Title

Evaluating the removal of paclobutrazol from captured irrigation runoff using slow sand filters

Author

Johnson, Grant Edward

Publication Date

2023

Peer reviewed|Thesis/dissertation

Evaluating the removal of paclobutrazol from captured irrigation runoff using slow sand filters

By

GRANT EDWARD JOHNSON
THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Horticulture and Agronomy

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

_____

Lorence Oki, Chair

_____

Astrid Volder

_____

Alessandro Ossola

Committee in Charge

2023

Abstract

In this study, a pilot scale slow sand filtration system was evaluated for biodegradation of the plant growth regulator paclobutrazol from recaptured irrigation leachate in a simulated greenhouse environment. Generated irrigation runoff containing 0.05 $mg \cdot L^{-1}$ paclobutrazol passed through 1-meter-deep slow sand filter sand beds for a period lasting 120 days. Evaluations through a broccoli hypocotyl length bioassay proved acutely sensitive to low concentrations of paclobutrazol, which is an affordable indicator of paclobutrazol presence in nursery runoff that can be utilized by nursery professionals. The SSF system proved ineffective in complete removal of PBZ as the bioassay indicated similar hypocotyl lengths grown with leachate before and after filtration.

Abbreviations:

PBZ – paclobutrazol

PRE – pre- filtration leachate

POST – post- filtration leachate

SSF – slow sand filtration

Acknowledgements

Receiving this momentous achievement has been a journey spurred through encouragement and mentorship. I would like to thank my thesis committee members, Dr. Astrid Volder and Dr. Alessandro Ossola who have all taught me patience, the pursuit of lifelong learning, and for instructing me through this degree.

I wanted to thank my committee chair, Dr. Lorence Oki, for supporting me through developing my scientific principles and allowing me to face countless opportunities for personal and professional growth. Without your persistence and dedication to my learning, I'm not sure whether I would have found the initiative which led me to this rewarding path.

I would also like to thank Dr. Bruno Pitton for the many intellectual conversations, technical guidance, encouragement, and many laughs under his mentorship.

Throughout my pursuit of higher education, I have Joanne Drake to thank for the love and encouragement she provides as the confidence she has given me continues to sustain me.

Finally, my family have been nothing but supportive throughout this process. I know how proud they are to watch me grow throughout my whole life and I'm just as excited to take the next steps towards my future.

Contents

1. Introduction

1.1 Nursery water recycling

Water utilization in nurseries and greenhouses continues to be scrutinized as runoff regulations, operating costs, and climate considerations play an increasingly significant role in the water management discussion (Pitton et al., 2018). Growers face increasing pressure to prevent and treat runoff, both to reduce the deterioration of water quality and to facilitate conservation through recycling (White et al., 2019). The California Irrigated Lands Regulatory Program has adopted regulations mandating runoff capture or control by agricultural operations such as plant production nurseries and greenhouses (CAEPA, 2023). A range of pollutants can be found in runoff, such as pesticides, herbicides, fertilizers, salts, and sediment. At high enough concentrations, these pollutants can harm aquatic organisms or make water unusable for drinking water or agricultural use (Park, 2005; Y. Yu & Wu, 2012). Although reused runoff reduces the consumption of high-quality water, it can lead to significant cost savings if the high-quality source water is expensive (Pitton et al., 2018; Redekar et al., 2020). It is advantageous for plant production nurseries to prevent discharge and remediate runoff on-site. Nurseries can reduce their water footprint by adopting water treatment technologies that encourage capture and reuse of irrigation runoff (Poudyal & Cregg, 2019).

1.2 Runoff contaminants

The quality of water being reused is a concern for growers as irrigating nursery crops with untreated runoff has the potential of introducing or spreading plant pathogens, synthetic

agrichemical residues, and nutrients carried through water movement to non-target crops (Abdi & Fernandez, 2019; Poudyal & Cregg, 2019; Redekar et al., 2020). Water borne pathogens found in nursery runoff can transfer a variety of plant diseases through connected runoff channels and the risk of spread is compounded through increased water reuse. Recent yield loss estimates attributed to the spread of root pathogens, *Phytophthora* and *Pythium*, on Pacific Northwest rhododendron nurseries averaged a 15% loss in container production systems with some sites experiencing 90% loss (Weiland et al., 2020). Without runoff remediation protocols, reused nursery water heavily infested with plant pathogens poses a significant risk to plant production nurseries and releasing contaminated runoff offsite could lead to environmental implications of downstream aquatic animals (Redekar et al., 2020).

Agrichemical residues can contaminate water sources with negative environmental impacts being well documented for pesticides, herbicides, and fungicides (Ahmed et al., 2021). Agrichemicals applied on nursery and floriculture nurseries included up to 350 different active ingredients in the chemical pesticide classifications of herbicides, insecticides, fungicides, and other (USDA, 2011).The most used class, 'other chemicals', accounted for 47% of the total weight of active ingredients applied. This category included plant growth regulators, rodenticides, disinfectants, biological pheromones, snail baits, and soil fumigants (USDA, 2011). Within this category, the second most used chemical was paclobutrazol (PBZ) with its application recorded at 9% of all nursery operations.

Nurseries applying PBZ increase the potential for residual chemical contamination in recycled runoff which can inhibit the growth of non-target crops leading to economic losses associated with stunted or deformed crops (Abdi & Fernandez, 2019; Poudyal et al., 2019).

Furthermore, PBZ has a persistent half-life of degradation in soil ranging from 43 to 618 days and it can retain and continue leaching with subsequent irrigations (Jiang et al., 2018). With its persistence in soil and risk of accumulating in runoff, paclobutrazol used at nurseries runs the risk of environmental threat as phytotoxic levels may be damaging to crops (Mahnken et al., 1999).

1.3 Paclobutrazol effect on plants

Plant growth regulators such as paclobutrazol (PBZ) [($C_{15}H_{20}ClN_3O$) [(2R,3R)-1-(4-chlorophenyl)- 4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol] are commonly used in nurseries for their plant growth regulating properties (Soumya et al., 2017). Paclobutrazol is one of the members of the triazole family of chemicals related to regulating growth characteristics by altering the level of plant hormones such as gibberellins, cytokinins, and abscisic acid (Soumya et al., 2017). Among the many properties, PBZ influences the isoprenoid pathway, changing phytohormones to interrupt gibberellin biosynthesis while promoting the production of abscisic acid and cytokinin (Ashraf & Ashraf, 2012; Soumya et al., 2017). The phytohormone disruption caused by PBZ application on developing plants is associated with increased chlorophyll content, soluble protein and mineral concentration in leaf tissue, increased root respiration, and reduced water use (Soumya et al., 2017). Additional physiological benefits of PBZ application have been reported, including improving crop productivity, fruit/grain quality, and disease protection (Shalaby et al., 2022).

Nurseries apply PBZ for its desirable properties, dependent on plant species, which include increasing flower density while reducing plant height by reducing internode elongation

to create compact growth (Ashraf & Ashraf, 2012). Furthermore, PBZ is effective not only in flower induction but it can also induce flowering in the early and off-seasons in some plants such as mango (Sharma & Awasthi, 2005). Paclobutrazol treatment on greenhouse grown seedlings produce greener, more compact seedlings that have better root systems that decrease transplant shock (Shalaby et al., 2022; Souza-Machado et al., 1997). Researchers evaluating water resource management of potted greenhouse geraniums found that water use efficiency increased when PBZ was applied to peat and coconut coir substrates (Bañón et al., 2009). Paclobutrazol also serves as a protection against abiotic stresses such as chilling, water deficit stress, and heat stress with effects on vegetative growth and crop quality (Desta & Amare, 2021).

Paclobutrazol is applied to a wide variety of crops as a foliar spray or soil/media drench at concentrations between 1 to 200 $mg \cdot L^{-1}$ and 0.1-8.0 $mg \cdot L^{-1}$, respectively (Grant, Fisher, Barrett, & Wilson, 2018). Varying degrees of crop sensitivity are reported to be dependent on species and cultivar with growth reduction occurring with as little as 0.5 $mg \cdot L^{-1}$ when applied to lantana (Currey et al., 2016). PBZ concentrations as low as 0.005 $mg \cdot L^{-1}$ leads to a 20% reduction in plant size of container grown begonia (J. B. Million et al., 1999) and concentrations of 100 $mg \cdot L^{-1}$ can lead to 50% reductions in chrysanthemums (Desta & Amare, 2021). The effects of growth reduction may be helpful for growers interested in maximizing return per unit land by increasing the quantity of plant populations of compact plants (Desta & Amare, 2021).

Runoff water containing pesticides from nurseries are usually diluted with other water sources, however, frequent daily irrigation increases the potential for chronic low-dose exposure to pesticides that may have phytotoxic effects (Poudyal et al., 2019). Furthermore, PBZ foliar sprays not intercepted by target plant foliage can contact other exposed surfaces, may dissolve

in irrigation water runoff, and be transported to holding tanks or retention ponds (Altland et al., 2015).

1.4 Treatment technologies

Technologies adopted by nurseries for treating captured runoff involve constructed wetlands, vegetative buffers, slow and rapid sand filtration, granular-activated carbon canisters, substrate filled filter socks, bioreactors, ultraviolet radiation, hydrogen peroxide, and electrochemical flocculation (Grant, Fisher, Barrett, Wilson, et al., 2018; Poudyal & Cregg, 2019). The treatment technology utilized are site-specific, but any number or combination of methods can be used to remediate pesticide, plant pathogen, or nutrient contaminants from recycled water and runoff (Majsztrik et al., 2017; Poudyal & Cregg, 2019; Taylor et al., 2006). Runoff contaminants can be removed, recycled on-site, volatilized, or transported off-site depending on the practices adopted by the nursery and site specifications (Majsztrik et al., 2017). Pesticide remediation is possible through constructed wetlands, vegetative buffers, granular activated carbon, and slow sand filtration (Grant et al., 2019; Majsztrik et al., 2017; Phu, 2014) . Each treatment technology that a nursery uses must balance concerns regarding costs of construction, maintenance, removal efficacy, energy, and consumable materials, among others. Slow sand filters often stand out due to their minimal inputs including not requiring chemicals, ease of maintenance, and are relatively inexpensive compared to more intensive remediation techniques (Clark et al., 2012; Majsztrik et al., 2017; Moran, 2018). Furthermore, slow sand filtration is a technology that is proven to reduce contamination from various sources of nursery

runoff through microbial degradation (Clark et al., 2012; Poudyal & Cregg, 2019; Ranjan & Prem, 2018) and may offer benefits in removing chemical pollutants (Casas & Bester, 2015; Phu, 2014).

1.5 Slow sand filters

Slow sand filters (SSF) are shallow beds filled with sand that consists of a water reservoir tank, a pump to distribute the water, the filter sand medium, and a gravel layer on the bottom to allow for treated water to exit the bed (Lee & Oki, 2013; Maurya et al., 2020; Moran, 2018). A one meter deep quartz sand bed is common with sand grain size ranging between 0.15 mm to 0.35 mm, (Clark et al., 2012; Logan et al., 2001; Maurya et al., 2020). The efficacy of slow sand filtration relies on the microbial community that develops on and within the sand bed called the "schmutzdecke", a German word for "filter skin" (Oki et al., 2017; Ranjan & Prem, 2018). The development of the schmutzdecke biofilm layer depends on the availability of microbes, available energy source, residence time of that energy source, and the constant wetting of the sand bed (Fitriani et al., 2020; Ranjan & Prem, 2018). This biofilm can form a layer up to 3 cm thick above the sand bed surface and is made up of a variety of microorganisms characterized by algae, bacteria, fungi, and protozoa with different community populations forming in each unique system (Fitriani et al., 2020; Maurya et al., 2020; Ranjan & Prem, 2018). The primary modes of organic matter removal are considered to be through physical adsorption within the media and biodegradation (Collins et al., 1992). As water passes through the sand media, the schmutzdecke can develop over a period of two weeks until it reaches maturity for contaminant removal.

The formation of the bacterial microcolonies and biofilms on the sand substrates is a major factor in SSF efficiency (Déniel et al., 2004). The microorganisms found within the schmutzdecke that are attributed to filtration removal include the genera *Pseudomonas, Bacillus, Acinetobacter*, and *Sphingomonas* which show capability in removal of both biological and chemical contaminants (Déniel et al., 2004; Haig et al., 2015). Studies of SSF column efficacy in removing nursery pathogens such as *Phytophthora spp.*, *Pythium spp., Fusarium spp., and Tobacco mosaic virus* have shown removal in as little as two to six weeks (Déniel et al., 2004; Lee & Oki, 2013; Oki et al., 2017).

