The Toxicity Effect of Bisphenol- A on Portulaca *oleracea*

By

Madison MacAndrew

To obtain the Degree “Master of Sciences”

Supervisor: Christine Stumpp (BOKU)

Co-Supervisors: Giuesppe Brunnetti (BOKU), Bjarne Strobel (UCPH)

Filed on

February 22, 2021

Institute for Soil Physics and Rural Water Management

University of Natural Resources and Applied Life Sciences, Vienna

STATUATORY DECLARATION

I hereby declare that I am the sole author of this work. No assistance other than that which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part

ABSTRACT

In der heutigen modernen Gesellschaft wurde ein großer Teil der natürlichen Landschaft der Welt von Grünflächen in urbanisierte Grundstücke umgewandelt. Dies hat dazu geführt, dass ehemals poröse Medien in undurchlässige Oberflächen umgewandelt wurden. Dadurch wurde der Wasserkreislauf so verändert, dass in städtischen Gebieten ein unnatürlicher Anstieg von Menge und Intensität des Regenwasserabflusses zu verzeichnen ist. Unter den vielen Maßnahmen, die ergriffen wurden, um dieses Ereignis abzumildern, sind Gründächer eine attraktive Option. Bekannt für ihre vielen ökologischen Vorteile, sind Gründächer auch weithin anerkannt für ihre Fähigkeit, städtische Regenwasserspitzen und -abflüsse abzumildern, um ungünstige Abflussereignisse zu reduzieren. Obwohl begrünte Dächer bereits eine Fülle von ökologischen Vorteilen bieten, ist die Hinzufügung eines neuen Vorteils durch grüne Stadtentwicklung in letzter Zeit eine Idee von wachsender Popularität. Durch die Integration von Pflanzenschutzmitteln können begrünte Dächer nicht nur die Menge und Intensität des Abflusses verringern, sondern auch die Qualität des häuslichen Abwassers gegen verschiedene Schadstoffe verbessern. Eine Verunreinigung, die zunehmend Anlass zur Sorge gibt, ist eine Verbindung, die bei der Herstellung und Zersetzung von Kunststoffprodukten entsteht, Bisphenol-A (BPA). Das als krebserregend bekannte BPA ist eine Verunreinigung, die in vielen Wasserressourcen in der Nähe von verdichteter Bevölkerung gefunden wird. Um BPA zu bekämpfen, kann eine bestimmte Pflanze, die für ihre Fähigkeit bekannt ist, BPA effektiv zu beseitigen, bekannt als Portulaca oleracea, auf Gründächern eingesetzt werden. Die aktuelle Studie kombiniert daher dieses Wissen, um die Materialisierung jeglicher Toxizitätseffekte von BPA auf P. oleracea in einer begrünten Dachumgebung in Bezug auf die Fähigkeit des begrünten Daches, seine Wasserschutzfunktionen fortzusetzen, zu analysieren. Um mögliche Toxizitätseffekte zu untersuchen, wurden drei Boxen erstellt, um verschiedene Konzentrationen von bewässertem BPA in dieser Art von Umgebung zu vergleichen. Diese Konzentrationen umfassten 0 mg/L (Box 1), 2 mg/L (Box 2) und 5 mg/L (Box 3). Die Daten aus diesen Boxen wurden über den Zeitraum von einem Monat im Sommer des Jahres 2020 gesammelt. Während dieser Zeit wurden die Pflanzen in einer Klimakammer bebrütet, die allen Boxen gleiche Variablen (d.h. Temperatur & Feuchtigkeit) aufzwang. Um einen eventuellen toxischen Effekt von BPA auf P. oleracea zu überwachen, wurde die Evapotranspiration durch Messungen des Blattwasserpotentials (LWP) analysiert. Druckhöhe, Bodenwassergehalt, Bewässerungsabfluss und Verdunstungstrends wurden ebenfalls gemessen und mit den LWP-Daten verglichen. Hinsichtlich der Druckhöhe, des Bodenwassergehalts und der Verdunstung gab es keine erkennbaren Trends oder Muster, die mit den Variationen der LWP-Messungen korrelierten. In Kasten 2 und Kasten 3 wurden jedoch morphologische Veränderungen in der Blattdichte und der Größe einzelner Blätter dargestellt. Box 2 zeigte auch einen verringerten Gesamtabfluss (12 259 mm Niederschlag), während Box 3 einen höheren Gesamtabfluss (15 284 mm Niederschlag) über den Untersuchungszeitraum zeigte, mit Box 1 als Kontrolle mit 14 561 mm Niederschlag. Trotz dieser Unterschiede wurden auf täglicher Basis keine eindeutigen Muster oder Trends zwischen den Boxen oder in Bezug auf Änderungen der LWP-Messungen beobachtet.

In current modern society, much of the natural landscape of the world has been altered from green areas to urbanized plots of land. This has led to what was once porous media being transformed to impervious surfaces. Thus, the water cycle has been modified in such a way that urban areas are experiencing unnatural increases, in both quantity and intensity, of stormwater runoff. Among many interventions that have been put in place to mitigate this event, green roofs are an attractive option. Known for their many ecological benefits, green roofs are also widely recognized for their ability to mitigate urban stormwater peak and flow to reduce adverse runoff events. Although green roofs already provide a plethora of ecological benefits, the addition of a new benefit through green urban development has, of recent, been an idea of growing popularity. By incorporating phytoremediation, green roofs can not only mitigate runoff quantity and intensity, but also provide a method of improving domestic wastewater quality against various contaminants. One contaminant of emerging concern is a compound produced in the production and decomposition of plastic products, bisphenol-A (BPA). Known as a carcinogen, BPA is a contaminant found in many water resources located near condensed populace. In order to combat BPA, A particular plant that is known for its ability to effectively remediate BPA, known as Portulaca *oleracea*, can be utilized on green roofs. The current study therefore combines this knowledge to analyze the materialization of any toxicity effects of BPA on P. *oleracea* in a green roof environment in relation to the ability of said green roof to continue its water mitigation functions. To examine any possible toxicity effects, three boxes were created to compare different concentrations of irrigated BPA in this kind of environment. These concentrations included 0 mg/L (Box 1), 2 mg/L (Box 2) and 5 mg/L (Box 3). The data from these boxes was collected over the period of one month in the summer of the year 2020. During this time, the plant subjects were incubated in a climate chamber which forced equal variables (i.e. temperature & humidity) on all boxes. To monitor any toxicity effect of BPA on P. *oleracea*, evapotranspiration was analyzed through leaf water potential (LWP) measurements. Pressure head, soil water content, irrigation outflow and evaporation trends were also measured and compared with LWP data. Regarding pressure head, soil water content and evaporation, there were no recognizable trends or patterns that correlated with variations in LWP measurements. Box 2 and Box 3, however, did portray morphological changes in foliage density and individual leaf size. Box 2 also showed a decreased overall outflow (12 259 mm rainfall) while box 3 showed a higher overall outflow (15 284 mm rainfall) over the study period, with box 1 as the control at 14 561 mm rainfall. Despite these differences, on a daily basis, no distinct patterns or trends were observed between the boxes or in relation to changes in LWP measurements.

