BD**$	$0.10$ bc	0.06a

Table 4-2. Average Initial Leachate Values for Different Containers

\*Means followed by the same letter within columns are not significantly different (P≤0.05; Fisher's LSD).

\*\*BD = Below Detection

The final leachate measurements for EC yielded similar results to the initial test. Leachate from plastic containers again contained significantly higher EC levels than both the Peat Pots and BioPots. The pH value of the leachate from the plastic containers was also significantly higher than that of the biocontainers, though still at a level unlikely to impact plant health. Average EC and pH of the final leachability test are shown in Table 4-1b.

<b>Container Type</b>	$EC$ (dS/m)	рH
Plastic	$0.231a*$	$7.46a*$
Peat	$0.040$ bc	7.29c
2:1 Cellulose	$0.039$ bc	7.35 bc
2:1 Cellulose + Starch	0.033c	7.32 bc
2:1 Cardboard	0.056 b	7.38 abc
2:1 Cardboard + Starch	0.025c	7.39 abc
5:1 Cardboard	0.027c	7.38 ab

**Table 4-1b. EC and pH Results of Final Leachability Test**

\*Means followed by the same letter within columns are not significantly different (P≤0.05; Fisher's LSD).

Average final leachate measurements for the different biocontainers and control plastic container are shown in Table 4-2b. The plastic container's leachate did not have significantly different Ortho-P levels than the leachate from Peat Pot, 2:1 Cardboard + Starch, or 5:1 Cardboard containers. Ortho-P levels were significantly higher in leachate from 2:1 Cellulose, 2:1 Cellulose + Starch, and 2:1 Cardboard than the other containers. The majority of the Ortho-P measurements were in the same magnitude as the initial leachability tests. No significant differences in  $NO<sub>3</sub>$ -N readings occurred between the leachate from the different containers. Surprisingly, leachate from the plastic container had significantly greater  $NH<sub>3</sub>-N$  readings than the other biocontainers.

With respect to metals, Ni was significantly greater in leachate from 2:1 Cardboard containers. Previously, Ni was greatest in the 2:1 Cellulose + Starch container's leachate, however, now leachate from 2:1 Cellulose + Starch is not significantly different from all other containers' leachate, with the exception of 2:1 Cardboard. As with the initial leachability test, leachate from 2:1 Cellulose contained a significantly greater concentration of Cu than other containers. Zn was significantly greatest in the Peat Pot leachate, whose reading was a magnitude higher than other containers, at 217.30 ppb. All other containers' leachate did not have significantly different Zn concentrations. As with Zn, As was significantly greatest in the leachate from the plastic containers, followed by the Peat Pot. All BioPots did not differ significantly in leachate As. Mo was significantly greatest in leachate from 2:1 Cardboard, which was a change from the initial leachability test when Mo was not significantly different from the other containers. Leachate from plastic containers contained significantly greater concentrations of Cd than all

other biocontainers. Lead was significantly greatest in leachate from Plastic and Peat Pots compared to the BioPots, which were not significantly different from each other. Leachate from the 2:1 Cellulose + Starch contained a significantly greater concentration of Hg, but still at very low levels.

<b>Container</b> <b>Type</b>	Ortho- P (mg/L)	$NO3 - N;$ (mg/L)	$NH3-N$ $mg/L$ )	Ni (ppb)	Cu (ppb)	Zn (ppb)	As (ppb)	Se (ppb)	Mo (ppb)	C <sub>d</sub> (ppb)	Pb (ppb)	Hg (ppb)
<b>Plastic</b>	$0.01 b*$	0.79a	0.21a	1.39 <sub>b</sub>	4.72 bc	33.44 b	0.93a		0.47c	0.06a	0.73a	$0.03$ ab
Peat	0.02 <sub>b</sub>	0.13a	0.12 <sub>b</sub>	1.97ab	6.98 bc	217.30 a	0.43 <sub>b</sub>		0.31c	$0.03$ bc	0.64a	$0.04$ ab
2:1 <b>Cellulose</b>	0.06a	0.07a	0.09 <sub>bc</sub>	1.32 <sub>b</sub>	12.05a	22.26 <sub>b</sub>	0.20c		2.17ab	$0.02$ cd	0.07 <sub>b</sub>	$0.03$ ab
2:1 <b>Cellulose</b> + Starch	0.06a	0.06a	0.05c	0.94 <sub>b</sub>	5.40 bc	28.80 b	0.15c	Below	1.28 bc	0.02 <sub>d</sub>	0.02 <sub>b</sub>	0.06a
2:1 <b>Cardboard</b>	0.09a	0.06a	0.12 <sub>b</sub>	2.90a	9.52ab	26.77 b	0.24c	Detection	2.69a	0.04 <sub>b</sub>	0.14 <sub>b</sub>	$0.01$ ab
2:1 Cardboard + Starch	0.01 <sub>b</sub>	0.05a	0.02c	0.83 <sub>b</sub>	2.39c	17.27 b	0.08c	Limit	0.29c	$0.03$ cd	0.05 <sub>b</sub>	$0.01$ ab
5:1 <b>Cardboard</b>	0.02 <sub>b</sub>	0.07a	0.04c	1.64ab	4.20c	19.91 b	0.11c		0.26c	0.02 <sub>d</sub>	0.11 <sub>b</sub>	$BD**$

**Table 4-2b. Average Final Leachate Values for Different Containers**

\*Means followed by the same letter within columns are not significantly different (P≤0.05; Fisher's LSD).

\*\*BD = Below Detection

## Algal/Fungal Growth

The biosolids containers experienced some discoloration, but it was not due to algal or fungal growth. Several of the containers showed whitish areas that were determined to be gypsum or lime from the potting soil, which remained when the water evaporated through the container walls. None of the containers were discolored greater than 5%, as measured using "Percent of Area Covered" (Schoeneberger et al., 2012).

## Strength Testing

The inflation test developed for this experiment proved to be a consistent and representative measure of biocontainer strength. Table 4-3 shows the average pressures upon breakage for new containers and the containers that were put through the resiliency study. Of the new containers, 2:1 Cellulose, 2:1 Cellulose + Starch, and 5:1 were all significantly stronger than the 2:1 Cardboard and 2:1 Cardboard + Starch. Although the 2:1 Cellulose mix was the strongest of the new containers, its strength was no longer significantly different from the weaker  $2:1$ Cardboard or 2:1 Cardboard + Starch containers after the resiliency phase. Of the different biosolids container mixes, the 2:1 Cellulose + Starch and 5:1 Cardboard were the strongest of the biosolids containers, with the used containers recording average pressure readings at failure of 22.41 and 25.03 kPa, respectively. Note that these pressure readings are not significantly different than that of the used Peat Pot. The addition of starch did not appear to make a significant difference in terms of strength. However, these results are still promising because most of the containers were able to survive the resiliency study, and yet, would likely breakdown easily when planted.

<b>Container Type</b>	Average New (kPa)	<b>Average Used</b> (kPa)
Peat		35.37 a
2:1 Cellulose	35.37 a*	10.34 bc
2:1 Cellulose + starch	37.09 a	22.41 abc
2:1 Cardboard	23.30 b	8.62c
2:1 Cardboard + starch	26.75 b	13.79 bc
5:1 Cardboard	35.37 a	25.03 ab

**Table 4-3. Strength of New and Used Containers**

\*Means followed by the same letter within columns are not significantly different (P≤0.05; Fisher's LSD).