1.6 Paclobutrazol degradation

The removal of pesticides through slow sand filtration has been less studied for horticultural purposes, but does show promising ability in agrichemical degradation (Casas & Bester, 2015). More importantly, slow sand filtration shows degradation of triazole compounds, similar in structure to PBZ, such as the fungicides tebuconazole and propiconazole (Casas & Bester, 2015). The 1,2,4-triazole ring of PBZ is susceptible to degradation by *Pseudomonas* spp. in soil conditions, which is the same genera found in the schmutzdecke layer that is attributed to removal efficiency (Déniel et al., 2004; Jackson et al., 1996). Additionally, a study has determined that certain strains of *Pseudomonas putida* can degrade PBZ concentrations up to 50 $mg \cdot L^{-1}$ (Kumar et al., 2021).

Nurseries that reuse irrigated runoff and apply PBZ risk contamination of the entire nursery, as well as the environment through off-site exposure, caused by persistent irrigations and frequent applications of PBZ. Treatment technologies such as slow sand filtration may

remediate runoff before levels of PBZ reach phytotoxic levels or levels that can affect non-targeted crops. Several soil microorganisms are capable of utilizing PBZ as source of carbon and energy and since SSF sand beds develop unique microbiomes in response to the chemical energy sources available (Déniel et al., 2004; Kumar et al., 2021; Ranjan & Prem, 2018) then the establishment of biofilms capable of degrading PBZ may occur. Chemical studies evaluating the same triazole family as PBZ exhibited partial removal in slow sand filtration (Casas & Bester, 2015) so the potential of PBZ degradation is possible.

This study aims to answer whether slow sand filtration systems can be an effective method to remove and degrade paclobutrazol from nursery runoff.

2. Materials and Methods

2.1 Leachate

The experiment setting was located inside the Environmental Horticulture greenhouse located at the University of California, Davis (N 38° 32' 9.402'', W 121° 44' 49.1424''). Chrysanthemums are one of the most cultivated potted flowering plants in the state of California that PBZ is used (CDFA, 2021; Kentelky et al., 2021). Chrysanthemum (*Chrysanthemum indicum 'Newport Bronze Bicolor'*) plugs were potted 3 per pot in UC mix soilless substrate in #1 size pots and top dressed with 10-20-10 (NPK) Osmocote Smart Release (Bloomington Brands LLC, Bloomington, IN, USA) fertilizer and grown on 3 greenhouse benches. There were 90 pots in total and the plants were trimmed periodically to keep foliage from overgrowing. High pressure sodium lighting (PR-2011, Plusrite, Ontario, CA, USA) above the benches ran for 6 hours starting

at 5:00 PM and prevented the chrysanthemums from flowering during the duration of the experiment and kept the plants in vegetative growth (Blanchard & Runkle, 2009). Each pot was fitted with one 2 gph drip emitter (SW20-30PS, Rainbird, Azusa, CA, USA) and the plants were irrigated daily with the greenhouse modified Hoagland's fertilizer solution (Appendix Z).

2.2 Water flow

The leachate that passed through the irrigated plants was collected into one of three 210 L holding tanks (Ronco Plastics, Tustin, CA) underneath each bench that were connected in series and served as the leachate reservoir (Oki et al., 2017). Between each bench drain and holding tank was a mesh screen (RHAD100, Rain Harvest Systems, Cumming, GA, USA) that prevented benchtop debris of plant clippings and large media particles from collecting inside the tanks. A pump (2E-N Series, Little GIANT, Oklahoma City, OK, USA) passed leachate through a 200-mesh filter (RBY100MPTX, Rain Bird, Azusa, CA, USA) and into a 1.27 cm (1/2 in) PVC distribution manifold where a water meter (HC100Flow, Hunter Industries, San Marcos, CA, USA) and an electromagnetic counter (CSK6-YKW, Keenso, Amazon, Seattle, WA, USA) quantified and recorded the amount of leachate being distributed.

The distribution manifold contained individual valves used for supplying eight 190 L barrels. Inside each barrel was an air stone (5289729, Spectrum Brands Holding, Inc., Middleton, WI, USA) connected to an air pump (Optima A-807, Hydrotech Hydroponics, Markham, ON, CAN) that provided agitation and an aerobic environment necessary for the development of the SSF column schmutzdecke (Moran, 2018). Each barrel was fitted with a 1.27 cm (1/2 in) PVC ball valve (QVC1015SSEW, Hayward, Clemmons, NC, USA) on the bottom. Filling each barrel with the

reservoir leachate occurred every three to four days as needed to replenish the amount used by the SSF columns. Before refilling, all barrels were isolated by closing the bottom valves and the supply manifold valve situated above the respective barrel to be replenished was opened. When filling an isolated barrel with leachate, a portable float switch (Model M8000, Madison Company, Brandford, CT, USA) was installed in the barrel to activate the filling pump (2E-N Series, Little GIANT, Oklahoma City, OK, USA) and turned it off when the barrel was full. After a barrel was filled, the supply manifold valve was closed and the amount of leachate added to the barrel was recorded. After filling all of the barrels, the bottom valves of each barrel were opened.

The eight barrels were split into two distinct groups such that four of the barrels contained the leachate supplying the control sand filters. The other four barrels were grouped and dosed with PBZ and contained the treatment water. The water pathway from this point was isolated between groups such that each grouping was connected by 1.27 cm polyethylene tubing (B37, DIG Corporation, Vista, CA, USA) leading to a pump (2E-N Series, Little GIANT, Oklahoma City, OK, USA) that supplied leachate to their respective SSF supply manifold (Figure 1).

2.3 PBZ dosing

A standard solution of 400 $mg \cdot L^{-1}$ paclobutrazol was prepared from a 4,000 $mg \cdot L^{-1}$ PBZ commercial source (Bonzi, Syngenta, Greensboro, NC USA). The commercial container was shaken by hand for one minute before 100 mL was pipetted into a 1 L glass amber bottle and the remaining volume was filled with 900 mL reverse osmosis water. A magnetic stir bar (14-512-126, Thermo Fisher Scientific, Waltham, MA, USA) was then placed into the bottle and the standard solution was stored in the dark at 23° C.

After refilling, each of the four barrels selected for supplying the treatment water were dosed with PBZ. Since the amount of leachate each barrel received was recorded, the quantity of PBZ solution required to maintain 0.05 $mg \cdot L^{-1}$ PBZ within the barrel was determined. The PBZ standard solution was placed on a stir plate (PC-351, Corning Life Sciences, Durham, NC, USA) for ten minutes and the appropriate amount of PBZ solution for each treatment barrel was pipetted from the bottle into a 50 mL Erlenmeyer flask and poured into the top of each barrel respectively. Each flask was rinsed with no more than 50 mL of leachate from the distribution manifold. The barrels then underwent 30 seconds of mixing using a power drill (PCL220B, Ryobi, Fuchu, Hiroshima, JP) fitted with a paint paddle mixer (Model 192500, Home Depot, Atlanta, GA, USA). After mixing, the bottom valves of the barrels were opened to supply the treatment water to the respective sand filters.

2.4 Slow sand filter design

Ten sand filters were installed standing ~2 meters tall constructed of 11.4 cm (4 in) Schedule 40 PVC pipe with a 10.2 cm inner diameter (Figure 1) (Lee & Oki, 2013; Oki et al., 2017).

The base of the sand filter was filled with a substrate gradient starting with the largest aggregate at the bottom followed by aggregate decreasing in size until the particle size of the top-most layer matched the grain size used for filtration (Oki et al., 2017). The lowest and largest substrate to the final substrate were: ~2 cm diameter pieces of gravel, 1 cm coarse aquarium pebbles (Kordon LLC, Hayward, CA), #3 Lapis Lustre quartz sand (RMC Pacific Materials, Pleasanton, CA, USA), #2/16 Lapis Lustre quartz sand, and the final layer of #1/20 Lapis Lustre quartz sand (Lee &

Oki, 2013; Oki et al., 2017). Each gradient texture was applied in a layer sufficient to cover the previous layer. The combined depth of the gradient layers was roughly 5 cm. The #1/20 Lapis Lustre quartz sand filled the body of the sand column to 1 meter in height.

A pump (2E-N Series, Little GIANT, Oklahoma City, OK, USA) supplied the leachate to the columns through a 1.27 cm PVC manifold that distributed leachate through a 0.625 cm (1/4 in) polyethylene tube to each filter supply port. Five centimeters below the supply port was a 1.27 cm overflow port. To ensure that the columns remained full of water, the supply manifold provided leachate at a slightly faster flow rate than the column flow rate, so excess water would flow out of the column through the overflow port, into a 1.27 cm polyethylene tube, through a 1.27 cm PVC return manifold, and was recirculated back into the group barrels exiting through a 1.27 cm PVC ball valve (QVC1015SSEW, Hayward, Clemmons, NC, USA) (Lee & Oki, 2013; Oki et al., 2017). The location of the overflow ports maintained a consistent water head of 1 meter above the sand bed within each column, providing pressure to push the leachate through the sand bed. Water flow rate through each column was controlled separately by individual rotameters (Model C-32461-32, Cole-Parmer, Vernon Hills, IL) set to 20 $mL \cdot min^{-1}$ (Lee & Oki, 2013).

2.5 Sample collection

There were five replicates of each control and treatment column, totaling ten sand columns in the experiment. Each column had a sampling port situated above the sand bed where the pre-filtration (PRE) water was collected (Oki et al., 2017). The pre-filtration water was collected in 2-50 mL centrifuge tubes by fully opening the valve and letting the water fill the

tube. Post-filtration (POST) water was collected in 2-50 mL centrifuge tubes after passing through the rotameter and freely dripped into the collection tubes at 20 $mL \cdot min^{-1}$. Water sampling occurred at: day 0, day 1, day 7 and then at two-week intervals until the last sampling at 119 days. The day 0 sampling occurred 4 hours after leachate was introduced into the sand filters and prior to the introduction of PBZ. Immediately after the sample collection on day 0, PBZ was dosed into the appropriate barrels. The day 1 sample collection occurred 24 hours after PBZ was introduced into the SSF column system. After each sampling period, the filled sample tubes were placed in a freezer at -40° C.

After the final leachate samples were collected, the supply manifold pumps were turned off. Each SSF column was dismantled and the sand bed surface was visually examined for schmutzdecke establishment (Appendix A). Photographs were taken of each sand bed and were analyzed with ImageJ software (Schneider et al., 2012). The biofilm area was traced and compared to the sand bed area of each respective column to determine percent coverage.

2.6 Bioassays

Bioassays were conducted using *Brassica oleracea* var. botrytis 'Waltham 29' seedlings for their sensitivity to paclobutrazol as the hypocotyl length is reduced in response to increased chemical presence (Grant, Fisher, Barrett, & Wilson, 2018; Jeff B. Million et al., 1999). A platform was assembled by wrapping half of a coffee filter (Basket Filter Paper White, Melitta North America, Inc., Clearwater, FL USA) around a 6 cm^2 grid of polystyrene lighting panel (1199232A, Plaskolite, Columbus, OH). This platform was then placed inside a plant culture box (Magenta GA-7, Magenta LLC., Lockport, IL, USA). The leachate samples were thawed at room temperature

and 20 mL aliquots were pipetted into the plant culture boxes. Forceps were used to evenly place 9 seeds onto the platform, the culture box lid was secured, and the boxes were placed inside a growth chamber for 14 days with minimum and maximum temperatures of 23° and 25° C, respectively. An experimental unit consisted of nine seeds evenly spaced on a platform. In total, there were 60 bioassay seedling boxes for each sampling period. This represented aliquots from all 10 columns consisting of the 5 column replicates of each control and treatment, 3 culture box replicates for the 2 sampling ports of the PRE and POST filtration water. There were 3 shelves in the growth chamber and each shelf held one seedling box from each treatment randomly placed, serving as a block. The plants were grown under fluorescent lighting at an average of 125 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 18 $hrs \cdot day^{-1}$ resulting in a daily light interval of 8.1 $mol \cdot m^{-2} \cdot day^{-1}$. After 14 days, the boxes were removed from the growth chamber. Aided by forceps, the platforms were removed from each culture box and the seedlings were removed from the platforms. The seedlings were separated and placed on the lab bench for measuring. Hypocotyl length from the base to the hypcotyledonary node was measured to the nearest mm using a caliper (Model DCLR-0605, Clockwise Tools Inc, Valencia, CA, USA) (Jeff B. Million et al., 1999).