LIST OF TABLES

LIST OF FIGURES

FIGURE **PAGE**

LIST OF ACRONYMS

BPA	Bisphenol-A
EDC	Endocrine Disrupting Compound
LWP	Leaf Water Potential

GLOSSARY

Bisphenol-A	An emerging contaminant of concern A human carcinogen Produced during plastic manufacturing and breakdown
Green Roof	The introduction of vegetation onto a roof surface Often installed for water management purposes
Leaf Water Potential	A measure of the potential energy of water in a plant Dictates if and where water will move
Portulaca oleracea	Also known as common purslane Able to remediate bisphenol-A Exhibits C3 and C4 carbon fixation methods
WP4C	Potentiameter produced my MeterGroup An instrument used to measure water potentials

TABLE OF CONTENTS

INTRODUCTION

With an ever-developing society, it is inevitable that contaminants will become more prevalent in the environment and it is essential to minimize human exposure to these hazardous substances. Contaminants such as bisphenol-A (BPA), which is a human carcinogen, can be found present globally in both naturally occurring and man-made waterways; especially where anthropogenic activity is high, concentrations of BPA are ever present. In such urban areas with dense population, a decentralized approach to mitigating human exposure to BPA is a relevant option. Through the installation of green roofs, BPA in cities can be combated in this way. However, despite this novel approach, the already established ecological benefits of green roofs should not be sacrificed. Aside from their aesthetic appeal, green roofs provide an array of benefits. One of the main essential benefits of a green roof is its ability to manage stormwater runoff.

As the natural landscape of the world has been slowly transformed from green areas to urbanized plots, there has been an increase in the number of impervious surfaces covering what was once porous soil (Anwar et al., 2012; Locatelli et al., 2014). The rate at which industrialization and urbanization is occurring is unsustainable in the natural world and is causing significant problems in many areas (Anwar et al., 2012). Surfaces such as roads, sidewalks and buildings modify the water cycle by increasing storm water runoff volumes and peaks, and by reducing the delay between peak rainfall and peak runoff (Bengtsson, 2006; Locatelli et al., 2014). Although cities are designed to drain and remove water from urban areas, these mechanisms are not always sufficient, especially in cases of intensive rainfall. Stormwater exceeding the capacity of municipal drainage systems can cause flooding, economic loss and can be detrimental to human health (Qin et al., 2013). Areas that are prone to flooding are at an even higher risk with the combined effects of urbanization and climate change.

Green roofs have been around for over 100 years and are now an important aspect of modern urban planning (Li & Yeung, 2014). The purpose of a green roof is to establish vegetation on what would otherwise be an impermeable roof surface. This introduces a range of ecological and economic benefits, or ecosystem services, including reduction of urban heat island effect, purification of air pollution, reduction of building energy consumption (Shafique, 2018) and increased stormwater retention (Getter & Rowe, 2006). However, not all green roofs contain the same variables. Factors such as depth of the substrate, slope, age, soil moisture characteristics, and vegetation type can greatly affect rainfall runoff dynamics such as the extent of the reduction of runoff volume, peak flow and the delay of peak flow (Anwar et al., 2012; Li & Babcock, 2014). For this reason, one of the main priorities of green roof installation has been to combat and manage urban stormwater runoff. While this has been one of the prime focuses of green roof design, a new idea has been proposed to further functionality. With an increase in global population and modernization, many new contaminants are becoming increasingly present in common everyday life. Many emerging contaminants of concern, particularly endocrine disrupting compounds (EDC's), are widely prevalent in urban areas. One such EDC known as bisphenol-A (BPA) has become widespread due to its global production and is recognized for its potential as a human carcinogen among other human health deficiencies (Seachrist et al., 2016; Syranidou et al., 2017). Many emerging contaminants (such as BPA) at trace levels (ng/L & ug/L) are considered micropollutants and are often able to bypass conventional methods of remediation such as sewage plants (Gorito et al., 2017). Wastewater treatment plants treating industrial effluents, sewage sludge and wastewater landfill leachates are therefore considered to be the main sources of BPA release into the environment (Syranidou et al., 2017). It is for this reason that new on-site technologies, such as phytoremediation, are quickly becoming recognized cost-effective methods to combating contaminated sites (Ruby & Appleton, 2010). While phytoremediation can be used to remediate natural ecosystems, it can also be applied to urban environments. Green roofs have thus been proposed in various countries as an efficient and practical tool to combat contaminants in urbanized areas (Vijayaraghavan et al., 2016). Therefore, green roofs can be considered a practical decentralized approach to remediate urban exposure to BPA.