## Growth and Plant Health

Upon visual examination of the biosolids containers, roots were seen growing through the walls. This was not apparent on the peat pots. Rooting into the walls is actually desirable because it shows that the container walls were weak enough to allow for penetration by the roots and confirms a lack of root limiting chemical conditions. Figure 4-3 shows roots breaking through the container walls.



**Figure 4-3. Roots Penetrating the Walls of a 5:1 Cardboard Container**

Visually, all plants grown in the BioPots grew as well, or better, than the soybeans grown in the Peat Pots. Figure 4-4 shows how soybeans grew in all containers; note that the two biosolids containers are on the right. It was apparent that soybeans grown in the BioPots were healthy and significantly outperformed those in the Peat Pots for the 5:1 Cardboard. Table 4-4 shows the average soybean stem heights (cm) by type of container. The soybeans grown in the 2:1 Cellulose  $+$  Starch container were significantly taller than the soybeans in the Peat and 5:1 Cardboard containers. However, the soybeans grown in the Peat and 5:1 Cardboard containers were not significantly different, indicating a plant growth advantage for the 2:1 Cellulose  $+$ starch pots.





\*Means followed by the same letter within columns are not significantly different (P≤0.05; Fisher's LSD).



**Figure 4-4. Soybeans Grown in Greenhouse**

Figure 4-5 provides a visual comparison for the growth of Romaine lettuce in the different containers. The Romaine grown in biosolids containers were more robust, with larger leaf sizes and increased plant height, as compared to the romaine grown in peat pots.



**Figure 4-5. Romaine Lettuce Grown in Greenhouse**

A photo of Cosmos in the different test containers is provided in Figure 4-6. Compared to the Cosmos grown in the peat pot controls, the Cosmos in the biocontainers were larger, with fuller leaves and blooms. One of the 5:1 cardboard containers broke during the study. Table 4-5 shows the average cosmos stem heights (cm) sorted by type of container. Both the 2:1 Cellulose + Starch and 5:1 Cardboard produced cosmos plants that were significantly taller than in the Peat Pots.





\*Means followed by the same letter within columns are not significantly different (P≤0.05; Fisher's LSD).



**Figure 4-6. Cosmos Grown in Greenhouse**

Table 4-6 shows the average dry weight of plants grown in each container. The visual growth of the soybeans, romaine lettuce, and cosmos in the BioPots was very encouraging. Not only was the improved growth apparent, but biomass production was significantly higher. The dry weight results reported below for all three plants confirmed both the plant height and the visual comparison observations.

<b>Container</b>	Soybean (g)	Lettuce $(g)$	Cosmos (g)
Peat	$0.98 b*$	1.15 <sub>b</sub>	2.75 <sub>b</sub>
2:1 Cellulose + starch	1.79a	3.3a	5.65a
5:1 Cardboard	1.46a	3.04a	4.77 ab

**Table 4-6. Dry Weight of Plants in Different Containers**

\*Means followed by the same letter within columns are not significantly different (P≤0.05; Fisher's LSD).

Dry weight of soybeans grown in BioPots was significantly greater than the soybeans grown in the Peat Pot. Though the 5:1 Cardboard did not produce a soybean significantly different from the Peat Pot in terms of height, the dry weight of the biomass produced was significantly

greater. As with soybeans, the dry weights of lettuce grown in the BioPots was also significantly greater than the lettuce grown in the Peat Pot. In several cases, the dry weight of plants grown in the biosolids containers were twice as heavy as the plants grown in peat pots. In the case of romaine lettuce, the dry weight of the plants grown in the biosolids containers was almost 3 times that of the control plants. The dry weight of the cosmos grown in the 2:1 Cellulose  $+$ Starch container was significantly greater than the cosmos grown in the Peat Pot. The cosmos grown in the  $2:1$  Cellulose + Starch containers produced biomass greater than twice that of the cosmos in the Peat Pots.

### **Conclusions**

The conclusions derived from this study are as follows:

- Under the conditions of this study, biosolids used as a base ingredient in biocontainers enhanced plant growth. In all cases, plants grown in BioPots produced significantly more biomass than plants grown in Peat Pots. In the case of the Cosmos and Romaine, the plants grown in BioPots were about two and three times heavier, respectively.
- A consistent and easily reproducible strength test was developed as part of this study. It is believed that this procedure will enable researchers to have a better metric for the comparison of the strength of biocontainers made from different types of materials.
- In most cases, container material did not affect the concentrations of nutrients or metals in the leachate.

In summary, plant growth seen in the biosolids containers confirmed the potential of using biosolids as a base ingredient for biocontainers. Biosolids are proven to increase plant yield and health as a viable organic fertilizer. Though it was not quantified, it was observed that BioPots retained moisture longer than Peat Pots. Visually, the water evaporated through the walls of the Peat Pots quicker, causing the soil in those containers to dry before the soil in the BioPots. The capability of the BioPot to retain water longer could be a defining characteristic that allowed the plants in the BioPots to outgrow their Peat Pot counterparts. Additional research is needed to determine the scale-up of operations for the production of BioPots and marketability potential. The production process for this study was time consuming and only intended to generate containers on a small-scale. The characteristics of the biosolids feedstock (liquid vs. dewatered cake) will likely have a significant impact on the mix required for the biocontainers. The type and dosage of additives will ultimately affect the structural integrity and durability of the biosolids containers, which need to be evaluated under rigorous transportation and handling conditions. Since many utilities in urban settings dewater their biosolids, research to optimize the blend recipe using dewatered cake is recommended. Once durability and greenhouse studies are conducted as presented herein, scale-up challenges can be addressed. Marketability as a sustainable alternative to floriculture's plastic pots should be evaluated. A high quality biosolids-derived biocontainer would provide utilities with a beneficial product and brand to offer customers.

The RRWPCP does not currently produce Class A biosolids, but by producing biodegradable transplant pots, the Authority hopes to produce a high-value, sustainable product that meets Class A requirements and diversifies their current biosolids management program.

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# Chapter 5: Conclusions and Recommendations

With many wastewater utilities seeking alternative biosolids management methods to become more sustainable, biocontainer production could be a viable alternative. Based on this research, it appears that lagooned biosolids with the correct mix of additives can yield solid biocontainers that could be marketed based on performance parameters. The fiber sources provided rigidity, and help to increase marketability as a legitimate commercial product.

The conclusions derived from this study are as follows:

- Biosolids can be beneficially used and implemented as a base ingredient along with other fiber additives for increased support. Additional research using a dewatered product could explore the possibility of using 100% biosolids with no additional fiber for support.
- Biosolids for use as a base ingredient in biocontainers similar to those produced in this study will not inhibit plant growth and in fact appear to significantly enhance plant growth. In all cases, plants grown in BioPots produced more biomass than plants grown in Peat Pots. In the case of the Cosmos and Romaine, the plants grown in BioPots were about two and three times heavier, respectively.
- A consistent and easily reproducible strength test using pressure on containers was developed. This test should allow other researchers conducting testing on various biocontainers to have a common metric to compare strengths across materials.