2.7 Statistical analysis

Statistical analysis was conducted using RStudio (v. 2022.02.2+485) (RStudio Team, 2022) and figures were produced using the 'ggplot2' package (Wickham, 2016). A linear mixed model was performed once model assumptions for heteroskedasticity and normality were met by performing log transformation of the data. Interpretation of results was aided by computing contrasts over estimated marginal means using the 'emmeans' package.

3. Results

3.1 Bioassays

The results of the samples retrieved from the control ports indicated no significant difference ($p>0.05$) between the PRE and POST filtration leachates (Figure 3). Comparison of the hypocotyl lengths grown with the control column leachate showed that passing through the sand filter bed did not affect growth. The PBZ dosed leachate samples collected from the PRE and POST sampling ports were not significantly different ($p>0.05$) as hypocotyl lengths were similar (Figure 3). This indicates the SSF columns receiving PBZ dosed leachate were not effective in the removal of PBZ as the POST filtered leachate contained similar levels of PBZ as the PRE filtered leachate (Figure 3). However, pairwise comparison between control and PBZ of both PRE and POST filtration revealed significant differences throughout ($p<0.001$) for all comparisons except day 0 ($p>0.058$) when PBZ was absent from the system (Figure 4). The hypocotyl seedling lengths grown with the PBZ dosed leachate were shorter compared to the controls.

At the start of the experiment, the broccoli hypocotyl lengths were similar for both control and treatment columns as PBZ was not present in the system (Figure 4). There was no significant difference ($p>0.05$) between the PRE and POST lengths of the hypocotyl lengths grown with day 0 leachate. As PBZ was introduced into the system starting from day 1 to the experiment completion at day 119, PBZ was detectable at levels high enough for the bioassay to capture. The POST filtration PBZ water samples produced hypocotyl lengths less than that of

both the PRE and POST controls for the duration of the experiment showing incomplete removal of PBZ (Figure 4).

Comparisons of samples from the PBZ PRE filtration to the PBZ POST filtration did indicate longer hypocotyl lengths after filtration for Day 0. However, hypocotyl lengths on day 63 and Day 77 showed increased PBZ in the POST filtration samples as lengths were shorter than PRE filtration (Figure 5). Both the PRE and POST PBZ dosed SSF columns resulted in slightly increased hypocotyl lengths starting on day 50 until the experiment completion on day 119.

Visual inspection of the SSF column sand beds revealed the presence of a reddish-brown biofilm on the surface of the sand beds that was ~1 cm thick. The biofilm was present in all treatment column sand beds and mostly absent on the control sand beds (Appendix A). The percent coverage of the treatment column biofilms was 45.49%, 43.25%, 56.26%, 47.85%, and 69.98% for replicates one through five, respectively (Table 1). The control column biofilms had the percent coverage of 0.72%, 0.34%, 0.40%, 0.52%, and 1.69%, for replicates one through five, respectively (Table 1).

4. Discussion

4.1 Treatment Effect

The ability of slow sand filters in removing PBZ at concentrations of 0.05 $mg \cdot L^{-1}$ was ineffective over the duration of the experiment lasting 119 days of constant flow. The treatment columns were unable to fully remove PBZ or reduce the concentration, as evidenced by the comparison between the control SSF columns POST filtration hypocotyl lengths to the treatment

POST filtration SSF column hypocotyl lengths, as seen in Figure 3. The control POST filtration hypocotyl growth showed no difference in length compared to the control PRE filtration hypocotyl lengths which was expected as there was no PBZ present (Figure 3). The comparison between the control PRE and POST leachate was necessary to confirm that the action of leachate passing through the sand bed had no effect on hypocotyl length.

The treatment columns received PBZ, so the effect of PBZ appears as expected which produced shorter hypocotyl lengths grown with the PRE filtered leachate (Figure 3). The presence of PBZ was confirmed by the comparison between the control PRE to the treatment PRE hypocotyl lengths. The PBZ dosed leachate collected from the treatment PRE port resulted in significantly shorter hypocotyls than the control PRE port leachate. The difference in hypocotyl lengths between the control and treatment leachate indicated that the leachate supplied to the treatment SSF columns contained high enough PBZ concentrations to stunt growth.

The treatment POST filtration water was not statistically different ($p<0.05$) compared to the PRE filtration water indicating that removal of PBZ was ineffective (Figure 3). If the sand columns were effective in removing the PBZ, then the water that had passed through the sand bed would result in longer hypocotyl lengths, but this did not occur. Given the bioassay results, partial removal could have been a possibility, although this was not seen, therefore indicating that the effects of PBZ are too acute at 0.05 $mg \cdot L^{-1}$ or that a combination of factors such as flow rate, schmutzdecke biological establishment, and substrate size contributed to the performance of the schmutzdecke.

4.2 Growth trend

It is important taking note of growth trends of the PBZ treated hypocotyl lengths in the data. The average growth showed elevated growth starting at day 63 that continued this trend during the remainder of the experiment (Figure 4). Initially this may represent the potential for growth linked degradation whereas the schmutzdecke has advanced the ability to remove PBZ (Kumar et al., 2021). However, since the increase in length appears in both the PRE and POST filtration seedlings during the same time interval, this suggests that the trend is not associated with biological filtration. The trend must be attributed to conditions other than treatment effects such as abiotic conditions (Pompei et al., 2019). The initial PBZ standard solution used in the experiment was insufficient to last the entire experimental period. The bottle had depleted its contents after the day 49 samples were collected. A second stock solution bottle of PBZ was created and was used for dosing prior to day 63 and was used for the duration of the experiment. The treatment barrels were dosed with the new PBZ stock solution prior to the day 63 sample collection. This coincided with the increased growth of the seedlings starting on day 63, indicating that the concentration of PBZ may have been slightly lower than the first stock solution, therefore partially elevating the hypocotyl seedling lengths.

4.3 Saturated sand bed binding sites

The PBZ treated columns had hypocotyl lengths that were significantly different ($p<0.05$) between the PRE and POST filtered water in few instances. In those instances, hypocotyl lengths grown using the POST filtration water were shorter than the PRE filtered water indicating residual PBZ held within the sand bed PBZ was being leached (Figure 5).

An explanation for this independent phenomenon that occurred on Day 63 and Day 77 may be that a saturation threshold of binding sites within the filter was reached. This also coincided with the new stock solution that may have had a slightly lower PBZ concentration which was dosed starting on Day 63. If the concentration of PBZ entering the SSF system was at a lower concentration, then the residual chemical may have resided within the sand bed such that PBZ leached into the POST filtration water. The PBZ residing within the sand bed may have accumulated and once binding sites were saturated, the PBZ leached through the sand bed leading to a higher concentration in the POST filtered water. The residual leaching effect was seen between the two sampling periods and dissipated 4 weeks later by day 91 and did not occur for the remainder of the experiment.

4.4 Sand bed particle size

The Lapis Lustre sand filling the SSF columns had a grain size of #1/20, which has a particle size range of 0.600 mm to 1.18 mm. This size distribution deviated from SSF designs using finer sized sand used in pathogen removal (Clark et al., 2012; Lee & Oki, 2013; Logan et al., 2001; Maurya et al., 2020; Oki et al., 2017). The grain size used in these studies has a particle range of 0.06 mm to 0.3 mm. The fine particle size provides a larger surface area for the schmutzdecke to form (Maurya et al., 2020) and the distance between the grains of very fine sand trap bacteria, which contribute to the development of the schmutzdecke (Clark et al., 2012). Filling the columns with a finer sand may have increased the microbial abundance of the schmutzdecke as more binding sites would be available, increasing the potential for the biological degradation of PBZ.

4.5 Lack of clogging

Most SSF systems require routine maintenance as the development of the schmutzdecke layer causes eventual filter clogging of the surface sand causing a reduction in flow rate (Hammes et al., 2011). Mechanical scraping and removal of the clogged biofilm layer is typical which restores flow rate but reduces efficacy until the schmutzdecke reforms (Fitriani et al., 2020). The larger sand particle size used in the experiment did not clog nor required cleaning as the flow rate was maintained at 20 $ml \cdot min^{-1}$ without noticeable reduction. This may be an indication that the schmutzdecke did not form a robust biofilm in the SSF columns. Schmutzdecke establishment on the surface of the sand bed can form within two to three weeks and is considered an important process of SSF purification (Ranjan & Prem, 2018). The lack of clogging during the 17-week experiment may be an indicator that the biofilm was still developing and had not formed a thick enough layer to be effective in removing PBZ.

4.6 Schmutzdecke establishment

Further evidence of an underdeveloped biofilm layer was seen in the visual inspection of the sand layers after the termination of the experiment (Appendix A). A visual indicator of a schmutzdecke has been described as a reddish-brown layer covering the surface of the sand bed (Ranjan & Prem, 2018) and each treatment column exhibited biofilm coloration on the surface of the sand bed, which indicated the presence of a schmutzdecke. However, the area that the biofilm covered the sand bed of each respective column was incomplete, ranging from 45.49% to 69.98% percent coverage (Table 1). The schmutzdecke may have needed more maturation time

to develop entirely over the sand bed (Paranychianakis et al., 2006). Leachate may have bypassed untreated through the uncovered portion of the sand bed, which may explain why PBZ was still present after filtration. The presence of air-bubbles trapped within the sand may have contributed to altered water flow hydraulics (Casas & Bester, 2015). The control column sand beds did not exhibit an apparent schmutzdecke. The microbial biomass on the control SSF column sand beds may have differed in species diversity compared to the treatment columns (Campos et al., 2002; Haig et al., 2015). The lack of PBZ serving as a microbial energy source may have produced a slower rate of development on the control sand beds or perhaps the sand beds exhibited spatially different microbial growth that resided within the sand bed (Campos et al., 2002; Haig et al., 2015). Measuring additional water parameters such as dissolved organic carbon or fertilizers (Haig et al., 2015) originating from the chrysanthemum leachate may have provided indication of schmutzdecke establishment when visual indicators are not apparent.

4.7 Duration of project

The experiment length progressed over 17 weeks, which was thought to be sufficient time for the schmutzdecke to establish on the sand bed of the SSF columns for experiments involving biological contaminants (Fitriani et al., 2020; Lee & Oki, 2013; Oki et al., 2017; Ranjan & Prem, 2018). However, timing is variable and a waste water treatment study has shown a 48 week long maturation period for fecal coliform removal (Paranychianakis et al., 2006). Chemical pollutant removal in SSF systems has been less studied and varied success in removal of chemicals has been reported (Fitriani et al., 2020; Phu, 2014). Although, in a laboratory sand filter study evaluating the removal potential of the herbicide atrazine, there was no removal

during the entirety of the 262 day experiment (Ho et al., 2011). Since PBZ degradation in soil has shown biological reduction between 43 to 618 days with an average of 182 days (Sharma & Awasthi, 2005) then the experiment may have needed to run for a longer period of time. If residual PBZ degradation may occur longer than this SSF system was installed, then a longer experimental duration may be necessary for the schmutzdecke community to mature the proper microbial community that would be able to remove PBZ (Kumar et al., 2021).

4.8 Bioassays grown without substrate

The bioassay seedlings were grown in constant contact with leachate through the wicking action of the filter paper platform (Sattar et al., 2010). This differed from previous PBZ bioassay studies that used media (Grant et al., 2019; Grant, Fisher, Barrett, & Wilson, 2018) because it has been reported that the type of growing media affects PBZ activity with the highest activity in the upper 2.5 cm of the media (Jeff B. Million et al., 1999). By not using media, the activity of PBZ was kept uniform to reduce the effects of media variability on seedling growth.

4.9 Caveats and limitations

Although bioassays were effective indicators of PBZ presence, it would have been beneficial submitting leachate collected in this study to determine the concentrations of PBZ. Extraction using liquid chromatography and mass spectrometry can determine the concentration of PBZ dosed to the columns (Grant, Fisher, Barrett, & Wilson, 2018; Jiang et al., 2019; Liu et al., 2015). More importantly, the potential for laboratory analysis may have indicated to what extent removal of PBZ was achievable and whether secondary metabolites were formed. Future

experimentation of paclobutrazol using slow sand filtration systems should incorporate quantitative techniques to determine percent removal (Y. Yu & Wu, 2012; Z. Yu et al., 2008).