Despite the practicality of using green roofs for this purpose, there is much disparity in the knowledge acquired for this particular exploitation. Little is known about how a green roof environment might affect the process of phytoremediation. Due to limitations found in the unique environment of a green roof such as growth space, shallow substrate material, high exposure to UV radiation, wind effects and storm events, it may be difficult choosing an appropriate vegetation coverage. It is for this reason that this study decided to use Portulaca *orleacea* as the chosen green roof vegetation. While already a popular choice for green roof coverage in different countries, it is also well recognized for its ability to phytoremediate BPA

(Imai et al.,2007; Matsui et al., 2011; Kaneda et al., 2012; Okuhata et al., 2013). While never thoroughly studied in a green roof environment, it is obvious that P. *oleracea* would be a suitable choice for the examination of remediation potential of BPA on a green roof. However, as previously mentioned, one of the main functions of a green roof is to mediate stormwater runoff. Even as an already established green roof vegetation, in order to be an effective option for phytoremediation of contaminants such as BPA, survivability and prior hydrological functions must also be upheld. To this aim, we conducted an experimental campaign to analyze the potential toxicity effect of BPA on P. *oleracea* while emulating a green roof environment.

LITERATURE REVIEW

Green Roof Design

A green roof is composed of multiple layers (Figure 1); a waterproofing membrane & root barrier, growing medium, drainage layer and a vegetation layer (Anwar et al., 2012). The waterproofing membrane sits on top of the roof material in order to prevent moisture from entering the underlying building. The root barrier lies above the waterproofing membrane and is designed to prevent the roots of the vegetation from penetrating lower layers (Anwar et al., 2012). The drainage layer is responsible for the quick removal of runoff water form the roof area and must therefore be composed of a highly permeable material (United States Environmental Protection Agency, 2009). The ability of a green roof to maintain aerated and support plant growth is dependent on the drainage layer, making it a vital component of a green roof (Vijayaraghavan, 2019). The growing medium, on the other hand, must be able to retain enough water for suitable growth of vegetation while still retaining necessary drainage properties (United States Environmental Protection Agency, 2009).

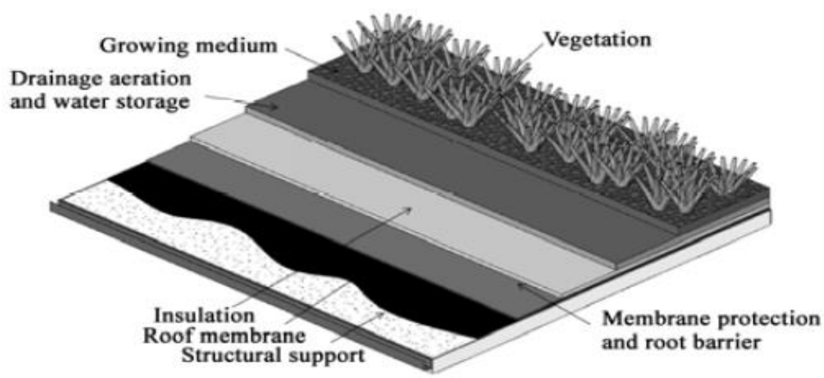


Figure 1. A typical green roof system and its associated layers (modified from Anwar et al., 2012).

There are generally two types of green roofs: intensive and extensive. Intensive green roofs have deep soil layers to support complex vegetation such as trees and shrubs, while extensive green roofs have a thin soil layer and typically use sedum or lawn as a vegetation cover (Anwar et al., 2012). Characteristics such as number of layers, type of materials, soil thickness, soil type and geometry (i.e. slope angle and length) can affect functions of a green roof (Anwar et al., 2012). Another important factor in green roof design is the vegetation type. For example, drought tolerant *Sedum* spp. are a popular choice for extensive green roofs due to their ability to store water and their process of crassulacean acid metabolism (CAM) (VanWoert et al., 2005). Other drought resistant species that would be suitable where irrigation is minimal or not present are succulent species such as *Sempervivum* and *Delosperma* (Getter & Rowe, 2006). Other factors that should be considered include aesthetic appeal, environmental conditions on both the macro- and micro- scale, installation methods and maintenance (Getter & Rowe, 2006).

Green Roof Vegetation Type

Vegetation is a key element in green roof design and should be selected to suit the green roof environment and meet runoff quality criteria (Li & Babcock, 2014). Plants on green roofs should generally be drought resistant, tolerant to solar radiation and have a cooling ability (Li & Yeung, 2014). Plants can be divided into three types based on their photosynthetic mechanism: C3, C4 and CAM (Nagase & Dunnett, 2012). The CAM photosynthetic pathway is characterized by net nighttime CO_2 uptake (Kluge & Ting, 1978; Ting, 1985; Winter & Smith, 1996). Other criteria that can confirm the CAM pathway include diurnal acid fluctuation, changes in malate content and increase in PEPC activity (Cushman & Bohnert, 1997). Many *sedum* are succulents and exhibit a CAM pathway, meaning their stomata open during the night and close during the day to prevent water loss from transpiration (Li & Yeung, 2014). Dvorak & Volder (2010) stated that plants in the *Sedum* genus have been shown to be reliable green roof vegetation as they have a high drought tolerance. Due to their ability to store water, *Sedum* would be a typical choice for an extensive green roof (VanWoert et al., 2005). Despite their ability to survive episodes of drought, however, *Sedum* species may be lacking as the preferred vegetation cover of a green roof. For example, in a study done in Quebec, Canada, *Sedum* grown in a shallow substrate were not successful due to root freezing (Boivin et al., 2001). *Sedum* may also lack other functions significant to maximizing green roof benefits. C3 plants (the majority of plants are in this category) and C4 plants (characterized by high transpiration and growth rates) have been hypothesized to be more effective at reducing the quantity of runoff water from green roof surfaces due to their increased water uptake as compared to CAM plants (Nagase & Dunnett, 2012). Species that have an accelerated rate of evapotranspiration can remove water from the growing medium at a faster rate, allowing more space for future water capture (MacIvor & Lundholm, 2011; Nagase & Dunnett, 2012). Graminoids, for example, were found to remove more water at a quicker rate from the soil substrate than succulents, even though they are not as drought tolerant (Wolf & Lundholm, 2008; Dvorak & Volder, 2010). In a study by Nagase &

Dunnett (2012), it was found that grass species were more effective for reducing water runoff than forbs and *Sedum*. It was also observed in this study that the low evapotranspiration rate of *Sedum* could decrease the ability of a green roof to mitigate stormwater retention. Therefore, green roof vegetation can not be chosen by a strict standard; unique environmental variables and desired ecosystem goals must be considered.