The biocontainer construction process for this study was time-consuming and only intended to generate a product for research testing. The characteristics of the biosolids feedstock (liquid vs. dewatered cake) will likely have a significant impact on the mix required for the biocontainers. Perhaps a dewatered cake product, at a higher total solids concentration, would be a preferred feedstock for producing potentially marketable biocontainers, and would require less additional fibers. The type and dosage of additives will ultimately affect the structural integrity and durability of the biosolids containers, which need to be evaluated under rigorous transportation and handling conditions. Since many utilities in urban settings dewater their biosolids, research to optimize the blend recipe using dewatered cake is recommended. To meet regulatory requirements for the final biocontainer product, Class A biosolids should be considered for use as a feedstock.

Plant growth seen in the biosolids containers confirmed the potential of using biosolids as a base ingredient for a biocontainer. Biosolids are proven to increase plant yield and health as an organic fertilizer. This research was performed with biosolids from a single source and solids handling process. Additional research is needed to determine scale-up of operation to produce BioPots and marketability potential. Biosolids from different sources might also contain different constituents, such as metals, organics, and added polymers that would need to be considered. Thus, any product derived from biosolids would need to meet applicable biosolids regulations, namely the Class A requirements stipulated in the federal Part 503 rule.

Once durability and greenhouse studies are conducted as presented herein, additional research to address scale-up challenges is recommended. Potential marketability and demand as a sustainable alternative to floriculture plastic pots should be evaluated. This would necessarily need to include an evaluation of public perception issues related to the use of biosolids in a home or garden direct contact environment. A high quality biosolids-derived biocontainer would provide utilities with a beneficial product and brand to offer customers.

# Appendix A



#### **Table A1-Tensile Strength Data (N)**

#### **Output A1-Analysis of Tensile Test Results**

#### **One-way ANOVA: Load at failure versus TRT**

Source DF SS MS F P TRT 17 21669 1275 2.44 0.006 Error 57 29814 523 Total 74 51483

 $S = 22.87$   $R-Sq = 42.09%$   $R-Sq(adj) = 24.82%$ 

 Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ----+---------+---------+---------+----- 1to1CB 4 50.75 16.11 (-------\*-------) 1to1CBP 4 48.12 25.38 (-------\*-------)<br>1to1CBST 4 75.50 32.52 (------\*---------) 1to1CBST 4 75.50 32.52 (------\*-------) 1to1Cell 4 62.98 3.39 (-------\*-------) 1to1cellP 4 57.25 15.11 (-------\*-------)<br>1to1CellST 4 60.50 30.92 (------\*-------) 1to1CellST 4 60.50 30.92 2to1CB 5 77.42 40.52 (------\*------) 2to1CBP 4 39.95 15.76 (------\*-------) 2to1CBST 6 81.50 25.19 (-----\*-----) 2to1Cell 4 69.50 14.82 (------\*-------) 2to1cellP 4 47.63 20.95 (-------\*-------)



Pooled StDev = 22.87

Grouping Information Using Fisher Method



Means that do not share a letter are significantly different.

**Figure A1- Broken BioPot Coupon**



Sample ID	pH	$SC$ (dS/m)
2:1 Cardboard	7.47	2.06
2:1 Cardboard	7.54	2.02
2:1 Cardboard	7.52	2.03
$2:1$ Cardboard + st	7.59	1.68
$2:1$ Cardboard + st	7.60	1.68
$2:1$ Cardboard + st	7.60	1.68
2:1 Cellulose	7.51	2.6
2:1 Cellulose	7.50	2.61
2:1 Cellulose	7.49	2.61
$2:1$ Cellulose + st	7.60	1.56
$2:1$ Cellulose + st	7.60	1.54
2:1 Cellulose + st	7.60	1.57
5:1 Cardboard	7.81	0.88
5:1 Cardboard	7.81	0.89
5:1 Cardboard	7.80	0.88

**Table A2. pH and Electrical Conductivity Measurements of Ground Mixture**

## **Table A3. Germination Results**



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# **Figure A2-3D Printed BioPot Mold**





**Figure A3-Lined BioPot Mold Cup**

# Appendix B

**Figure B1-Conducting Media Capacity Test** 





**Figure B2-Broken BioPot During Resiliency Testing**



<b>Name</b>	Mix	Weight (g)	Ortho-P (mg/L)	<b>Nitrate</b> (mg $N/L$ as $NO3$ )	Ammonia (mg N/L) as $NH3$ )	60Ni (ppb)	<b>65Cu</b> (ppb)	66Zn (ppb)	<b>75As</b> (ppb)	<b>78Se</b> (ppb)	95Mo (ppb)	111Cd (ppb)	<b>208Pb</b> (ppb)	202Hg (ppb)
$\mathbf{1}$	Plastic	18	0.0083	0.893	0.3376	2.3	3.5	18.5	$\mathbf{1}$	0.9	0.4	0.4	0.6	0.039
$\overline{2}$	Plastic	18	0.004	0.7916	0.3247	2.1	2.5	13.7	0.7	0.5	0.4	0	0.1	0.073
$\mathbf{3}$	Plastic	18	0.009	0.934	0.2647	4.8	2.8	12.8	0.9	$\mathbf{0}$	0.3	0	0.4	0.038
4	Plastic	18	0.0203	0.9807	0.4746	1.5	2.8	14.2	0.9	0.3	0.3	0	0.4	0.044
5	Peat	15.4	0.0473	0.0524	0.5136	$1.2\,$	4.5	294.5	0.3	0.2	0.3	0	0.4	0.049
6	Peat	15	0.0262	0.0532	0.3514	1.7	4.3	147.6	0.5	0.2	0.3	$\mathbf 0$	0.4	0.053
$\overline{7}$	Peat	15	0.0132	0.0401	0.3342	2.3	2.6	27	0.1	0.2	0.3	0	0.1	0.04
8	Peat	15.4	0.0123	0.0473	0.2837	1	3.3	36.8	0.1	0.4	0.3	0	0.1	0.043
9	2:1 Cell	51.2	0.1057	0.0898	1.3101	8.7	25.6	32.2	0.5	0.5	16.9	0	0	0.079
10	2:1 Cell	42.3	0.0956	0.0834	1.2384	4.8	11.9	21.8	0.3	0.3	10.1	0	$\Omega$	0.058
11	2:1 Cell	41.7	0.1919	0.0631	3.0479	11.7	33.9	66.4	0.8	$\mathbf{1}$	19.7	0.1	0.1	0.068
12	2:1 Cell	40.6	0.0636	0.012	0.0801	2.1	5.5	8.4	0.2	0.4	3.3	0	0	0.048
13	2:1 Cell St	46.2	0.1682	0.0465	1.8272	16	21.2	284.1	1.2	1.2	26.3	0.1	0.2	0.132
14	2:1 Cell St	41.6	0.1429	0.0311	1.159	2.6	7.5	44.6	0.2	0.1	3.5	0	0.1	0.053
15	2:1 Cell St	44.6	0.1299	0.0246	1.0699	2.9	6.8	31.8	0.1	0.3	3.2	0	0.1	0.042
16	2:1 Cell St	46.8	0.0875	0.0538	0.8265	10.5	10.1	24.6	0.3	0.5	6.8	0	0.1	0.054
17	2:1CB	31.7	0.0367	0.0201	0.1128	3.5	7.8	18.7	0.2	0.4	5.3	0	0	0.051
18	2:1CB	32.2	0.0571	0.031	0.0347	2.4	6.3	14.3	0.1	0.1	3.4	0	0	0.044
19	2:1CB	28.5	0.0321	0.0228	0.0701	4.3	11.1	21.6	0.2	0.3	6.4	0.4	0.1	0.048
20	2:1CB	32.1	0.0758	0.0507	0.3732	6.8	16.7	41.9	0.5	0.1	8.5	$\Omega$	0.2	0.073
21	$2:1$ CB st	34.5	0.0076	0.0448	0.3257	4.4	5.7	28.6	0	0.2	2.3	0.1	0.1	0.067
22	$2:1$ CB st	37.1	0.0217	0.025	0.1892	1.8	4.1	14.5	$-0.1$	0.1	$\mathbf{1}$	$\pmb{0}$	0.1	0.036
23	$2:1$ CB st	30.3	0.0227	0.0285	0.3524	1.6	4.9	31.4	0	0.4	1.3	0	0.1	0.052
24	$2:1$ CB st	30.1	0.0088	0.0393	0.7744	11.4	23.8	126.2	0.6	0.2	10.5	0.1	0.1	0.143