The SSF columns relied on the natural development of the schmutzdecke. Characterizing the microbial diversity and composition living within the schmutzdecke may help identify the capability of biodegradation and metabolism of chemical contaminants within the SSF system (Haig et al., 2015). Illumina 16S rRNA gene sequencing is an effective technique in identifying key genera within SSF sand beds that characterize filter performance (Haig et al., 2015). Genera found in SSF sand bed studies, such as *Acinetobacter* and *Sphingomonas*, are known to be capable of metabolism and biodegradation of a wide range of chemicals including aromatic hydrocarbons and herbicides (Haig et al., 2015). Furthermore, isolated strains of *pseudomonas putida* cultured in liquid broth utilized PBZ as a carbon and energy source with removal of 50 $mg \cdot L^{-1}$ PBZ in 15 days (Kumar et al., 2021). It may be possible to culture genera capable of PBZ biodegradation and inoculate the SSF columns to increase the establishment of the schmutzdecke (Casas & Bester, 2015; Kumar et al., 2021). By evaluating the microbial composition of the schmutzdecke, identifying the presence of these genera may indicate the maturity of the schmutzdecke and the potential of microbial degradation of PBZ.

4.10 Conclusion

Bioassays were chosen for their sensitivity and cost effectiveness and could be adopted by nursery professionals as a method of field testing PBZ concentrations in irrigated runoff. Bioassays detected PBZ in concentrations able to reduce plant growth, however, quantifying concentration levels of PBZ was not possible using this method. Growers may not need to

precisely know the concentration of PBZ, but merely that its presence for the potential phytotoxicity of the irrigated captured runoff would have on their target crops (Poudyal & Cregg, 2019). The two-week period between water collection and bioassay results indicating chemical pollutants found in runoff is relatively short and requires minimal equipment and time. Adopting bioassays as a preventative check on the residual chemical load of recycled irrigation runoff is a practice worth pursuing.

The SSF columns relied on the natural formation of the schmutzdecke, however, methods of seeding the sand bed with an established microbial community adapted to targeted chemical removal would be a worthwhile next step (Casas & Bester, 2015; Sniegowski et al., 2012). Casas and Bester used wastewater treatment sludge previously exposed to chemical pollutants as an inoculum source to establish quicker schmutzdecke development. Inoculating sand beds with the microbial communities that are found in successful SSF columns, such as the genera *Pseudomonas, Acinetobacter,* and *Sphingomonas* may jumpstart the formation of the schmutzdecke and influence the degradation power of the columns for quicker establishment (Haig et al., 2015; Kumar et al., 2021; Lee & Oki, 2013).

Although the removal of paclobutrazol did not occur within the experimental period, there is evidence that SSF systems are capable of partially degrading other agrichemicals, including few triazole family compounds similar in composition to PBZ (Casas & Bester, 2015). Slow sand filtration is a proven solution for pathogen removal, and additional chemical pollutant removal should be studied further depending on agricultural needs (Lee & Oki, 2013; Oki et al., 2017; Poudyal & Cregg, 2019; Redekar et al., 2020).

5. Figures

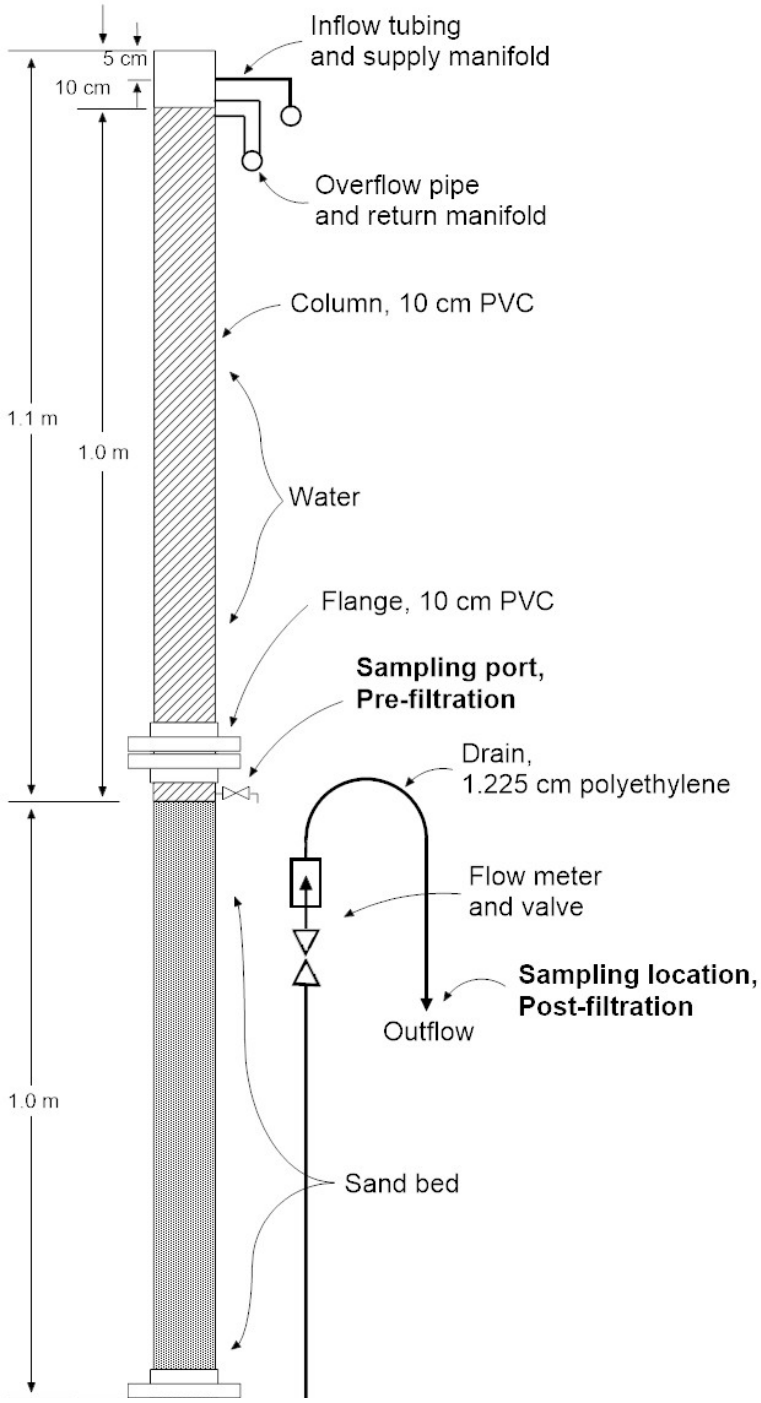

Figure 1. Slow Sand Filter. The slow sand filter schematic was constructed of 10 cm PVC. Pretreatment leachate (PRE) was collected just above the sand bed and post treatment leachate (POST) was collected after passing through the flow meter.

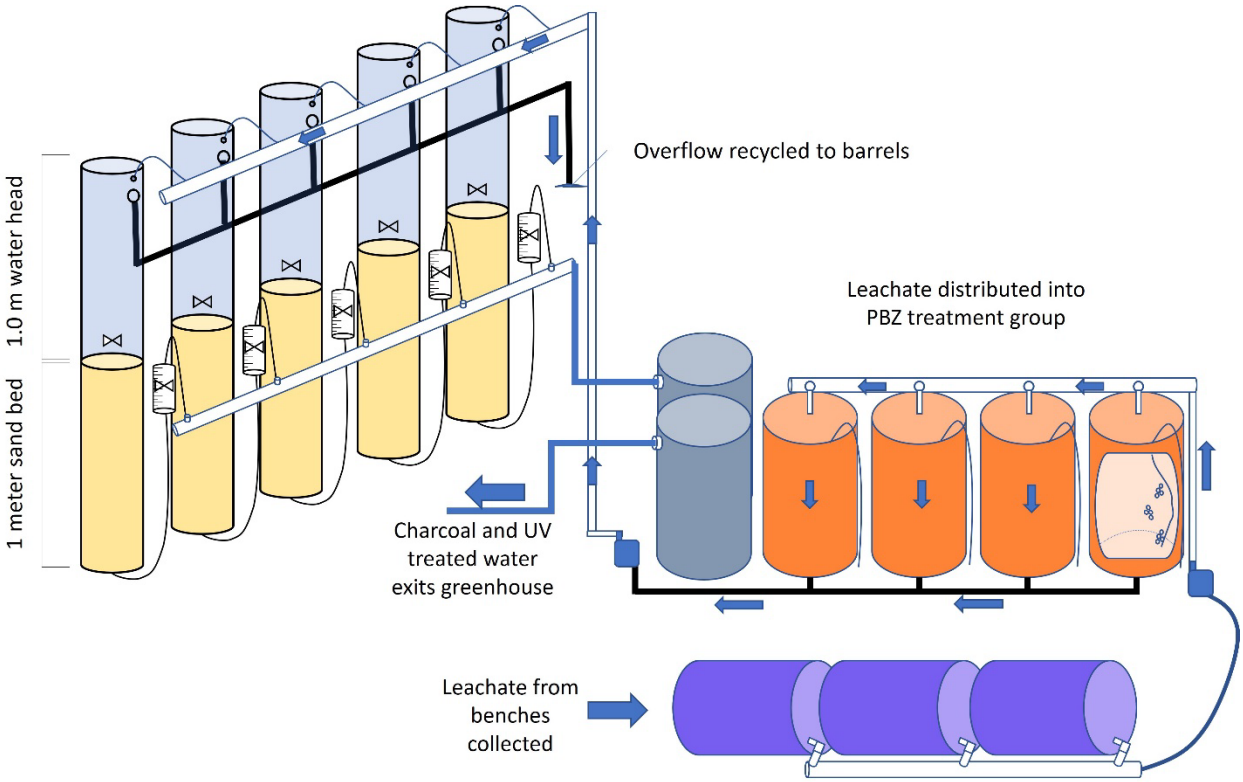

Figure 2. The design schematic of water storage and one set of treatment sand columns is depicted. The 5 sand columns not shown were duplicated following the same schematic. Leachate from irrigated chrysanthemum benches was collected in 3- 210 L tanks and pumped through a distribution manifold. The manifold contained individual valves used for filling the 190 L barrels. The four treatment barrels (orange) were dosed to maintain a concentration of 0.05 $mg \cdot L^{-1}$ paclobutrazol during each filling session. Each barrel had an air stone to mix and oxygenate the water. Following the pathway for the PBZ dosed barrels, the water was pumped through a supply manifold that continually filled the columns allowing the overflow manifold to return the water back to the treatment barrels. The PRE filtration sampling valve was located above the sand bed. The POST filtration sample was collected after passing through a rotameter controlling the flow rate at 20 mL/min. The water exiting the rotameters for all columns passed through barrels (gray) containing charcoal, UV filter, and pumped out of the greenhouse.

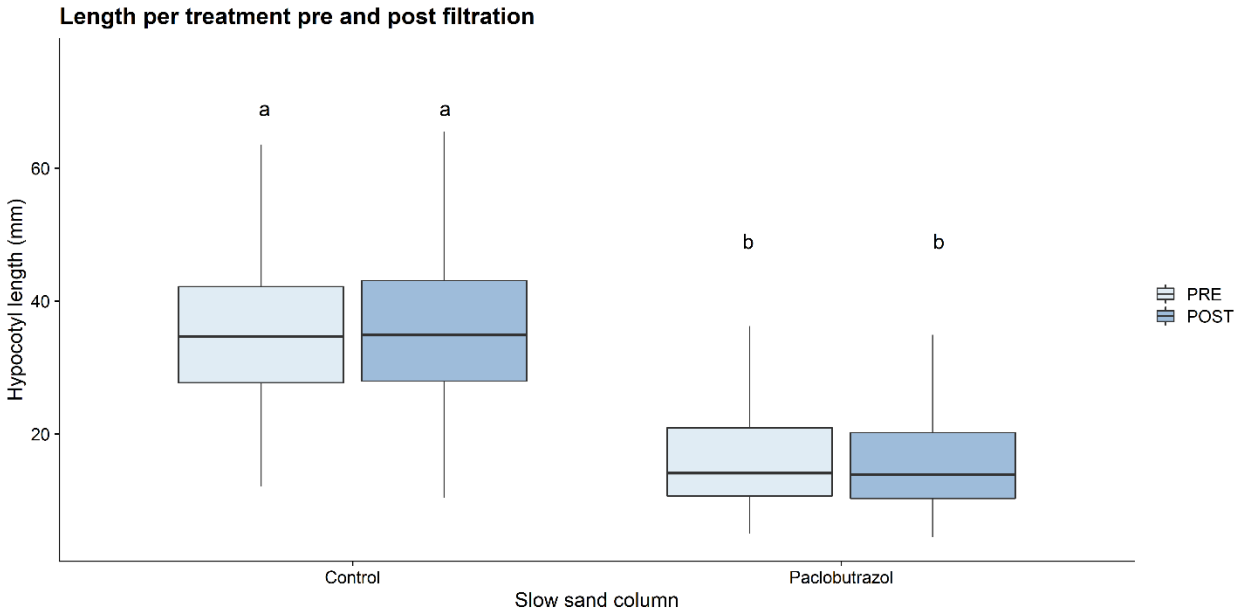

Figure 3. Boxplot summarizing mean treatment differences between filtration location and treatment type. Letters indicate significance ($p < 0.05$) between filtration locations.