Bisphenol-A

Bisphenol-A, or BPA, is an organic compound composed of two phenol rings connected by a methyl bridge, with two methyl functional groups attached to the bridge as seen in Figure 2 (Kang et al., 2006). BPA is an estrogenic environmental contaminant used in the manufacturing of many common plastics (Gould et al., 1998) such as engineering plastics, food cans and dental sealants (Huang et al., 2011). As BPA is not a naturally occurring substance, its presence in the environment can be considered a result of anthropogenic activities; it is released during the process of disposal and manufacturing of polycarbonates/epoxy resins (Fürhacker et al., 2000; Watanabe et al., 2001; Huang et al., 2011). As an endocrine disruptor, BPA is able to mimic hormones of the human body, resulting in a vast array of human health deficiencies. Exposure to BPA is suspected to affect fetal development (Rubin & Soto, 2009), to be a potential human carcinogen in the breast and prostate (Seachrist et al., 2016), to reduce the efficacy of chemotherapeutic agents (LaPensee et al., 2009) and has been associated with cardiovascular disease and diabetes (Lang et al., 2008). Other adverse effects include, but are not limited to, behavioural changes, insulin resistance, obesity, early puberty, reduced sperm count, impaired immune function, brain damage and miscarriage (Chouhan et al., 2014).

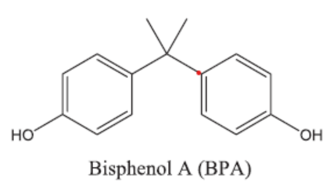


Figure 2. The chemical structure of bisphenol-A (BPA) (modified from Imai et al., 2007).

Due to the toxicity of BPA, its release into the environment is of great concern. Once released, BPA can discharge to air, water and soil/sediments (Cousins et al., 2002, Lintelmann et al., 2003) with aqueous routes being the predominant pathway of distribution (Lintelmann et al., 2003; Ignatius et al., 2010). Studies conducted in urban environments have found concentrations of BPA in aqueous environments such as wastewater treatment effluent (Lee & Peart, 2000; Drewes et al., 2005; Jackson & Sutton, 2008; Petrie et al., 2019), urban runoff (Zafra et al., 2003; Petrie et al., 2019), surface waters (Gonzalez-Casado et al., 1998; Staniszewska et al., 2014) and even tap water (Shao et al., 2008; Zhou et al., 2009; Colin et al., 2014) and bottled water (Colin et al., 2014). As seen in Table 1, aqueous BPA concentrations are widely prevalent across the globe and have been found in a variety of environments: both treated water for human consumption and naturally existing surface and subsurface dwellings.

Location	BPA Concentrations	Medium	References
*Baltic Sea	193 ng/L	Surface Waters	Staniszewska et al., 2014
	39 ng/L	Subsurface & bottom waters	
Canada (Southern Ontario)	193-2440 ng/L	Municipal sewage treatment plants effluents	Lee & Peart, 2000a
Canada (Southern Ontario)	31-223 ng/L	Municipal sewage treatment plants effluents	Lee & Peart, 2000a
Canada (Toronto)	80-4980 ng/L	Influent sewages	Lee & Peart, 2000b
Canada (Toronto) China (Beijing)	10-1080 ng/L	Effluent sewages	Lee & Peart, 2000b Zhou et al., 2009
	230-149200 ng/L	Industrial effluents	
	1194 – 1574 ng/L	River Effluents	
China (Beijing) China (Guangzhou – Pearl River Estuary)	15-63 ng/L	Tap Water	Zhou et al., 2009 Dong et al., 2009a
	950-392 ng/L	River Water & Pond Water	
China (Harbin - Songhua River)	29-64 ng/L	River Water	Shao et al., 2008
China (Harbin - Songhua River) *France	15-63 ng/L	Tap Water	Shao et al., 2008 Colin et al., 2014
	14 ng/L (mean) 1.3 ug/L (max)	Potable tap water samples	
*Ghana (Nakwantakese)	0.009 ng/L	Drinking water	Karalius et al., 2014
*Jamaica (Kingston)	0.003 ng/L	Drinking water	Karalius et al., 2014 Yamamoto et al., 2001
*Jamaica (Kingston) *Japan	0.016 ng/L	Stream	
	0.008 ng/L	Bottled water	
	17.2 mg/L	Hazardous waste landfill leachates	
Japan (Shizuoka)	8000-370,000 ng/L	Effluents – 8 paper recycling plants	Fukazawa et al., 2001
Spain	10-2500 ng/L	Urban Wastewaters	Zafra et al., 2003
	52.0 – 219 ng/L	River Waters	

Spain (Granada)			Gonzalez-Casado et al, 1998
Spain (Granada)	49.1 – 196 ng/L	Sea Waters	Gonzalez-Casado et al, 1998
	51.6 – 207 ng/L	Underground Waters	
*USA	12 ug/L	Effluents	Kolpin et al., 2002
USA	Max. 420 ng/L	Drinking water treatment plant waters	Stackelberg et al., 2004
*USA	21.5 ug/L	Industrial laundry wastewater	Jackson & Sutton, 2008
*USA	0.295 ug/L	Pharmaceutical manufacturer wastewater	Jackson & Sutton, 2008
	0.753 ug/L	Paper products manufacturer wastewater	
	0.38 ug/L	Treated wastewater	
USA	0.31 ug/L	Treated wastewater	Drewes et al., 2005
	281-3642 ng/L	Primary effluents – domestic wastewater treatment plants	
USA	6-50 ng/L	Secondary effluents – domestic wastewater treatment plants	Drewes et al., 2005
*USA (Maywood, Illinois)	0.011 ng/L	Drinking water	Karalius et al., 2014
*USA (Maywood, Illinois)	0.119 ng/L	Stream	Karalius et al., 2014
	0.003 ng/L	Bottled water	
UK (East of Sussex)	1105 ng/L	Sewage treatment plant influents	Hernando et al, 2004
UK (East of Sussex)	19.2 ng/L	Sewage treatment plant influents	Hernando et al, 2004
*UK (South West)	Up to ~100 ug/L	Municipal wastewaters	Petrie et al., 2019
*UK (South West)	62-892 ng/L	Treated wastewater treatment plant effluent	Petrie et al., 2019
	117 ng/L (max)	River Water	

Table 1. Modified from Huang et al. (2011). Entries marked with * were gathered separately and incorporated with the data provided from Huang et al. (2011). This table summarizes BPA concentrations reported in aquatic environments in countries around the world.