**Table B1-Initial Leachability Test Results**



Pot #	<b>Treatment</b>	Rep	$EC$ (dS/m)	pH
$\mathbf{1}$	PL	1	0.898	7.29
$\overline{2}$	PL	$\overline{2}$	0.883	7.25
$\overline{3}$	PL	3	0.916	7.10
$\overline{4}$	PL	4	0.670	7.19
5	PT	$\mathbf{1}$	0.107	6.77
6	PT	$\overline{2}$	0.068	6.61
$\overline{7}$	PT	3	0.035	7.30
8	PT	4	0.035	7.23
9	2:1 CELL	$\mathbf{1}$	0.135	6.99
10	$2:1$ CELL	$\overline{2}$	0.140	6.84
11	2:1 CELL	3	0.244	6.90
12	2:1 CELL	4	0.105	7.01
13	2:1 CELL ST	$\mathbf{1}$	0.334	7.04
14	2:1 CELL ST	$\overline{2}$	0.083	7.07
15	2:1 CELL ST	3	0.312	7.00
16	2:1 CELL ST	4	0.132	7.10
17	2:1CB	$\mathbf{1}$	0.115	7.06
18	2:1CB	$\overline{2}$	0.089	7.06
19	2:1CB	3	0.132	7.12
20	2:1CB	4	0.190	7.14
21	2:1 CB ST	$\overline{1}$	0.085	7.10
22	2:1 CB ST	$\overline{2}$	0.042	7.19
23	2:1 CB ST	3	0.054	7.43
24	2:1 CB ST	4	0.306	7.28
25	5:1CB	$\mathbf{1}$	0.162	6.85
26	5:1CB	$\overline{2}$	n/a	n/a
27	$5:1$ CB	3	0.209	7.14
28	5:1 CB	4	0.097	7.32

Table B2-pH and EC from Initial Leachability Test

# **Output B1-Initial Leachability Results**

### **RBP INITIAL pour-through leachate stats for Peyton (ANOVA with Fisher's LSD at 95% - run on Minitab 16) One-way ANOVA: Initial EC (dS/m) versus TRT**



Pooled StDev = 0.08955

Grouping Information Using Fisher Method



#### **One-way ANOVA: Initial pH versus TRT**

Source DF SS MS F P TRT 6 0.3105 0.0518 1.82 0.145 Error 20 0.5677 0.0284 Total 26 0.8782  $S = 0.1685$  R-Sq = 35.36% R-Sq(adj) = 15.97% Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev --+---------+---------+---------+------- 2:1 CB 4 7.0950 0.0412 (--------\*--------) 2:1 CB st 4 7.2500 0.1407 2:1 Cell 4 6.9350 0.0794 (--------\*--------) 2:1 Cell St 4 7.0525 0.0427 (--------\*-------) 5:1 CB 3 7.1033 0.2371 (---------\*---------)<br>Peat 4 6.9775 0.3395 (--------\*--------) 4 6.9775 0.3395 (--------\*--------) Plastic 4 7.2075 0.0826 (-------\*--------) --+---------+---------+---------+------- 6.80 7.00 7.20 7.40

Pooled StDev = 0.1685

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 CB st 4 7.2500 A Plastic 4 7.2075 A B<br>5:1 CB 3 7.1033 A B 3 7.1033 A B C 2:1 CB 4 7.0950 A B C 2:1 Cell St 4 7.0525 A B C Peat 4 6.9775 B C 2:1 Cell 4 6.9350 C

#### **One-way ANOVA: Initial Ortho-P (mg/L) versus TRT**

Source DF SS MS F P TRT 6 0.056734 0.009456 12.33 0.000 Error 21 0.016100 0.000767 Total 27 0.072834  $S = 0.02769$  R-Sq = 77.89% R-Sq(adj) = 71.58% Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ----+---------+---------+---------+----- 2:1 CB 4 0.05042 0.02010 (-----\*-----) 2:1 CB st 4 0.01520 0.00811 (-----\*-----) 2:1 Cell 4 0.11420 0.05482 (-----\*-----)<br>2:1 Cell St 4 0.13212 0.03373 (----\*------) 2:1 Cell St 4 0.13212 0.03373 (-<br>5:1 CB 4 0.07560 0.02095 (-----\*-----) 5:1 CB 4 0.07560 0.02095 Peat 4 0.02475 0.01632 (-----\*-----) Plastic 4 0.01040 0.00696 (-----\*-----) ----+---------+---------+---------+----- 0.000 0.050 0.100 0.150

Pooled StDev =  $0.02769$ 

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 Cell St 4 0.13212 A 2:1 Cell 4 0.11420 A B 5:1 CB 4 0.07560 B C<br>2:1 CB 4 0.05042 C D 2:1 CB 4 0.05042 C D Peat 4 0.02475 D 2:1 CB st 4 0.01520 D Plastic 4 0.01040 D

# **One-way ANOVA: Initial Nitrate (mg/L) versus TRT**



Grouping Information Using Fisher Method



#### **One-way ANOVA: Initial Ammonium (mg/L) versus TRT**

Source DF SS MS F P TRT 6 6.467 1.078 3.93 0.009 Error 21 5.757 0.274 Total 27 12.224  $S = 0.5236$  R-Sq = 52.90% R-Sq(adj) = 39.45% Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev -------+---------+---------+---------+-- 2:1 CB 4 0.1477 0.1537 (--------\*-----------) 2:1 CB st 4 0.4104 0.2530 (--------\*---------) 2:1 Cell 4 1.4191 1.2234 (--------\*--------)<br>2:1 Cell St 4 1.2207 0.4281 (--------\*--------) 2:1 Cell St 4 1.2207 0.4281 5:1 CB  $\begin{array}{cccc} 4 & 1.1623 & 0.3655 \\ 4 & 0.3707 & 0.0995 \end{array}$  (-------\*-------) 4 0.3707 0.0995 Plastic 4 0.3504 0.0887 (--------\*--------) -------+---------+---------+---------+-- 0.00 0.60 1.20 1.80