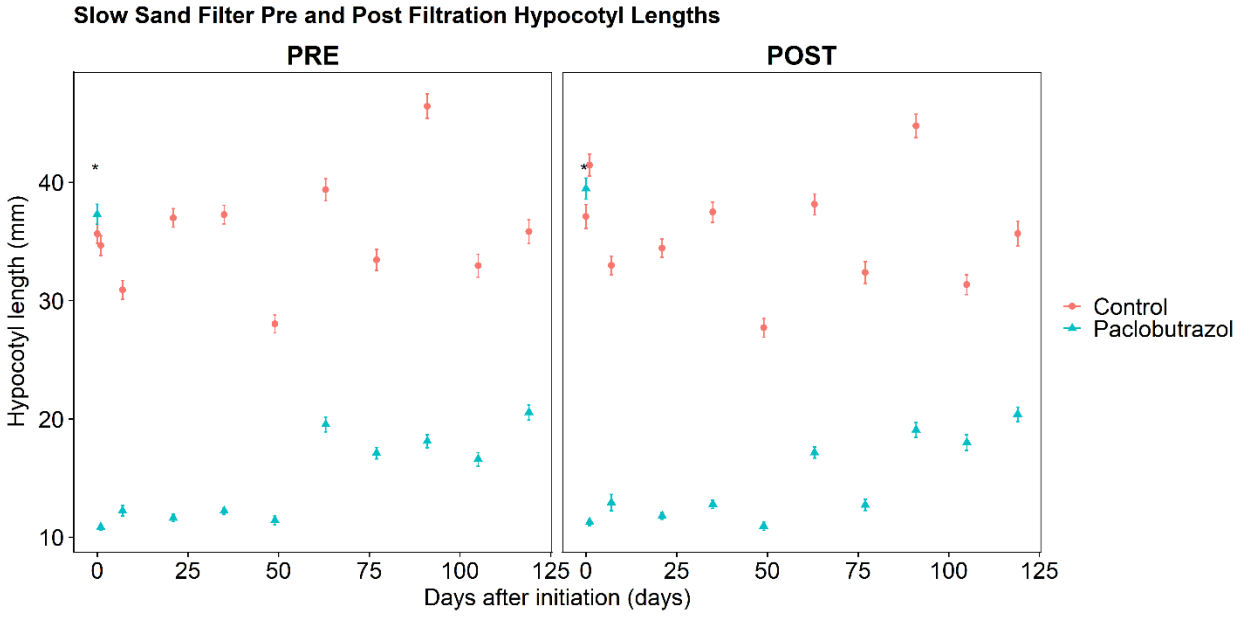

Figure 4. Mean broccoli hypocotyl lengths from sampling periods of PRE and POST sand filtered leachate. Control and paclobutrazol dosed leachate plotted together for each sample collection day. Asterisks indicate nonsignificant difference in hypocotyl lengths between control and paclobutrazol treatments at day 0.

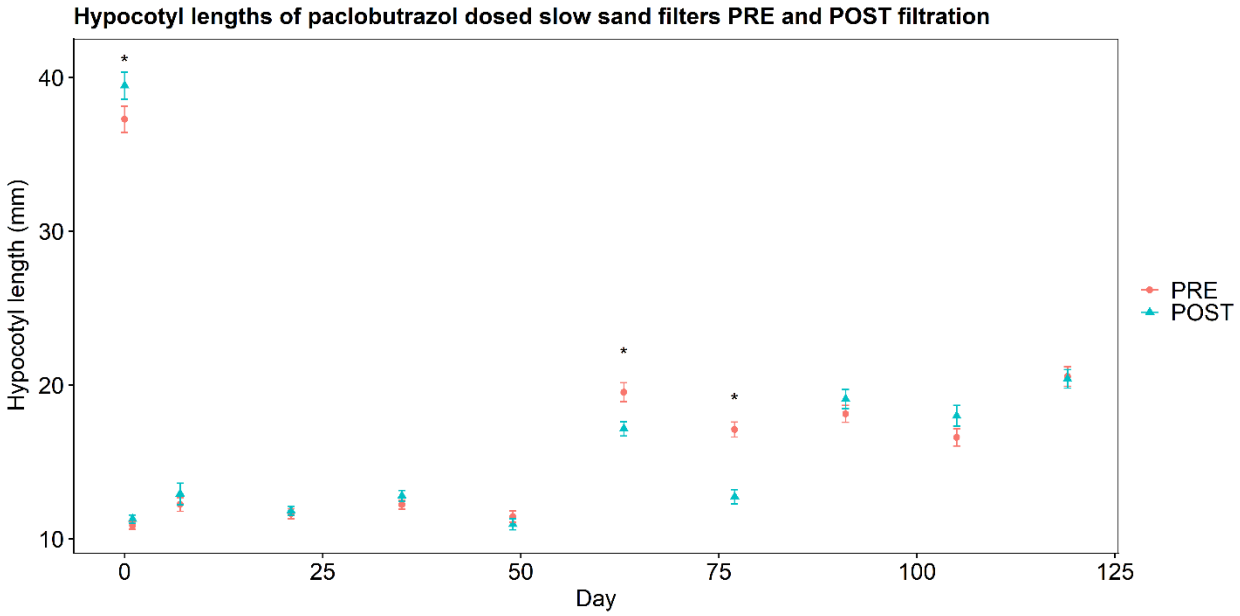

Figure 5. Mean lengths of broccoli hypocotyls exposed to samples from paclobutrazol treatment columns. Asterisks represent significant differences ($p < 0.05$) between the Pre and Post filtration samples.

Percent coverage of SSF column schmutzdecke

SSF column	Replicate	Sand bed area (cm^2)	Biofilm area (cm^2)	Percent coverage (%)
Control	1	530.7	3.81	0.72%
Control	2	530.7	1.81	0.34%
Control	3	530.7	2.15	0.40%
Control	4	530.7	2.74	0.52%
Control	5	530.7	8.98	1.69%
Treatment	1	530.7	241.40	45.49%
Treatment	2	530.7	229.55	43.25%
Treatment	3	530.7	298.59	56.26%
Treatment	4	530.7	253.92	47.85%
Treatment	5	530.7	371.39	69.98%

Table 1. The percent coverage of the schmutzdecke layer was determined for each respective SSF column.

7. References

Abdi, D. E., & Fernandez, R. T. (2019). Reducing water and pesticide movement in nursery production. *HortTechnology*, *29*(6), 730–735. https://doi.org/10.21273/HORTTECH04298-19

Ahmed, A., Abdelkhalek, S. T., Qureshi, S. R., & Wang, M.-Q. (2021). Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics*, *9*(42). https://doi.org/doi.org/10.3390/toxics9030042

Altland, J. E., Morris, L., Boldt, J., Fisher, P., & Raudales, R. (2015). Sample container and storage temperature for paclobutrazol monitoring in irrigation water. *HortTechnology*, *25*(6), 769–773. https://doi.org/10.21273/horttech.25.6.769

Ashraf, N., & Ashraf, M. (2012). Response of growth inhibitor paclobutrazol in fruit crops. *Intech*, 13. http://dx.doi.org/10.1039/C7RA00172J%0Ahttps://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics%0Ahttp://dx.doi.org/10.1016/j.colsurfa.2011.12.014

Bañón, S., Miralles, J., Navarro, A., & Sánchez-Blanco, M. J. (2009). Influence of paclobutrazol and substrate on daily evapotranspiration of potted geranium. *Scientia Horticulturae*, *122*(4), 572–578. https://doi.org/10.1016/j.scienta.2009.06.033

Blanchard, M. G., & Runkle, E. S. (2009). Use of a cyclic high-pressure sodium lamp to inhibit flowering of chrysanthemum and velvet sage. *Scientia Horticulturae*, *122*(3), 448–454. https://doi.org/10.1016/j.scienta.2009.06.016

CAEPA, California Environmental Protection Agency. (2023). State Water Resources Control Board. *Irrigated Lands Regulatory Program*. http://www.swrcb.ca.gov/water_issues/programs/agriculture/

Campos, L. C., Su, M. F. J., Graham, N. J. D., & Smith, S. R. (2002). Biomass development in slow sand filters. *Water Research*, *36*(18), 4543–4551. https://doi.org/10.1016/S0043-1354(02)00167-7

Casas, M. E., & Bester, K. (2015). Can those organic micro-pollutants that are recalcitrant in activated sludge treatment be removed from wastewater by biofilm reactors (slow sand filters)? *Science of the Total Environment*, *506–507*, 315–322. https://doi.org/10.1016/j.scitotenv.2014.10.113

CDFA, California Department of Food and Agriculture. (2021). *California Agricultural Statistics Review 2020-2021*. https://www.cdfa.ca.gov/Statistics/

Clark, P. A., Pinedo, C. A., Fadus, M., & Capuzzi, S. (2012). Slow-sand water filter: Design, implementation, accessibility and sustainability in developing countries. *Medical Science Monitor*, *18*(7), 105–117. https://doi.org/10.12659/MSM.883200

Collins, M. R., Eighmy, T. T., Fenstermacher, J. M., & Spanos, S. K. (1992). Removing natural organic matter by conventional slow sand filtration. *Journal / American Water Works Association*, *84*(5), 80–90. https://doi.org/10.1002/j.1551-8833.1992.tb07357.x

Currey, C. J., Walters, K. J., & McCabe, K. G. (2016). Quantifying growth control of lantana cultivars varying in vigor with ancymidol, flurprimidol, paclobutrazol, and uniconazole substrate drenches. *HortTechnology*, *26*(3), 320–326. https://doi.org/10.21273/horttech.26.3.320

Déniel, F., Rey, P., Chérif, M., Guillou, A., & Tirilly, Y. (2004). Indigenous bacteria with antagonistic and plant-growth-promoting activities improve slow-filtration efficiency in soilless cultivation. *Canadian Journal of Microbiology*, *50*(7), 499–508. https://doi.org/10.1139/w04-034

Desta, B., & Amare, G. (2021). Paclobutrazol as a plant growth regulator. *Chemical and Biological Technologies in Agriculture*, *8*(1), 1–15. https://doi.org/10.1186/s40538-020-00199-z

Fitriani, N., Eka, P., & Prasetyo, E. (2020). Behavior of schmutzdecke with varied filtration rates of slow sand filter to remove total coliforms. *Heliyon*, *6*(February), e03736. https://doi.org/10.1016/j.heliyon.2020.e03736

Grant, G. A., Fisher, P. R., Barrett, J. E., & Wilson, P. C. (2018). Removal of paclobutrazol from irrigation water using granular-activated carbon. *Irrigation Science*, *36*(3), 159–166. https://doi.org/10.1007/s00271-018-0572-1

Grant, G. A., Fisher, P. R., Barrett, J. E., & Wilson, P. C. (2019). Removal of Agrichemicals from Water Using Granular Activated Carbon Filtration. *Water, Air, and Soil Pollution*, *230*(1), 1–12. https://doi.org/10.1007/s11270-018-4056-y

Grant, G. A., Fisher, P. R., Barrett, J. E., Wilson, P. C., & Raudales, R. E. (2018). Paclobutrazol removal from irrigation water using a commercial-scale granular activated carbon system. *Scientia Horticulturae*, *241*(February), 160–166. https://doi.org/10.1016/j.scienta.2018.06.093

Haig, S., Quince, C., Davies, R. L., & Dorea, C. C. (2015). The Relationship between Microbial Community Evenness and FunctioHaig, S., Quince, C., Davies, R. L., & Dorea, C. C. (2015). The Relationship between Microbial Community Evenness and Function in Slow Sand Filters. American Society for Microbiology, 6(5), . *American Society for Microbiology*, *6*(5), 1–12. https://doi.org/10.1128/mBio.00729-15.Editor