According to the European Food and Safety Authority (EFSA, 2015), exposure to BPA should not exceed 4ug/kg of body weight per day. With BPA concentrations reported across a wide distribution of aqueous environments and geographical locations (refer to Table 1), there is an increased likelihood of human exposure to this EDC.

Methods of BPA Remediation

EDCs are a structurally diverse class of emerging contaminants of concern (Scruggs et al., 2005). As such, remediation techniques should be viewed as a necessity in combating these contaminants. Regarding BPA removal, one vastly studied approach is through process of adsorption (Bhatnagar & Anastopoulos, 2017) and is considered superior to other BPA remediation methods (Tsai et al., 2006; Dehghani et al., 2016). Due to their high hydrophobicity, BPs (including BPA) may adsorb onto materials such as sewage sludge (Hu et al., 2019) or other materials that are commonly used for removal of pollutants from aqueous solutions such as clays, zeolites and chitosan (Bhatnagar & Anastopoulos, 2017). Along with adsorption, biodegradation has been regarded as one of two major mechanisms involved in the removal of BPA from WWTPs (Sun et al., 2017). Biodegradation may even be considered the key factor of BPA removal in conventional WWTPs, although complete removal of micropollutants (such as BPA) remains incomplete through this process (Hu et al., 2019). Other methods used to remediate BPA from aqueous solutions include application of ozone (Umar et al., 2013), nanofiltration (Zielinska et al., 2016), photocatalysis and ultrasonic irradiation (Belgiorno et al., 2007) and various membrane bioreactors (Chen et al., 2008; Yang et al., 2013).

For a conventional WWTP, there are four recognized removal pathways for organic compounds: i) adsorption onto suspended solids, ii) Aerobic and anaerobic degradation, iii) chemical degradation (i.e. hydrolysis), and iv) volitalization (Belgiorno et al., 2007). Despite these removal pathways, many EDCs are only partially removed through conventional WWTPs (Belgiorno et al., 2007). The primary treatment of a WWTP utilizes sorption onto sludge through coagulation, flocculation and sedimentation (Joss et al., 2005; Guerra et al., 2015). Secondary treatment involves various treatment techniques such as membrane bioreactors, activated sludge process and biological aerated filter process. Advanced treatment processes are used to further improve removal efficiency and include membrane technique, ultraviolet disinfection and ozonation (Guerra et al., 2015). While micropollutants such as BPA often undergo incomplete removal during primary treatment of a WWTP, secondary and advanced treatments can be introduced to increase efficiency, although at high operation costs (Luo et al., 2014).

Phytoremediation of BPA

Phytoremediation is a cost-effective, non-invasive alternative for environmental remediation that utilizes plants and their unique functions (Salt et al., 1998; Alkorta & Garbisu, 2001; Pilon-Smits, 2005). By growing plants in a contaminated matrix, environmental contaminants can be removed through processes of sequestration and/or degradation (Paz-Alberto & Sigua, 2012). Phytoremediation is an *in situ* method of treating contaminated soils, water and sediments, and can be utilized at sites contaminated by organic, nutrient or metal pollutants (Dietz & Schnoor, 2001). As compared to mechanical or chemical methods of removing pollutants from the soil, phytoremediation is more cost-effective (Bollag et al., 1994) and is deemed an effective, non-intrusive and inexpensive means of remediation (Wiltse et al., 1998; Alkorta & Garbisu, 2001). Pilon-Smits (2005) described phytoremediation as being divided into the following areas: phytodegradation, phytovolatilization, phytoextraction, phytostabilization and phytostimulation (Figure.3).

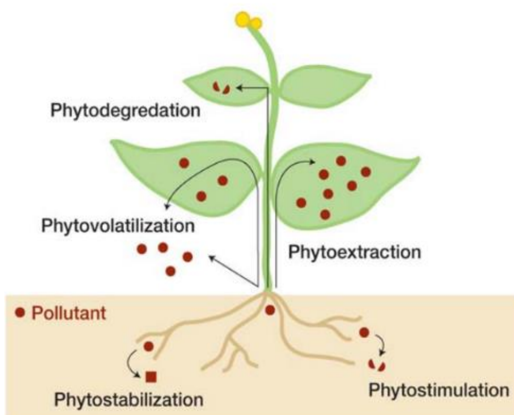


Figure 3. A diagram depicting the path of a pollutant through the process of phytoremediation. Phytostabilization uses plants to stabilize pollutants in the soil. Phytoextraction refers to the ability of plants to extract and accumulate pollutants in their biomass. Phytostimulation (or phytodegradation) is the ability of a plant to biodegrade pollutants by microbes in their rhizosphere. Phytovolatilization occurs when, after uptake by the plant, the pollutant(s) can leave the plant in volatile form. Phytodegradation is when the plant can degrade organic pollutants directly by their own enzymatic activities (modified from Pilon-Smits, 2005).