Pooled StDev = 0.5236

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 Cell 4 1.4191 A 2:1 Cell St 4 1.2207 A 5:1 CB 4 1.1623 A B 2:1 CB st 4 0.4104 B C Peat 4 0.3707 C Plastic 4 0.3504 C 2:1 CB 4 0.1477 C

#### **One-way ANOVA: Initial Ni (ppb) versus TRT**

Source DF SS MS F P TRT 6 119.5 19.9 1.53 0.216 Error 21 273.0 13.0 Total 27 392.5  $S = 3.606$  R-Sq = 30.45% R-Sq(adj) = 10.58% Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ------+---------+---------+---------+--- 2:1 CB 4 4.250 1.870 (----------\*----------)<br>2:1 CB st 4 4.800 4.581 (----------\*---------) 2:1 CB st 4 4.800 4.581 (----------\*---------) 2:1 Cell 4 6.825 4.231 (-----------\*---------)<br>2:1 Cell St 4 8.000 6.466 (-----------\*--------------) 2:1 Cell St 4 8.000 6.466 5:1 CB 4 4.150 2.086 (----------\*----------)  $4 \quad 1.550 \quad 0.580 \quad (-------+---+)$ Plastic 4 2.675 1.457 (-----------\*----------) ------+---------+---------+---------+--- 0.0 3.5 7.0 10.5

Pooled StDev = 3.606

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 Cell St 4 8.000 A 2:1 Cell 4 6.825 A B 2:1 CB st 4 4.800 A B 2:1 CB 4 4.250 A B 5:1 CB 4 4.150 A B Plastic 4 2.675 B Peat 4 1.550 B

#### **One-way ANOVA: Initial Cu (ppb) versus TRT**

Source DF SS MS F P TRT 6 955.7 159.3 2.73 0.041 Error 21 1226.6 58.4 Total 27 2182.2  $S = 7.642$  R-Sq = 43.79% R-Sq(adj) = 27.73% Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev -----+---------+---------+---------+---- 2:1 CB 4 10.475 4.609 (------\*-------)<br>2:1 CB st 4 9.625 9.473 (-------\*-------) 2:1 CB st 4 9.625 9.473 2:1 Cell 4 19.225 12.884 (-------\*-------)<br>2:1 Cell St 4 11.400 6.686 (-------\*-------) 2:1 Cell St 4 11.400 6.686 5:1 CB  $\begin{array}{cccc} 4 & 18.075 & 9.285 & (-----*-----* \n\end{array}$  Peat  $\begin{array}{cccc} 4 & 3.675 & 0.888 & (------*----- \n\end{array}$  $4 \quad 3.675 \quad 0.888 \quad (- \cdots + \cdots + \cdots - \cdots)$ Plastic 4 2.900 0.424 (-------\*-------) -----+---------+---------+---------+---- 0 10 20 30

Pooled StDev = 7.642

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 Cell 4 19.225 A 5:1 CB 4 18.075 A 2:1 Cell St 4 11.400 A B 2:1 CB 4 10.475 A B 2:1 CB st 4 9.625 A B Peat 4 3.675 B Plastic 4 2.900 B

Means that do not share a letter are significantly different.

-15 0 15 30

#### **One-way ANOVA: Initial Zn (ppb) versus TRT**

Source DF SS MS F P TRT 6 40490 6748 1.32 0.290 Error 21 107029 5097 Total 27 147519

 $S = 71.39$  R-Sq = 27.45% R-Sq(adj) = 6.72%

 Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev --------+---------+---------+---------+- 2:1 CB 4 24.13 12.22 (---------\*----------)<br>2:1 CB st 4 50.17 51.22 (---------\*-----------) 2:1 CB st 4 50.17 51.22 2:1 Cell 4 32.20 24.79 (----------\*---------) 2:1 Cell St 4 96.28 125.49 5:1 CB 4 73.75 31.56 (----------\*----------)<br>Peat 4 126.48 124.65 (-----------\*---(----------\*--------------) Plastic 4 14.80 2.53 (---------\*-----------) --------+---------+---------+---------+- 0 70 140 210

Pooled StDev = 71.39

Grouping Information Using Fisher Method

TRT N Mean Grouping Peat 4 126.48 A 2:1 Cell St 4 96.28 A B 5:1 CB 4 73.75 A B 2:1 CB st 4 50.17 A B 2:1 Cell 4 32.20 A B 2:1 CB 4 24.13 A B Plastic 4 14.80 B

#### **One-way ANOVA: Initial As (ppb) versus TRT**

Source DF SS MS F P TRT 6 1.4393 0.2399 2.94 0.031 Error 21 1.7150 0.0817 Total 27 3.1543  $S = 0.2858$  R-Sq = 45.63% R-Sq(adj) = 30.10% Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev -----+---------+---------+---------+---- 2:1 CB 4 0.2500 0.1732 (-------\*---------) 2:1 CB st 4 0.1250 0.3202 (--------\*-------)<br>2:1 Cell 4 0.4500 0.2646 (--------\*-------) 2:1 Cell 4 0.4500 0.2646 (--------\*-------)<br>2:1 Cell St 4 0.4500 0.5066 (--------\*-------) 2:1 Cell St 4 0.4500 0.5066 (--------\*----<br>5:1 CB 4 0.3000 0.2449 (--------\*-------) 5:1 CB  $\begin{array}{cccc} 4 & 0.3000 & 0.2449 \\ 4 & 0.2500 & 0.1915 \end{array}$ 4 0.2500 0.1915 (-------\*---------) Plastic 4 0.8750 0.1258 (-------\*-------) -----+---------+---------+---------+---- 0.00 0.35 0.70 1.05

Pooled StDev = 0.2858

Grouping Information Using Fisher Method

TRT N Mean Grouping Plastic 4 0.8750 A 2:1 Cell St 4 0.4500 B 2:1 Cell 4 0.4500 B 5:1 CB 4 0.3000 B Peat 4 0.2500 B 2:1 CB 4 0.2500 B 2:1 CB st 4 0.1250 B

#### **One-way ANOVA: Initial Se (ppb) versus TRT**

Source DF SS MS F P TRT 6 0.4921 0.0820 1.11 0.392 Error 21 1.5575 0.0742 Total 27 2.0496  $S = 0.2723$  R-Sq = 24.01% R-Sq(adj) = 2.30% Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev --+---------+---------+---------+------- 2:1 CB 4 0.2250 0.1500 (----------\*------------) 2:1 CB st 4 0.2250 0.1258 (----------\*----------) 2:1 Cell 4 0.5500 0.3109 (----------\*----------) 2:1 Cell St 4 0.5250 0.4787 5:1 CB  $\begin{array}{cccc} 4 & 0.2750 & 0.0500 & (-------*-------)- \\ \text{Peat} & 4 & 0.2500 & 0.1000 & (-------*-----------) \end{array}$  $9.2500 \quad 0.1000 \quad (-------+------)$ Plastic 4 0.4250 0.3775 (----------\*-----------) --+---------+---------+---------+------- 0.00 0.25 0.50 0.75