Hammes, F., Velten, S., Egli, T., & Juhna, T. (2011). Biotreatment of Drinking Water. In *Comprehensive Biotechnology, Second Edition* (Second Edi, Vol. 6). Elsevier B.V. https://doi.org/10.1016/B978-0-08-088504-9.00386-X

Ho, L., Grasset, C., Hoefel, D., Dixon, M. B., Leusch, F. D. L., Newcombe, G., Saint, C. P., & Brookes, J. D. (2011). Assessing granular media filtration for the removal of chemical contaminants from wastewater. *Water Research*, *45*(11), 3461–3472. https://doi.org/10.1016/j.watres.2011.04.005

Jackson, M. J., Line, M. A., & Hason, O. (1996). Microbial degredation of a recalcitrant plant growth retardant-paclobutrazol. *Soil Biol. Biochem.*, *28*(9), 1265–1267. https://doi.org/10.1163/187529293X00358

Jiang, X., Wang, Y., Xie, H., Li, R., Wei, J., & Liu, Y. (2019). Environmental behavior of paclobutrazol in soil and its toxicity on potato and taro plants. *Environmental Science and Pollution Research*, *26*(26), 27385–27395. https://doi.org/10.1007/s11356-019-05947-9

Jiang, X., Wang, Y., Xie, H., Li, R., Wei, J., Liu, Y., Grant, G. A., Fisher, P. R., Barrett, J. E., Wilson, P. C., Raudales, R. E., Sterrett, J. R., Kaminski, J. E., Dernoeden, P. H., Bigelow, C. A., Zhao, X., Mu, Y., Yang, M., Singh, V. K., & Bhattacherjee, A. K. (2018). A simple multi-residue method for determination of plant growth retardants in Ophiopogon japonicus and soil using ultra-performance liquid chromatography–tandem mass spectrometry. *Scientia Horticulturae*, *207*(1), 160–166. https://doi.org/10.21273/hortsci.39.5.1126

Kentelky, E., Szekely-Varga, Z., Bálint, J., & Balog, A. (2021). Enhance growth and flower quality of chrysanthemum indicum l. With application of plant growth retardants. *Horticulturae*, *7*(12), 1–15. https://doi.org/10.3390/horticulturae7120532

Kumar, G., Lal, S., Bhatt, P., Ram, R. A., Bhattacherjee, A. K., Dikshit, A., & Rajan, S. (2021). Mechanisms and kinetics for the degradation of paclobutrazol and biocontrol action of a novel Pseudomonas putida strain T7. *Pesticide Biochemistry and Physiology*, *175*(March), 104846. https://doi.org/10.1016/j.pestbp.2021.104846

Lee, E., & Oki, L. R. (2013). Slow sand filters effectively reduce Phytophthora after a pathogen switch from Fusarium and a simulated pump failure. *Water Research*, *47*(14), 5121–5129. https://doi.org/10.1016/j.watres.2013.05.054

Liu, H., Lin, T., Mao, J., Lu, H., Yang, D., Wang, J., & Li, Q. (2015). Paclobutrazol residue determination in potato and soil using low temperature partition extraction and ultrahigh performance liquid chromatography tandem mass spectrometry. *Journal of Analytical Methods in Chemistry*, *2015*. https://doi.org/10.1155/2015/404925

Logan, A. J., Stevik, T. K., Siegrist, R. L., & Rønn, R. M. (2001). Transport and fate of Cryptosporidium parvum oocysts in intermittent sand filters. *Water Research*, *35*(18), 4359–4369. https://doi.org/10.1016/S0043-1354(01)00181-6

Mahnken, G. E. ., Skroch, W. A., Leidy, R. B. ., & Sheets, T. J. . (1999). Metolachlor and Simazine in Surface Runoff Water from a Simulated Container Plant Nursery. *Weed Technology*, *13*(4), 799–806. https://www.jstor.org/stable/3989014

Majsztrik, J. C., Fernandez, R. T., Fisher, P. R., Hitchcock, D. R., Lea-Cox, J., Owen, J. S., Oki, L. R., & White, S. A. (2017). Water Use and Treatment in Container-Grown Specialty Crop Production: A Review. *Water, Air, and Soil Pollution*, *228*(4). https://doi.org/10.1007/s11270-017-3272-1

Maurya, A., Singh, M. K., & Kumar, S. (2020). Biofiltration technique for removal of waterborne pathogens. In *Waterborne Pathogens*. Elsevier. https://doi.org/10.1016/b978-0-12-818783-

8.00007-4

Million, J. B., Barrett, J. E., Nell, T. A., & Clark, D. G. (1999). Inhibiting growth of flowering crops with ancymidol and paclobutrazol in subirrigation water. *HortScience*, *34*(6), 1103–1105. https://doi.org/10.21273/hortsci.34.6.1103

Million, Jeff B., Barrett, J. E., Nell, T. A., & Clark, D. G. (1999). Paclobutrazol distribution following application to two media as determined by bioassay. *HortScience*, *34*(6), 1099–1102. https://doi.org/10.21273/hortsci.34.6.1099

Moran, S. (2018). Clean water unit operation design. In *An Applied Guide to Water and Effluent Treatment Plant Design*. https://doi.org/10.1016/b978-0-12-811309-7.00007-2

Oki, L. R., Bodaghi, S., Lee, E., Haver, D., Pitton, B., Nackley, L., & Mathews, D. M. (2017). Elimination of Tobacco mosaic virus from irrigation runoff using slow sand filtration. *Scientia Horticulturae*, *217*, 107–113. https://doi.org/10.1016/j.scienta.2017.01.036

Paranychianakis, N. V., Angelakis, A. N., Leverenz, H., & Tchobanoglous, G. (2006). Treatment of wastewater with slow rate systems: A review of treatment processes and plant functions. *Critical Reviews in Environmental Science and Technology*, *36*(3), 187–259. https://doi.org/10.1080/10643380500542756

Park, S. (2005). E Nergy and E Nvironmental I Mpacts of R Oadway G Rades. *Journal of Transportation Engineering*, *April*, 154–160.

Phu, N. (2014). *Evaluating Pilot Scale Slow Sand Filtration Columns to Effectively Remove Emerging Contaminants in Recycled Water*. *University of California*, *Riverside*, *CA*, M.S. Thesis.

Pitton, B. J. L., Hall, C. R., Haver, D. L., White, S. A., & Oki, L. R. (2018). A cost analysis for using recycled irrigation runoff water in container nursery production: a Southern California nursery case study. *Irrigation Science*, *36*(4–5), 217–226. https://doi.org/10.1007/s00271-018-0578-8

Pompei, M. C. M. E., Campos, L. C., Ferreira da Silva, B., Fogo, J. C., & Vieira, E. M. (2019). Occurrence of PPCPs in a Brazilian water reservoir and their removal ef fi ciency by ecological fi ltration. *Chemosphere*, *226*, 210–219. https://doi.org/10.1016/j.chemosphere.2019.03.122

Poudyal, S., & Cregg, B. M. (2019). Irrigating nursery crops with recycled run-off: A review of the potential impact of pesticides on plant growth and physiology. *HortTechnology*, *29*(6), 716–729. https://doi.org/10.21273/HORTTECH04302-19

Poudyal, S., Thomas Fernandez, R., Owen, J., & Cregg, B. (2019). Dose-dependent phytotoxicity of pesticides in simulated nursery runoff on landscape nursery plants. *Water (Switzerland)*, *11*(11). https://doi.org/10.3390/w11112354

Ranjan, P., & Prem, M. (2018). Schmutzdecke- A Filtration Layer of Slow Sand Filter. *International Journal of Current Microbiology and Applied Sciences*, *7*(07), 637–645.

https://doi.org/10.20546/ijcmas.2018.707.077

Redekar, N. R., Bourret, T. B., Eberhart, J. L., Johnson, G. E., Pitton, B. J. L., Haver, D. L., Oki, L. R., & Parke, J. L. (2020). The population of oomycetes in a recycled irrigation water system at a horticultural nursery in southern California. *Water Research*, *183*, 116050. https://doi.org/10.1016/j.watres.2020.116050

RStudio Team. (2022). RStudio: Integrated Development Environment for R. In *R Foundation for Statistical Computing*. http://www.rstudio.com/

Sanders, E. R. (2012). Aseptic laboratory techniques: Plating methods. *Journal of Visualized Experiments*, *63*, 1–18. https://doi.org/10.3791/3064

Sattar, S., Husnain, T., & Javaid, A. (2010). Effect of NACL salinity on cotton (Gossypium arboreum L .) grown on MS medium and in hydroponic cultures. *Jounral of Animal and Plant Sciences*, *20*(2), 87–89. https://www.researchgate.net/publication/236840138%0AEffect

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Shalaby, T. A., Taha, N. A., Taher, D. I., Metwaly, M. M., El-Beltagi, H. S., Rezk, A. A., El-Ganainy, S. M., Shehata, W. F., El-Ramady, H. R., & Bayoumi, Y. A. (2022). Paclobutrazol Improves the Quality of Tomato Seedlings to Be Resistant to Alternaria solani Blight Disease: Biochemical and Histological Perspectives. *Plants*, *11*(3). https://doi.org/10.3390/plants11030425

Sharma, D., & Awasthi, M. D. (2005). Uptake of soil applied paclobutrazol in mango (Mangifera indica L.) and its persistence in fruit and soil. *Chemosphere*, *60*(2), 164–169. https://doi.org/10.1016/j.chemosphere.2004.12.069

Sniegowski, K., Bers, K., Ryckeboer, J., Jaeken, P., Spanoghe, P., & Springael, D. (2012). Minimal pesticide-primed soil inoculum density to secure maximum pesticide degradation efficiency in on-farm biopurification systems. *Chemosphere*, *88*(9), 1114–1118. https://doi.org/10.1016/j.chemosphere.2012.04.057

Soumya, P. R., Kumar, P., & Pal, M. (2017). Paclobutrazol : a novel plant growth regulator and multi-stress ameliorant. *Indian Journal of Plant Physiology*, *22*(3), 267–278. https://doi.org/10.1007/s40502-017-0316-x

Souza-Machado, V., Pitblado, R., Ali, A., & May, P. (1997). *Paclobutrazol seed treatment in tomato for improved tolerance to early transplanting and early harvest maturity*. *487*, 17.

Taylor, M. D., White, S. A., Chandler, S. L., Klaine, S. J., & Whitwell, T. (2006). Nutrient Management of Nursery Runoff Water using Constructed Wetland Systems. *HortTechnology*, *16*(December), 610–614.

USDA, United States Department of Agriculture. (2011). *Agricultural chemical use nursery and floriculture crops 2009*.

Weiland, J. E., Scagel, C. F., Gr, N. J., Davis, E. A., Beck, B. R., Foster, Z. S. L., & Fieland, V. J. (2020). *Soilborne Phytophthora and Pythium Diversity From Rhododendron in Propagation ,*

Container , and Field Production Systems of the Pacific Northwest. *104*(June), 1841–1850. https://doi.org/10.1094/PDIS-08-19-1672-RE

White, S. A., Owen, J. S., Majsztrik, J. C., Oki, L. R., Fisher, P. R., Hall, C. R., Lea-Cox, J. D., & Thomas Fernandez, R. (2019). Greenhouse and nursery water management characterization and research priorities in the USA. *Water (Switzerland)*, *11*(11), 1–19. https://doi.org/10.3390/w11112338

Yu, Y., & Wu, L. (2012). Analysis of endocrine disrupting compounds, pharmaceuticals and personal care products in sewage sludge by gas chromatography-mass spectrometry. *Talanta*, *89*, 258–263. https://doi.org/10.1016/j.talanta.2011.12.023

Yu, Z., Peldszus, S., & Huck, P. M. (2008). Adsorption characteristics of selected pharmaceuticals and an endocrine disrupting compound-Naproxen, carbamazepine and nonylphenol-on activated carbon. *Water Research*, *42*(12), 2873–2882. https://doi.org/10.1016/j.watres.2008.02.020

8. Appendix A

This appendix includes images that were not included in the thesis body but are representative of information captured.