The ability of plants to remediate BPA has been extensively analysed using various plant species and methods of phytoremediation. Hamada et al (2002) found that cultured suspension of *Eucalyptus perriniana* cells were capable of regioselective hydroxylation and glycosylation of BPA. Tabei & Sakakibara (2006) analysed *Limnobium Laevigatum*, *Fantinalis Antipyretica*, *Nasturtium Officinale*, *Ricciocarpos natans*, *Riccia fluitans*, *Hydrilla verticillata*, and *Potamogeton oxyphyllus* and concluded that they were able to remove EDC's such as 2,4-

Dichlorophenol (DCP), 4-t-Octylphenol (OP), Nonylphenol (NP), and BPA. Syranidou et al (2017) determined that *Juncus acutus*, through rhizodegradation, was able to remediate BPA contaminated groundwater. In a review by Kang et al (2006), the results of multiple studies analyzing the remediation of BPA were summarized and the key enzymes were listed (Figure.4). Looking at the table, enzymes present in plants that are important in the phytoremediation of BPA include peroxidase and polyphenol oxidase (PPO).

Enzymes capable of biodegrading or metabolizing bisphenol A

Enzymes	Sources	References
Manganese peroxidase (MnP)	Fungi (*Pleurotus ostreatus* O-48, *Phanerochaete chrysosporium* ME-446, *Trametes versicolor* IFO-7043, *Phanerochaete chrysosporum* ME-446 and *Trametes versicolor* IFO-6482)	Hirano et al. (2000), Tsutsumi et al. (2001), Suzuki et al. (2003)
Laccase	Fungi (*Phanerochaete chrysosporium* ME-446, *Trametes versicolor* IFO-7043, *Trametes villosa*, *Phanerochaete chrysosporum* ME-446 and *Trametes versicolor* IFO-6482)	Tsutsumi et al. (2001), Fukuda et al. (2001), Uchida et al. (2001); Suzuki et al. (2003)
Peroxidase	Bacteria (*Coprinus cinereus*), plant [soybean and horseradish (*Armoracia rusticana*)]	Sakurai et al. (2001), Caza et al. (1999), Sakuyama et al. (2003)
Polyphenol oxidase	Plant (mushroom)	Yoshida et al. (2002)
Cytochrome P450	Bacteria (*Sphingomonas* sp. strain AO1), mammals (mouse and rat)	Atkinson and Roy (1995a,b), Sakurai et al. (2001), Yoshihara et al. (2001)
UDP-glucuronosyltransferase (UGT)	Fish [carp (*Cyprinus carpino*)], mammals (mouse, rat and human)	Yokota et al. (1999, 2002), Cappiello et al. (2000), Matsumoto et al. (2002), Strassburg et al. (2002)
Sulfotransferase	Mammal (human)	Suiko et al. (2000), Nishiyama et al. (2002)

Figure 4. A summary of enzymes that are capable of biodegrading or metabolizing BPA and where the enzymes can be found (modified from Kang et al., 2006).

PPO is an enzyme found in many plant tissues (Sherman et al., 1991) and acts to protect the plant against insects and microorganisms (Whitaker, 1995). This enzyme catalyzes the oxidation of mono-, di-, and polyhydric phenols to quinones (Felton & Gatehouse, 1996; Yamane et al., 2010) at the expense of O_2 (Steffens et al., 1994). Quinones generated by PPO are highly reactive, electrophilic molecules, which can covalently modify and crosslink a variety of cellular nucleophiles (Steffens et al., 1994), and is responsible for changes in pigmentation (i.e. fruit browning) as a result of formation of brown and black coloured melanin polymers from autoxidation (Steffens et al., 1994; Whitaker, 1995). Peroxidase is a heme-containing enzyme (Sumner & Dounce, 1937; Meunier, 2003; Vigneswaran et al., 2014) that catalyzes the oxidation of a variety of substrates by reacting with hydrogen peroxide (Meunier, 2003). In these reactions, hydrogen peroxide is decomposed into molecular oxygen and water as shown in Figure 5 (Vigneswaran et al., 2014; Padaki et al., 2015).

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Figure 5. The reaction caused by the decomposition of hydrogen peroxide into water and molecular oxygen as caused by peroxidase (taken from Vigneswaran et al., 2014)

PPO and peroxidase are enzymes known for the metabolization/degradation of BPA in many sources including fungi, fish, bacteria and plants. PPO and peroxidase are both enzymes that can be found in various plants. In P. *oleracea*, the vegetation chosen for the green roof experiment in this study, both enzymes have been reported active in the remediation of BPA.

Portulaca *oleracea*

The genus Portulaca L. consists of a wide distribution of about 100 species (McNeill 1974; Nyananyo, 1986), and is primarily a tropical and subtropical herbaceous genus (Matthews & Levins, 1985). Among the species of Portulaca L., a few have gained attention for their phytoremediation capabilities. In particular, P. *oleracea* (purslane) has demonstrated an innate ability for remediating contaminants. Purslane is a widely distributed weed that has often been used as a folk medicine in many countries for its vast range of pharmacological effects (Xiang et al., 2005), including antibacterial (Zhang et al., 2002), analgesic, anti-inflammatory (Chan et al., 2000) and wound healing (Rashed et al., 2003) capabilities. Besides medicinal uses, purslane has proven efficient at phytoremediation of contaminants such as endocrine disrupting compounds (EDCs) having a phenol group including BPA, nonylphenol (NP) and octylphenol (OP) (Imai et al., 2007). This process can occur through techniques such as the degradation of organic contaminants by plants (in this study by P. *oleracea*) with the help of enzymes (Ali et al., 2013). With P. *oleracea*, an enzyme mostly located in the roots is responsible for the removal of phenolic pollutants. In a study by Matsui et al., (2011), this enzyme was thought to be peroxidase; an enzyme that catalyses the oxidation of many organic compounds by hydrogen peroxide such as phenols (Lück, 1965). Matsui et al., (2011) states that phenol pollutants were removed by P. *oleracea* roots through cross-linking them to cell wall polysaccharides at the expense of reduced hydrogen peroxide (H_2O_2). In another study by Kaneda et al., (2012), it was found that, in the initial steps of detoxifying BPA in P. *oleracea* roots, polyphenol oxidase (PPO) was involved. This study also determined that PPO genes significantly contribute to the ability of P. *oleracea* to metabolize EDCs. In another study by Watanabe et al., (2012), it was also concluded that PPOs were a likely contributing factor in the removal of BPA by P. *oleracea*.