Pooled StDev = 0.2723

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 Cell 4 0.5500 A 2:1 Cell St 4 0.5250 A Plastic 4 0.4250 A<br>5:1 CB 4 0.2750 A 4 0.2750 A Peat 4 0.2500 A 2:1 CB st 4 0.2250 A 2:1 CB 4 0.2250 A

#### **One-way ANOVA: Initial Mo (ppb) versus TRT**

Source DF SS MS F P TRT 6 502.0 83.7 2.76 0.039 Error 21 636.2 30.3 Total 27 1138.2

 $S = 5.504$   $R-Sq = 44.10$   $R-Sq(adj) = 28.13$ 

 Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ---------+---------+---------+---------+ 2:1 CB 4 5.900 2.131 (---------\*--------) 2:1 CB st 4 3.775 4.518 (--------\*----------) 2:1 Cell 4 12.500 7.339 (---------\*--------)<br>2:1 Cell St 4 9.950 11.021 (---------\*--------) 2:1 Cell St 4 9.950 11.021 5:1 CB  $4$  5.400 3.432 (---------\*---------)<br>Peat  $4$  0.300 0.000 (--------\*--------)  $4 \quad 0.300 \quad 0.000 \quad (-------+---+)$ Plastic 4 0.350 0.058 (---------\*---------) ---------+---------+---------+---------+ 0.0 6.0 12.0 18.0

Pooled StDev = 5.504

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 Cell 4 12.500 A 2:1 Cell St 4 9.950 A B 2:1 CB 4 5.900 A B C 5:1 CB 4 5.400 A B C 2:1 CB st 4 3.775 B C Plastic 4 0.350 C Peat 4 0.300 C

Means that do not share a letter are significantly different.

Fisher 95% Individual Confidence Intervals

#### **One-way ANOVA: Initial Cd (ppb) versus TRT**



 Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev --+---------+---------+---------+------- 2:1 CB 4 0.1000 0.2000 (-----------\*-----------) 2:1 CB st 4 0.0500 0.0577 (-----------\*-------------) 2:1 Cell 4 0.0250 0.0500 (------------\*------------) 2:1 Cell St 4 0.0250 0.0500 (------------\*----------)<br>5:1 CB 4 0.0000 0.0000 (-----------\*------------)  $(-------+---+--------)$ Peat 4 0.0000 0.0000 (-----------\*-----------) Plastic 4 0.1000 0.2000 (-----------\*-------------) --+---------+---------+---------+-------  $-0.10$  0.00 0.10 0.20

Pooled StDev = 0.1123

Grouping Information Using Fisher Method

TRT N Mean Grouping Plastic 4 0.1000 A 2:1 CB 4 0.1000 A 2:1 CB st 4 0.0500 A 2:1 Cell St 4 0.0250 A 2:1 Cell 4 0.0250 A Peat 4 0.0000 A 5:1 CB 4 0.0000 A

#### **One-way ANOVA: Initial Pb (ppb) versus TRT**



 Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ------+---------+---------+---------+--- 2:1 CB 4 0.0750 0.0957 (-------\*-------) 2:1 CB st 4 0.1000 0.0000 (-------\*------) 2:1 Cell 4 0.0250 0.0500 (-------\*------) 2:1 Cell St 4 0.1250 0.0500 (------\*-------)<br>5:1 CB 4 0.1000 0.0000 (-------\*------) 4 0.1000 0.0000 Peat 4 0.2500 0.1732 (-------\*------) Plastic 4 0.3750 0.2062 (-------\*-------) ------+---------+---------+---------+---  $0.00$   $0.15$   $0.30$   $0.45$ 

Pooled StDev = 0.1113

Grouping Information Using Fisher Method

TRT N Mean Grouping Plastic 4 0.3750 A Peat 4 0.2500 A B 2:1 Cell St 4 0.1250 B C 5:1 CB 4 0.1000 B C 2:1 CB st 4 0.1000 B C 2:1 CB 4 0.0750 C 2:1 Cell 4 0.0250 C

#### **One-way ANOVA: Initial Hg (ppb) versus TRT**

Source DF SS MS F P TRT 6 0.002729 0.000455 0.64 0.694 Error 21 0.014817 0.000706 Total 27 0.017546  $S = 0.02656$  R-Sq = 15.55% R-Sq(adj) = 0.00% Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ---+---------+---------+---------+------ 2:1 CB 4 0.05400 0.01299 (----------\*-------------) 2:1 CB st 4 0.07450 0.04739 (----------\*-----------)<br>2:1 Cell 4 0.06325 0.01330 (----------\*------------) 2:1 Cell 4 0.06325 0.01330 (----------\*-----------)<br>2:1 Cell St 4 0.07025 0.04152 (----------\*-----------) 2:1 Cell St 4 0.07025 0.04152 5:1 CB  $\begin{array}{cccc} 4 & 0.05850 & 0.01777 & (-------*---------)-\\ \text{Peat} & 4 & 0.04625 & 0.00585 & (-------*---*-------)- \end{array}$  $4 \quad 0.04625 \quad 0.00585 \quad (-------+---+)$ Plastic 4 0.04850 0.01654 (-----------\*-----------) ---+---------+---------+---------+------ 0.025 0.050 0.075 0.100

Pooled StDev =  $0.02656$ 

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 CB st 4 0.07450 A 2:1 Cell St 4 0.07025 A 2:1 Cell 4 0.06325 A 5:1 CB 4 0.05850 A 2:1 CB 4 0.05400 A Plastic 4 0.04850 A Peat 4 0.04625 A