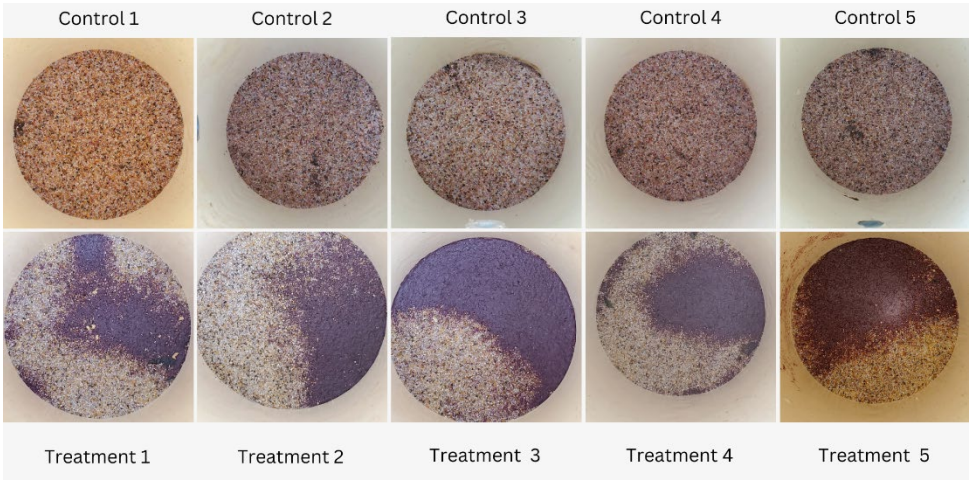

Figure A-1. The schmutzdecke of each column after the experimental period.

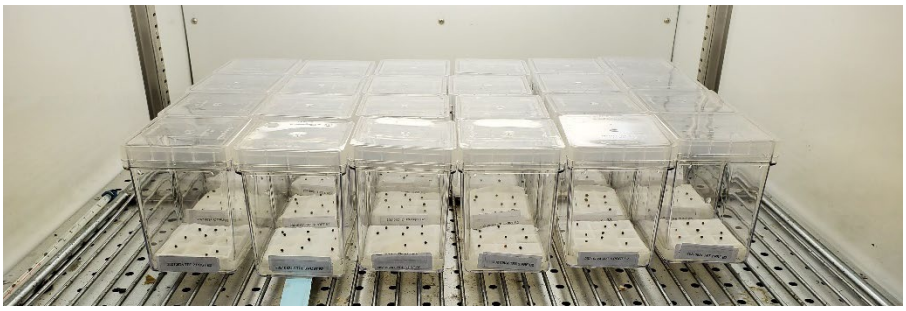

Figure A-2. Completed boxes placed in the growth chamber with seedlings placed on stage.

Figure A-3. Representation of the broccoli seedlings after 14 days of growth prior to being removed.

Figure A-4. Seedlings removed from platform after 14 days and lined up for hypocotyl measurement.

Figure A-5. The slow sand filtration system on the left, water storage in the background, and chrysanthemum beds on the right.

9. Appendix B

This appendix includes text, figures, and images regarding a follow-up experiment. This experiment was for exploratory purposes which may guide future research.

1. SSF column inoculation

1.1 Purpose

After the completion of the first experiment, a second pathfinder experiment was performed to evaluate whether microbial inoculation of the sand columns would help achieve removal of paclobutrazol (PBZ). The microbial inoculation involved the collection of soil from a nursery site that had previously applied PBZ into potted crops which was then incorporated into the experimental chrysanthemum bed media. The purpose was to establish a mature and diverse microbial community within the media that would sustain the SSFs through the generated runoff. Studies involved in biofiltration have inoculated biofilters with certain bacteria to seed and foster specific compound degradation (Ho et al., 2011; Sniegowski et al., 2012). One additional strategy was utilized which involved culturing *Pseudomonas spp.* in a concentrated PBZ solution. This technique has been effective in culturing bacterium capable of PBZ removal (Kumar et al., 2021). This culture was then added to the water provided to the SSF columns with the purpose of introducing more microbial diversity and establishment of biofilm with the increased potential for PBZ removal (Casas & Bester, 2015; Kumar et al., 2021).

2. Materials and Methods

2.1 Slow Sand System

The slow sand filter design used in the first experiment remained unchanged. Before starting the experiment, all leachate was drained from the system which included the holdings tanks, barrels, and sand columns. The SSF columns were dismantled, and the remaining sand was removed from each column. The SSF columns were reassembled and filled with new media.

Barrel dosing followed the same protocol, although a new PBZ standard solution was prepared. Twice the amount of PBZ standard solution was prepared in a 2 L Erlenmeyer flask containing the same PBZ concentration of 400 $mg \cdot L^{-1}$. The PBZ standard solution was used in barrel dosing and for bacterial culturing.

Water sampling occurred at: day 0, week 4 and then at two-week intervals until the last sampling at 20 weeks. The day 0 sampling occurred 4 hours after leachate was introduced into the sand filters and prior to the introduction of PBZ. Immediately after the sample collection on day 0, PBZ was dosed into the appropriate barrels. After each sampling period, the filled sample tubes were placed in a freezer at -40° C.

Similar to the first experiment, the ten SSF columns were split into two groups such that five were untreated controls receiving leachate without PBZ and five treatment columns that received PBZ dosed leachate. All treatment SSF columns were dosed with a microbial inoculum.

Bioassay procedures followed the same protocol as the first experiment including germination box preparation, 14-day growth chamber incubation, and hypocotyl length measurement.

2.2 Culturing media

The solution used to culture bacterial inoculum was mineral salt medium (MSM) with the recipe of ($g \cdot L^{-1}$) $MgSO4.7H_2O$ 0.2, $CaCl_2.2H_2O$ 0.01, $(NH_4)_2SO_4$ 2.0, $Na_2HPO_4.12H_2O$ 1.5, KH_2PO4 2.0, $FeSO_4.7H_2O$ 0.001 and glucose 2.5. This media was used for bacterial enrichment and was subsequently dosed into the slow sand filtration columns serving as a concentrated inoculum source.

2.3 Culturing *Pseudomonas putida*

Soil samples were collected by hand trowel from nursery beds at Everde Growers (Winters, CA, USA) to fill half of a 1-gallon resealable bag. The container plants placed on these beds had undergone PBZ treatment in the past and soil samples were taken within and along the runoff channels from these beds. An additional ten soil samples were taken with a soil core sampling tool (3KHF2, Grainger, Lake Forest, CA, USA) within planted landscapes at the University of California arboretum (N 38°32'13.1", W 121°44'51.2”) (Davis, CA, USA). Both location samples were homogenized via hand agitation in a 1-gallon resealable bag and the starter culture was prepared by adding 1 gram of soil into a 250 mL Erlenmeyer flask containing 50 mL sterile MSM broth (Kumar et al., 2021).

Culturing PBZ tolerant bacteria involved following an enrichment series of additions of increasing concentrations of PBZ. After five days, a 1 mL aliquot of the starter culture was pipetted into a 250 mL Erlenmeyer flask with 50 mL autoclaved MSM broth containing a concentration of 5 $mg \cdot L^{-1}$ PBZ. The concentration of PBZ within the MSM broth was increased subsequently after each five-day period, increasing to 5 $mg \cdot L^{-1}$, 10 $mg \cdot L^{-1}$, 20 $mg \cdot L^{-1}$ each period to the final concentration of 40 $mg \cdot L^{-1}$ PBZ. Five replicate flasks of the bacterial solution were kept at 40 $mg \cdot L^{-1}$ for the remainder of the study and was refreshed by transferring 1 gram of solution into new flasks containing 50 mL of MSM broth at the concentration of 40 $mg \cdot L^{-1}$ PBZ every five days. All flasks were placed on a shaker table that was programmed to suspend the bacteria in solution periodically over the entire day.

2.4 Inoculating the slow sand filters

A 400 mL composite sample of the five cultured bacterial solution flasks (40 $mg \cdot L^{-1}$ PBZ) was combined and inoculated into the treatment sand columns via the PRE filtration sampling port every five days. A 15 cm length of 0.635cm polyethylene tubing (B38P, DIG Corporation, Vista, CA, USA) was secured to a 50 mL syringe (BD309654, VWR International, Radnor, PA, USA) and attached to the PRE sampling port of the respective SSF column. The port was opened allowing for the solution to be injected, and then the valve was closed. The amount of injected solution was 40 mL into each treatment column. If there was a water collection event that coincided on the same day as bacterial injection, the water collection occurred first.

2.5 Evaluating *Pseudomonas* spp. presence

The presence of *Pseudomonas spp.* in the bacterial inoculation solutions was evaluated on prepared selective agar (Difco Pseudomonas isolation agar,90004-394, VWR International, Radnor, PA, USA). The agar was prepared on standard petri dishes (P1656, VWR International, Radnor, PA, USA) following plate preparation protocol of Sanders, and were stored in a refrigerator at 5° C until needed (Sanders, 2012). Periodic testing of the bacterial solution consisted of streak plating. A sterilized wooden stick was dipped into the bacterial solution and spread across the surface of one quadrant of the agar plate in a zigzag pattern. A new sterilized wooden stick touched the end of the previous pattern and was streaked across in the same fashion in the second quadrant. This was replicated until all 4 quadrants were streaked. The dishes were incubated inside a plastic container on the lab bench at 23° C for up to seven days (Blazevic et al.,1973). Plates were observed every 24 hours.

2.6 Data Analysis

Statistical analysis was conducted with the same methodology as the first experiment. The pathfinder experiment data analysis was processed in RStudio (v. 2022.02.2+485) (RStudio Team, 2022) and figures were produced using the 'ggplot2' package (Wickham, 2016).

3. Results

The 20-week period with microbial inoculation did not show PBZ degradation as there were no significant differences ($p>0.05$) between the hypocotyl lengths grown with POST

filtration leachate compared to PRE filtration leachate of the treatment columns (Figure B-1). The hypocotyl lengths growth with the control PRE and POST leachates were similar in length. (Figure B-1) indicating that leachate passing through the sand bed did not affect hypocotyl length. Although there was no PBZ present in the system, the day zero hypocotyl lengths of the control PRE leachate were significantly shorter ($p< 0.05$) than the treatment PRE lengths (Figure B-2). The treatment PRE leachate grew significantly ($p<0.05$) shorter hypocotyls than the hypocotyls grown with the control PRE leachate. This indicates that PBZ was effective in reducing hypocotyl length and the bioassays were responsive to low concentrations of PBZ.

Comparisons between the treatment column hypocotyl lengths varied during the experiment. Weeks 6, 10, 14, 16 and 20 showed significant differences in growth between the hypocotyl lengths grown with PRE and POST leachate (Figure B-3). Weeks 6, 10, and 16 had significantly ($p<0.05$) longer hypocotyl lengths grown with POST filtrate which indicated less PBZ presence (Figure B-3). Conversely, weeks 14 and 20 showed significantly less growth of the POST filtration hypocotyl lengths compared to PRE filtration leachate (Figure B-3). This indicated that PBZ concentrations in the leachate were elevated after filtration.

4. Discussion

The pathfinder experiment had some components that were missing that would have made it statistically more robust. The experiment consisted of control columns that did not receive PBZ and did not receive bacterial inoculation. It would have been advantageous to have columns that did not receive PBZ but did receive bacterial inoculation. This may have increased confidence that the treatment effect observed were caused by PBZ instead of the interactions of

the bacterial addition. The bacterial solution was cultured in a PBZ solution, which further complicates having an unaltered control.

To avoid complications of residual PBZ concentrations of the bacterial solution, the microbial solution could have been plated onto the *Pseudomonas* selective agar after undergoing the selective pressure of increasing PBZ concentrations. Once the final concentration was reached, the bacterial solution could have been plated onto the selective media and the colonies could have been cultured onto additional agar plates as a purification step or reintroduced into MSM broth lacking PBZ. The MSM broth would then contain *Pseudomonas spp.* that were pre-selected for PBZ tolerance, but the risk of PBZ contamination to control columns would be reduced. The resulting microbial inoculation would have consisted of mostly *Pseudomonas* and would lack the presence of PBZ. Since the bacterial solution originated from soil, there could have been any number of different species present.

Furthermore, the *Pseudomonas* selective agar does not identify which species, and the protocol was meant to culture *Pseudomonas putida* as this species was identified in reducing PBZ. Genetic analysis would be necessary to identify the species diversity of the inoculation solution. The species and diversity analysis may indicate whether the species present are associated with PBZ removal or are associated with efficient SSF columns.

The same limitations apply to this experiment as did the first experiment regarding leachate analysis using liquid chromatography and mass spectrometry (Grant, Fisher, Barrett, & Wilson, 2018; Jiang et al., 2019; Liu et al., 2015). This would lead to direct PBZ concentration analysis which may indicate whether there was partial degradation. Additionally, lab analysis could evaluate whether PBZ degradation was occurring in the culture flasks, and perhaps a

preliminary benchtop study would preclude injection into the SSF system. Bioassays performed with the bacterial solutions may have also indicated whether PBZ was degraded as a two-week check.