This study even went on to formulate a proposed pathway for the metabolism of BPA as demonstrated in Figure 6.

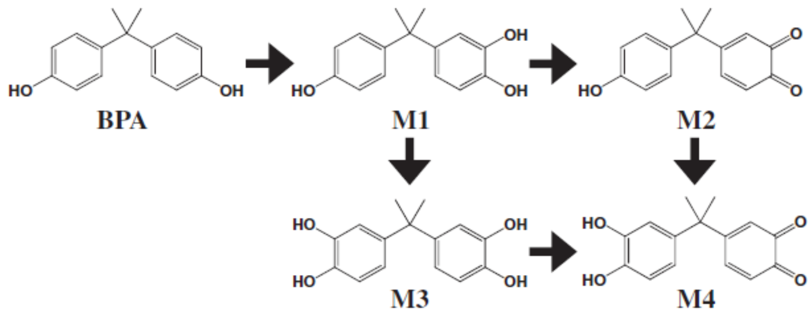


Figure 6. The steps in the Pathway of BPA metabolism by P. *oleracea* (modified from Watanabe et al., 2012).

According to Watanabe et al., (2012), and referring to Figure 6, P. *oleracea* metabolizes BPA through the following steps: 1) BPA is hydroxylated to M1, 2) M1 is oxidized to give M2 or hydroxylated to give M3, 3) M3 is converted into M4 through further oxidation of the catechol group, or, by another pathway, M2 is hydroxylated to produce M4. Furthermore, this process occurs independent of BPA concentration (up to 250 uM), cultivation in the dark, temperatures ranging from 15°C to 30°C and pH ranging from 4 to 7 (Imai et al., 2007). Such results suggest that P. *oleracea* is a practical choice for phytoremediation of sites affected by sewage sludge (Suchkova et al., 2014) as well as landfill leachates and industrial wastewater contaminated with EDCs (Imai et al., 2007).

P. *oleracea* is a C4 plant that, under drought conditions, can change its carbon fixation method into CAM (Koch & Kennedy, 1980; Koch & Kennedy, 1982). It is one of the only C4 species known to exhibit CAM metabolism (Koch & Kennedy, 1980; Ku et al., 1981; Guralnick et al., 2002; Lara et al., 2003). Plants exhibiting a C4 pathway restrict the C3 pathway to the interior cells within the leaf and use an enzyme in the mesophyll cells to produce a C4 acid which is used to increase the CO2/O2 ratio in the C3 pathway and limit photorespiratory activity (Ehleringer & Cerling, 2002). During at least part of the day, plants exhibiting the CAM pathway will close their stomata, utilize malate, and maintain high CO_2 levels for photosynthesis, decreasing the amount of water loss from the plant due to transpiration (Guralnick et al., 2002). Koch and Kennedy (1981) watered two groups of P. *oleracea* (one group every day and one group every 3 to 4 weeks) and found that well watered plants relied on C4 photosynthesis, while plants subjected to drought showed CAM activity. Lara et al (2003) found that, after 21-23 days of drought conditions, P. *oleracea* shifted its photosynthetic metabolism from C4 to CAM. Danin et al. (1978) analysed the distribution of subspecies of P. *oleracea*, showing its ability to flourish in many biogeographical zones throughout the globe and exhibited the ability of the species to survive extreme conditions such as drought, heat, saline and nutrient scarcity. The survivability of P. *oleracea* gives it a competitive edge over other vegetative species (Alam et al., 2014).

MATERIALS AND METHODS

Laboratory Method

Leaf Water Potential

Water potential indicates how tightly water is bound within a substance (Meter Group, 2019). It refers to the potential energy of water per unit volume, or the energy required to transport a quantity of water. In terms of vegetation, water potential can be applied to individual areas of a plant to examine hydrological functions. Leaf water potential, for example, is the water potential of the leaves of plants, and indicate whether water will move and where it will go in the plant (Kanemasu & Tanner, 1969). Leaf water potential can provide many indicators about the health and general hydrologic performance of the plant examined. In a study by Garnier et al. (1988), it was determined that the water potential of vegetation could provide a good estimation of transpiration. Factors that can affect transpiration rates include temperature, light intensity, relative humidity and wind. Therefore, Nobel (1999) showed that transpiration can be defined by Equation (1). In this equation, T represents transpiration, Ψ represents water potential and r is defined as resistance (Nobel, 1999). Many studies over the years have exploited the relationship between leaf water potential and plant transpiration to further understand the complexity of the soil-plant-atmosphere continuum. In general, understanding leaf water potential can provide insight to soil moisture (Denmead & Shaw, 1962), hydrology of the soil-plant-atmosphere system (Elfving et al., 1972) and plant physiology such as stomatal conductance and flow of water through the plant (Jarvis, 1976).

$$T = \frac{[\Psi_{\text{leaf}} - \Psi_{\text{atm}}]}{r} \qquad (1)$$

Leaf water potential can be measured through a variety of methods. Some studies chose a thermocouple psychrometer (Ehlig 1962; Boyer & Knipling, 1965), a pressure chamber (Boyer, 1967) and a dye method (Knipling, 1967). The thermocouple psychrometer infers water potential by matching a solution of known vapour pressure to the vapour pressure of the water in the sample (Rawlins & Campbell, 1986; Boyer, 1990). Although difficulties can arise from fluctuating temperatures, temperature gradients in the psychrometer, proper sealing of the chamber and instances of resistance to water vapour diffusion (Savage et al., 1982), the thermocouple psychrometer is one of the most widely used and versatile methods of measuring plant water status (Boyer, 1965). By method of a pressure chamber, pressure is increased around the plant material (i.e. leafy shoot) until xylem sap appears at the cut end of the shoot, whereby the pressure required to force water out of the leaf cells is a function of leaf water potential (Boyer, 1967). The dye method utilizes the osmotic potential by submerging leaves in solutions with different densities and using the rise and fall of dye drops to indicate changes in osmotic water exchange (Knipling & Kramer, 1967). Another method is the utilization of a dew point potentiometer. With this device, a sample is placed in a sealed chamber where the dew point temperature of the head space in the sealed chamber is measured through the detection of condensation (monitored by infrared thermometry) on a cooling mirror (Campbell et al., 2007). This technique can be used for fast and accurate water potential measurements (Gubiani et al., 2013). One such device that uses the dew point method is the WP4C Potentiameter. As stated by MeterGroup (2019), Equation (2) shows the determination of water potentials as calculated by the WP4C. Referring to this equation, Ψ is the sample water potential, R is the gas constant (8.31 J/mol·K), e_sT_s is the saturation vapour pressure at sample temperature, e_sT_d is the saturation vapour pressure of the air at dew point temperature, T is the temperature in Kelvin and M is the molecular mass of water (MeterGroup, 2019).