Mix	Ortho-P (mg/L)	<b>Nitrate</b> $(NO3-N)$ $mg/L$ )	Ammonia $(NH_3-N)$ mg/L)	Ni (ppb)	Cu (ppb)	Zn (ppb)	As (ppb)	Se (ppb)	Mo (ppb)	Cd (ppb)	Pb (ppb)	Hg (ppb)
<b>Plastic</b>	0.01	0.16	0.30	1.49	10.03	39.09	0.88	$-0.82$	0.56	0.06	0.71	0.04
<b>Plastic</b>	0.00	0.19	0.19	1.39	3.26	31.52	0.89	$-1.20$	0.49	0.05	0.41	0.02
<b>Plastic</b>	0.00	0.19	0.19	1.19	2.39	32.80	0.83	$-0.97$	0.42	0.05	0.82	0.03
<b>Plastic</b>	0.03	2.61	0.16	1.50	3.18	30.36	1.10	$-1.03$	0.42	0.06	0.98	0.01
Peat	0.03	0.21	0.12	1.96	13.02	302.30	0.59	$-0.63$	0.42	0.04	0.84	0.04
Peat	0.03	0.11	0.13	2.26	7.82	329.60	0.68	$-0.77$	0.53	0.04	0.88	0.05
Peat	0.01	0.09	0.06	0.74	3.13	122.70	0.24	$-1.17$	0.15	0.02	0.42	0.03
Peat	0.01	0.12	0.17	2.90	3.93	114.60	0.22	$-0.86$	0.15	0.02	0.42	0.04
2:1 Cell	0.06	0.06	0.03	1.16	12.98	18.99	0.18	$-1.15$	1.49	0.02	0.09	0.04
2:1 Cell	0.06	0.07	0.15	1.70	13.64	18.83	0.22	$-0.64$	3.03	0.02	0.03	0.02
$2:1$ Cell	0.05	0.08	0.09	1.10	9.52	28.95	0.21	$-0.95$	1.99	0.02	0.08	0.02
2:1 Cell St	0.09	0.09	0.11	1.13	9.64	45.94	0.21	$-0.85$	2.06	0.02	0.03	0.17
2:1 Cell St	0.07	0.05	0.01	1.16	6.05	24.99	0.18	$-0.86$	1.86	0.02	0.03	0.02
2:1 Cell St	0.04	0.05	0.05	0.51	3.38	22.66	0.11	$-0.99$	0.58	0.01	0.00	0.01
2:1 Cell St	0.04	0.06	0.04	0.96	2.51	21.61	0.10	$-1.06$	0.62	0.02	0.03	0.03
2:1CB	0.07	0.05	0.11	3.20	9.55	22.00	0.33	$-0.90$	4.01	0.03	0.18	0.01
2:1CB	0.06	0.04	0.15	1.86	9.44	29.14	0.23	$-0.64$	3.36	0.04	0.04	0.02
2:1CB	0.14	0.10	0.11	3.64	9.56	29.18	0.16	$-1.00$	0.70	0.05	0.19	0.01
2:1CBst	0.01	0.04	0.00	0.91	2.22	15.14	0.07	$-0.89$	0.21	0.03	0.05	0.01
2:1CBst	0.01	0.05	0.03	0.75	2.56	19.41	0.09	$-0.90$	0.36	0.02	0.04	0.01
5:1CB	0.04	0.06	0.05	0.96	6.72	22.60	0.15	$-0.31$	0.39	0.02	0.05	0.00
5:1 CB	0.01	0.06	0.05	0.57	2.11	19.02	0.07	$-1.10$	0.08	0.01	0.02	0.00
5:1 CB	0.02	0.05	0.02	0.72	4.26	15.90	0.13	$-1.07$	0.40	0.01	0.01	0.00
5:1 CB	0.02	0.10	0.04	4.29	3.71	22.11	0.10	$-1.03$	0.17	0.02	0.34	0.01

**Table B3-Final Leachability Test Data**

Pot				
#	<b>Treatment</b>	Rep	$EC$ (dS/m)	pH
$\overline{1}$	PL	1	0.223	7.57
$\overline{2}$	PL	$\overline{2}$	0.252	7.48
3	PL	3	0.252	7.46
4	PL	4	0.205	7.34
5	PT	$\overline{1}$	0.048	7.23
6	PT	$\overline{2}$	0.050	7.17
$\overline{7}$	PT	3	0.031	7.34
8	PT	4	0.032	7.40
9	2:1 CELL	1	0.035	7.36
10	2:1 CELL	$\overline{2}$	0.042	7.34
11	2:1 CELL	3	0.042	7.35
12	2:1 CELL	4	no pot	no pot
13	2:1 CELL ST	$\overline{1}$	0.038	7.32
14	2:1 CELL ST	$\overline{\mathbf{c}}$	0.040	7.33
15	2:1 CELL ST	3	0.027	7.34
16	2:1 CELL ST	4	0.027	7.29
17	2:1CB	$\overline{1}$	0.062	7.38
18	2:1CB	$\overline{2}$	0.062	7.40
19	2:1CB	3	no pot	no pot
20	2:1CB	4	0.044	7.36
21	2:1 CB ST	$\overline{1}$	no pot	no pot
22	2:1 CB ST	$\overline{2}$	0.022	7.39
23	2:1 CB ST	3	0.028	7.38
24	2:1 CB ST	$\overline{4}$	no pot	no pot
25	5:1CB	$\overline{1}$	0.032	7.39
26	5:1CB	$\overline{2}$	0.023	7.40
27	5:1CB	3	0.029	7.36
28	5:1CB	4	0.022	7.36
	DI blank		0.004	6.63

Table B4-pH and EC from Final Leachability Test

# **RBP FINAL pour-through leachate stats for Peyton (ANOVA with Fisher's LSD at 95% - run on Minitab 16) REVISED 2-27-16**

**Note: no stats on Se because values were all below the limit of detection**

## **One-way ANOVA: EC (dS/m) versus TRT**



Pooled StDev = 0.01187

Grouping Information Using Fisher Method

TRT N Mean Grouping Plastic 4 0.23300 A



## **One-way ANOVA: pH versus TRT**





Pooled StDev =  $0.0609$ 

Grouping Information Using Fisher Method

TRT N Mean Grouping Plastic 4 7.46250 A 2:1 CB st 2 7.38500 A B C 2:1 CB 3 7.38000 A B C 5:1 CB 4 7.37750 A B 2:1 Cell 3 7.35000 B C 2:1 Cell St 4 7.32000 B C Peat 4 7.28500 C

#### **One-way ANOVA: Ortho-P (mg/L) versus TRT**

```
Source DF SS MS F P
TRT 6 0.018054 0.003009 7.16 0.001
Error 17 0.007142 0.000420
Total 23 0.025196
S = 0.02050 R-Sq = 71.66% R-Sq(adj) = 61.65%
                              Individual 95% CIs For Mean Based on
                             Pooled StDev
Level N Mean StDev ------+---------+---------+---------+---
2:1 CB 3 0.09000 0.04359
2:1 CB st 2 0.01000 0.00000 (--------*--------)
2:1 Cell 3 0.05667 0.00577 (------*------)<br>2:1 Cell St 4 0.06000 0.02449 (-----*------)
2:1 Cell St 4 0.06000 0.02449
5:1 CB 4 0.02250 0.01258 (-----*------)
Peat 4 0.02000 0.01155 (-----*-----)
Plastic 4 0.01000 0.01414 (-----*-----)
                              ------+---------+---------+---------+---
                                 0.000 0.035 0.070 0.105
```
Pooled StDev =  $0.02050$ 

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 CB 3 0.09000 A 2:1 Cell St 4 0.06000 A 2:1 Cell 3 0.05667 A 5:1 CB 4 0.02250 B Peat 4 0.02000 B Plastic 4 0.01000 B 2:1 CB st 2 0.01000 B

Means that do not share a letter are significantly different.