What was most inciteful about the experiment was the comparisons of the treatment column hypocotyl lengths. There were three sample days that showed reduced PBZ concentrations after undergoing filtration (Figure B-3). More notably, the sixth week showed the largest range between the PRE and POST hypocotyl lengths which is encouraging. However, a major error in the methodology of the experiment may have interfered with schmutzdecke progress. The bacterial inoculation contained a higher level of PBZ than the treatment columns were receiving from the leachate. The inoculation solution contained 40 $mg \cdot L^{-1}$ PBZ, while the treatment leachate entering the SSF columns was 0.05 $mg \cdot L^{-1}$ PBZ. The large difference in concentrations meant that the SSF columns were receiving a larger dose of PBZ at periods that were not uniform to leachate sampling days. This may have resulted in the POST filtration hypocotyl lengths being shorter on weeks 14, 16, and 20. The bacterial inoculation with the higher concentration of PBZ may have leached into the POST sample leachate.

5. Conclusions

The pathfinder experiment highlighted an area of study involving inoculation techniques for slow sand filtration systems. As a pathfinder, there were few highlights that indicate the presence of PBZ removal within the SSF system, however the methodology needed to be altered to account for statistical robustness and checks in place to reduce introducing higher concentrations of PBZ that may have obscured treatment effects. The bacterial culturing

technique would be a likely branching point to study prior to introducing into a SSF system. Bioassay testing was effective and still offers a fast and inexpensive method to evaluate the presence of PBZ. Coupled with lab analysis of various PBZ concentrations, a dilution series could be designed to indicate hypocotyl lengths with precise concentrations of PBZ.

6. Figures

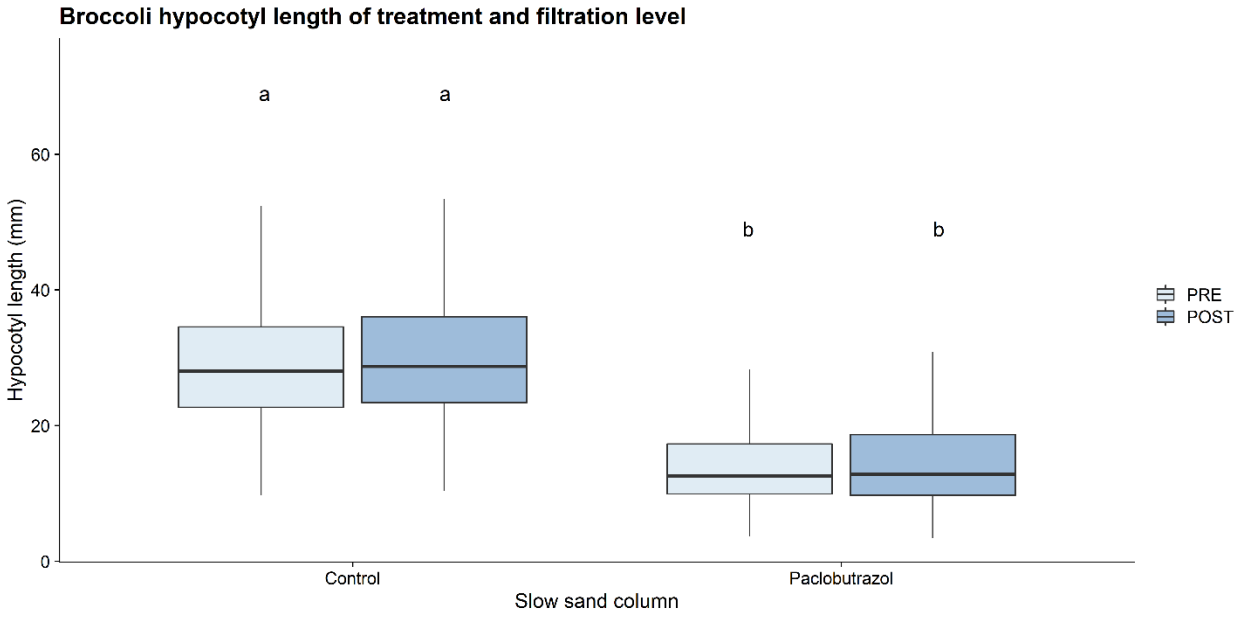

Figure B-1. Boxplot summarizing mean treatment differences between filtration location and treatment type. Letters indicate significance ($p < 0.05$) between filtration locations.

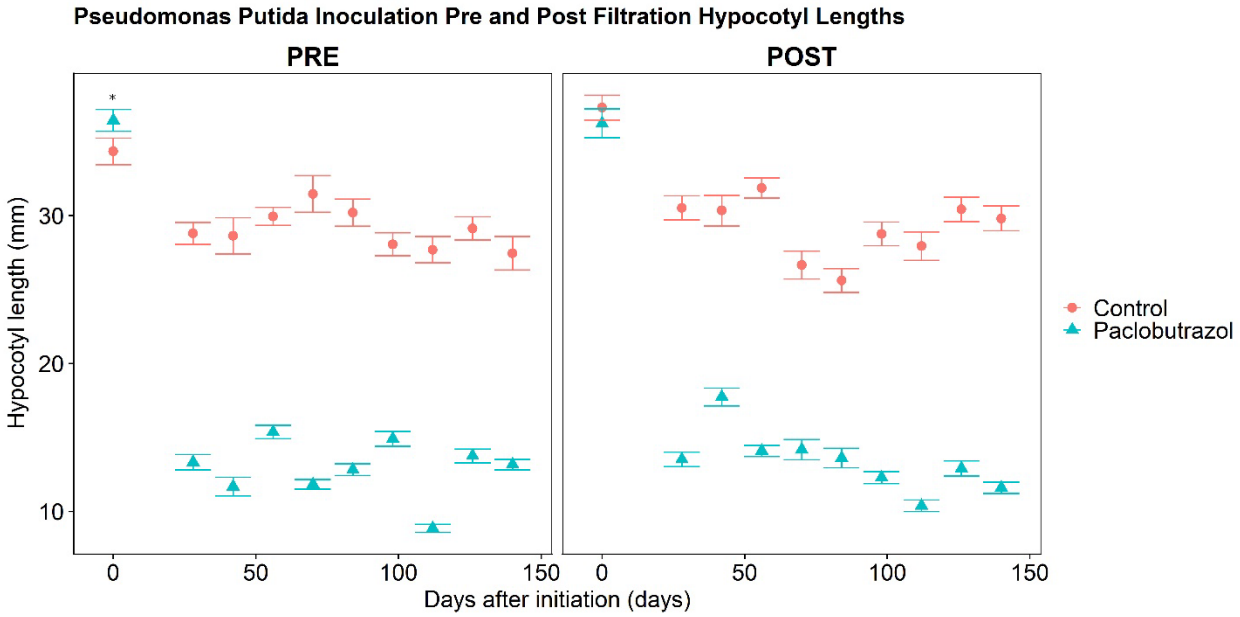

Figure B-2 Mean lengths of broccoli hypocotyls exposed to samples from paclobutrazol treatment columns. Day 0 represents significant differences ($p < 0.05$) between the Pre and Post filtration samples.

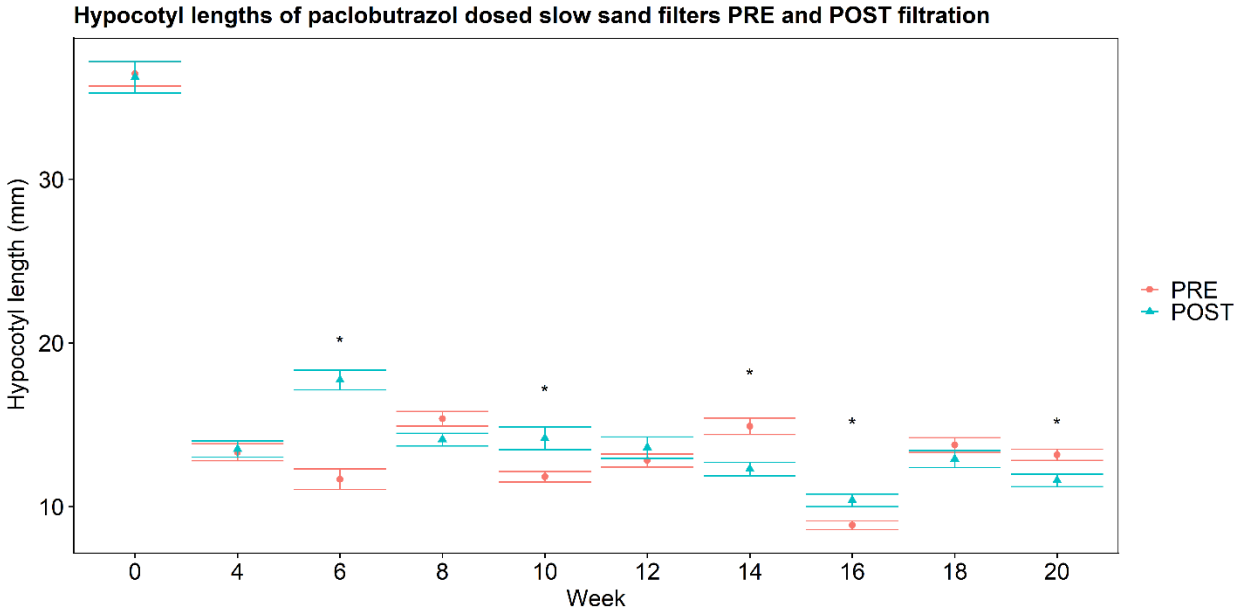

Figure B-3. Mean lengths of broccoli hypocotyls exposed to samples from paclobutrazol treatment columns. Asterisks represent significant differences ($p < 0.05$) between the Pre and Post filtration samples of week 6, 10, 14, 16, and 20.

7. Literature Cited

Blazevic, D. J., Koepcke, M. H., & Matsen, J. M. (1973). Incidence and identification of Pseudomonas fluorescens and Pseudomonas putida in the clinical laboratory. *Applied microbiology*, *25*(1), 107–110. https://doi.org/10.1128/am.25.1.107-110.1973

Casas, M. E., & Bester, K. (2015). Can those organic micro-pollutants that are recalcitrant in activated sludge treatment be removed from wastewater by biofilm reactors (slow sand filters)? *Science of the Total Environment*, *506–507*, 315–322. https://doi.org/10.1016/j.scitotenv.2014.10.113

Grant, G. A., Fisher, P. R., Barrett, J. E., & Wilson, P. C. (2018). Removal of paclobutrazol from irrigation water using granular-activated carbon. *Irrigation Science*, *36*(3), 159–166. https://doi.org/10.1007/s00271-018-0572-1

Ho, L., Grasset, C., Hoefel, D., Dixon, M. B., Leusch, F. D. L., Newcombe, G., Saint, C. P., & Brookes, J. D. (2011). Assessing granular media filtration for the removal of chemical contaminants from wastewater. *Water Research*, *45*(11), 3461–3472. https://doi.org/10.1016/j.watres.2011.04.005

Jiang, X., Wang, Y., Xie, H., Li, R., Wei, J., & Liu, Y. (2019). Environmental behavior of paclobutrazol in soil and its toxicity on potato and taro plants. *Environmental Science and Pollution Research*, *26*(26), 27385–27395. https://doi.org/10.1007/s11356-019-05947-9

Kumar, G., Lal, S., Bhatt, P., Ram, R. A., Bhattacherjee, A. K., Dikshit, A., & Rajan, S. (2021). Mechanisms and kinetics for the degradation of paclobutrazol and biocontrol action of a novel Pseudomonas putida strain T7. *Pesticide Biochemistry and Physiology*, *175*(March), 104846. https://doi.org/10.1016/j.pestbp.2021.104846

Liu, H., Lin, T., Mao, J., Lu, H., Yang, D., Wang, J., & Li, Q. (2015). Paclobutrazol residue determination in potato and soil using low temperature partition extraction and ultrahigh performance liquid chromatography tandem mass spectrometry. *Journal of Analytical Methods in Chemistry*, *2015*. https://doi.org/10.1155/2015/404925

R Development Core Team. (2019). A Language and Environment for Statistical Computing. In R Foundation for Statistical Computing

Sanders, E. R. (2012). Aseptic laboratory techniques: Plating methods. *Journal of Visualized Experiments*, *63*, 1–18. https://doi.org/10.3791/3064