Fisher 95% Individual Confidence Intervals All Pairwise Comparisons among Levels of TRT

Simultaneous confidence level = 61.00%

#### **One-way ANOVA: Nitrate (NO3-N) versus TRT**

Source DF SS MS F P TRT 6 1.699 0.283 1.08 0.411 Error 17 4.443 0.261 Total 23 6.142  $S = 0.5112$  R-Sq = 27.66% R-Sq(adj) = 2.13% Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev --+---------+---------+---------+------- 2:1 CB 3 0.0633 0.0321 (---------\*----------) 2:1 CB st 2 0.0450 0.0071 (------------\*-------------) 2:1 Cell 3 0.0700 0.0100 (---------\*----------)<br>2:1 Cell St 4 0.0625 0.0189 (-------\*--------) 2:1 Cell St 4 0.0625 0.0189 5:1 CB 4 0.0675 0.0222 (--------\*--------) Peat 4 0.1325 0.0532 (--------\*--------) Plastic 4 0.7875 1.2151 (--------\*--------) --+---------+---------+---------+------- -0.60 0.00 0.60 1.20

Pooled StDev = 0.5112

Grouping Information Using Fisher Method

TRT N Mean Grouping Plastic 4 0.7875 A Peat 4 0.1325 A 2:1 Cell 3 0.0700 A 5:1 CB 4 0.0675 A 2:1 CB 3 0.0633 A 2:1 Cell St 4 0.0625 A 2:1 CB st 2 0.0450 A

## **One-way ANOVA: Ammonia (NH3-N) versus TRT**

Source DF SS MS F P TRT 6 0.08974 0.01496 7.90 0.000 Error 17 0.03219 0.00189 Total 23 0.12193  $S = 0.04352$  R-Sq = 73.60% R-Sq(adj) = 64.28%



Pooled StDev =  $0.04352$ 

Grouping Information Using Fisher Method



#### **One-way ANOVA: 60Ni (ppb) versus TRT**



 $S = 0.9146$  R-Sq = 38.60% R-Sq(adj) = 16.92%

 Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ----+---------+---------+---------+----- 2:1 CB 3 2.9000 0.9271 (--------\*--------) 2:1 CB st 2 0.8300 0.1131 (----------\*----------)<br>2:1 Cell 3 1.3200 0.3305 (--------\*--------) 2:1 Cell 3 1.3200 0.3305 2:1 Cell St 4 0.9400 0.2999 (-------\*-------)<br>5:1 CB 4 1.6350 1.7773 (-------\*--- $(----+---+---)$ Peat 4 1.9650 0.9059 (-------\*-------)<br>Plastic 4 1.3925 0.1438 (-------\*-------) Plastic 4 1.3925 0.1438 ----+---------+---------+---------+----- 0.0 1.2 2.4 3.6

Pooled StDev = 0.9146

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 CB 3 2.9000 A Peat 4 1.9650 A B 5:1 CB 4 1.6350 A B Plastic 4 1.3925 B 2:1 Cell 3 1.3200 B 2:1 Cell St 4 0.9400 B 2:1 CB st 2 0.8300 B

#### **One-way ANOVA: 65Cu (ppb) versus TRT**



 $S = 2.981$  R-Sq = 56.09% R-Sq(adj) = 40.59%

 Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ----+---------+---------+---------+----- 3 9.517 0.067 2:1 CB st 2 2.390 0.240 (--------\*---------) 2:1 Cell 3 12.047 2.213 (------\*------) 2:1 Cell St 4 5.395 3.206 (-----\*-----) 5:1 CB 4 4.200 1.912 (-----\*------) Peat 4 6.975 4.521 (-----\*-----)<br>Plastic 4 4.715 3.565 (-----\*------) Plastic 4 4.715 3.565 ----+---------+---------+---------+----- 0.0 5.0 10.0 15.0

Pooled StDev = 2.981

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 Cell 3 12.047 A 2:1 CB 3 9.517 A B Peat 4 6.975 B C 2:1 Cell St 4 5.395 B C Plastic 4 4.715 B C 5:1 CB 4 4.200 C 2:1 CB st 2 2.390 C

#### **One-way ANOVA: 66Zn (ppb) versus TRT**



 $S = 48.46$  R-Sq = 75.53% R-Sq(adj) = 66.89%

 Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ------+---------+---------+---------+--- 3 26.77 4.13 (-----\*-----)<br>2 17.27 3.02 (-------\*------) 2:1 CB st 2 17.27 2:1 Cell 3 22.26 5.80 (-----\*-----) 2:1 Cell St 4 28.80 11.51 (----\*----)<br>5:1 CB 4 19.91 3.11 (----\*----) 4 19.91 3.11 Peat 4 217.30 114.50 (----\*----) Plastic 4 33.44 3.89 (----\*----) ------+---------+---------+---------+--- 0 100 200 300

Pooled StDev = 48.46

Grouping Information Using Fisher Method

TRT N Mean Grouping Peat 4 217.30 A Plastic 4 33.44 B 2:1 Cell St 4 28.80 B 2:1 CB 3 26.77 B 2:1 Cell 3 22.26 B<br>5:1 CB 4 19.91 B 5:1 CB 4 19.91 2:1 CB st 2 17.27 B

#### **One-way ANOVA: 75As (ppb) versus TRT**



 Individual 95% CIs For Mean Based on Pooled StDev Level N Mean StDev ---+---------+---------+---------+------ 2:1 CB 3 0.2400 0.0854 (----\*----) 2:1 CB st 2 0.0800 0.0141 (-----\*-----)<br>2:1 Cell 3 0.2033 0.0208 (----\*----) 2:1 Cell 3 0.2033 0.0208 2:1 Cell St 4 0.1500 0.0535 (---\*---)<br>5:1 CB 4 0.1125 0.0350 (---\*---) 5:1 CB 4 0.1125 0.0350 (---\*---)<br>Peat 4 0.4325 0.2368 (---\*----) Peat 4 0.4325 0.2368 Plastic 4 0.9250 0.1196 (---\*---) ---+---------+---------+---------+------ 0.00 0.30 0.60 0.90

Pooled StDev = 0.1186

Grouping Information Using Fisher Method



# **One-way ANOVA: 95Mo (ppb) versus TRT**



 $S = 0.7463$  R-Sq = 66.97% R-Sq(adj) = 55.31%



Pooled StDev =  $0.7463$ 

Grouping Information Using Fisher Method



## **One-way ANOVA: 111Cd (ppb) versus TRT**

Source DF SS MS F P TRT 6 0.0046583 0.0007764 14.27 0.000 Error 17 0.0009250 0.0000544 Total 23 0.0055833  $S = 0.007376$  R-Sq = 83.43% R-Sq(adj) = 77.59%



Pooled StDev = 0.007376

Grouping Information Using Fisher Method


### **One-way ANOVA: 208Pb (ppb) versus TRT**



 $S = 0.1643$   $R-Sq = 81.51%$   $R-Sq(adj) = 74.99%$ 



Pooled StDev =  $0.1643$ 

Grouping Information Using Fisher Method



Means that do not share a letter are significantly different.

#### **One-way ANOVA: 202Hg (ppb) versus TRT**





Pooled StDev = 0.03270

Grouping Information Using Fisher Method

TRT N Mean Grouping 2:1 Cell St 4 0.05750 A Peat 4 0.04000 A B 2:1 Cell 3 0.02667 A B Plastic 4 0.02500 A B 2:1 CB 3 0.01333 A B 2:1 CB st 2 0.01000 A B 5:1 CB 4 0.00250 B

Means that do not share a letter are significantly different.



#### **Table B5-Inflation Test Data**



## **Table B6. Soybean Heights (cm)**

# **Table B7. Cosmos Heights (in)**