$$\Psi = \left(\frac{\mathrm{RT}}{\mathrm{M}}\right) \ln\left(\frac{e_s T_d}{e_s T_s}\right) \tag{2}$$

The WP4C works by sealing the sample in a chamber with a dew point mirror that measures the dew point temperature of the air, while an infrared thermometer measures the air temperature. When the water potential and the air are in equilibrium, the vapour pressure and temperature give the water potential of the sample being measured (Meter Group, 2019). The water potential calculated in equations 2, or as measured by the WP4C, can then be plugged in to equations 1 as Ψ_{leaf} to estimate the transpiration of a leaf. In this way, when factors of resistance and atmospheric potentials are minimized, leaf water potential is a good estimation of transpiration.

WP4C Potentiameter

Total water potential can be quantified by a sum of four potentials: gravitational, matric, osmotic and pressure. The WP4C measures the osmotic and matric potentials to quantify the water potential of a sample. To measure these potentials, the WP4C uses the chilled-mirror dew point method. The sample is equilibrated with the headspace in a sealed chamber, and a mirror is used as a means of detecting condensation. When the water potential of the air is the same as that of the sample, equilibrium has been reached. (MeterGroup, 2019)

According the MeterGroup (2019), the three reading modes available by the WP4C are precise, continuous and fast. Precise mode is the default, where sample readings will be repeated until successive readings agree within a pre-set standard. Continuous mode takes continuous measurements of a sample until the sample drawer is placed into open/load and is recommended for samples that take a long time to come to vapor equilibrium (20-30mins) such as plant samples. Fast mode measures a sample once and is typically less precise than the other two modes. As plant material is the main sampling focus of this study, continuous mode was used to measure all leaf water potentials. Although the WP4C is designed to measure soil water potential, with special steps, it can accurately measure LWP.

As confirmed by MeterGroup (2019), these steps include the following:

1) Apply a drop of water to the leaf surface

2) Abrade the leaf surface with a 5cm x 2cm piece of 600-grit sandpaper (abrasion is utilized to speed up rate of equilibration)

3) Dry the leaf surface with a lint-free tissue to remove any excess water and excise the leaf from the plant

4) Immediately seal the sample with a moist towel in a plastic bag (if transport is required) and seal the sample in the chamber as soon as possible

Initial Testing

As the WP4C is a relatively new method to measuring LWPs compared to more conventional techniques, an initial testing period was required to verify quality of results. For this reason, during October and November of 2019, LWPs of two different plants were measured using the WP4C. The two plants used were Helleborus *niger* and Sedum *telephium*. These species were chosen for their availability at the time of year, as well as for their different carbon fixation methods. This particular difference in the functioning of the plants results in unique evaporation patterns throughout the day of each species. These expected patterns were used to verify the credibility and functionality of the WP4C for LWP measurements during this project.

As Sedum *telephium* uses a CAM carbon fixation method, its stomata remain closed during the day and open instead during the night. Helleborus *niger* performs oppositely in this regard, meaning its stomata open during the day and close at night. In other words, Helleborus *niger* will lose moisture due to evaporation throughout the day while Sedum *telephium* perspires at night and will therefore retain water during the day. Due to these differences, the predicted patterns are as follows; LWP trends for Sedum *telephium* should become less negative throughout the day while LWP trends for Helleborus *niger* should become more negative.

For this initial testing period, both plants were watered equally and kept in the same environment. Measurements were taken on multiple days in October and November of 2019 at different times of the day. The data was then compiled to identify any trends or patterns observed. These trends and patterns were observed graphically through analyzing LWPs at equilibrium and coming to equilibrium in the WP4C.

Case Study Description

Growth of P. *oleracea* with regular watering occurred over the course of December 2019 through to March 2020. Three boxes, each containing 6 specimens of P. *oleracea*, were placed in a climate chamber and subjected to the same conditions (i.e., temperature, humidity, wind resistance, irrigation quantity, UV exposure). After the growth period, plants were exposed to irrigation by three varying solutions: water and two concentrations of BPA (2mg/L & 5mg/L). These concentrations were chosen based on two main factors: previously reported effects of BPA on vegetation and concentrations of BPA found in urban environments. Huang et al. (2011) summarized a vast array of studies analysing concentrations of BPA in both naturally occurring waters and those treated for human consumption. These concentrations tended to remain with the range of ng/L to ug/L. When extended beyond the scope of this study, some cases were found to report concentrations beyond this range, veering into the mg/L, such as with hazardous waste landfills in Japan (Yamamoto et al., 2001). It was also reported by many studies that exposure to low concentrations of BPA have shown a potentially beneficial effect on various plant types (Terouchi et al., 2004; Pan et al., 2013; Qiu et al., 2013; Ali et al., 2016; Jiao et al., 2017; Li et al., 2018). For this reason, it was determined that concentrations should be selected to both reflect the environment of this study while also considering potential future developments and extended uses while encompassing the range of reported BPA concentrations. Although many cases did report concentrations on the ng/L to ug/L scale, these were not optional due to a coinciding MSc. project that was taking place along with the presently described project. It was therefore decided that concentrations on the mg/L scale would be used. Therefore, the concentrations 2 mg/L and 5 mg/L were chosen to analyze any potential benefits induced by concentrations below, as opposed to above 3 mg/L.

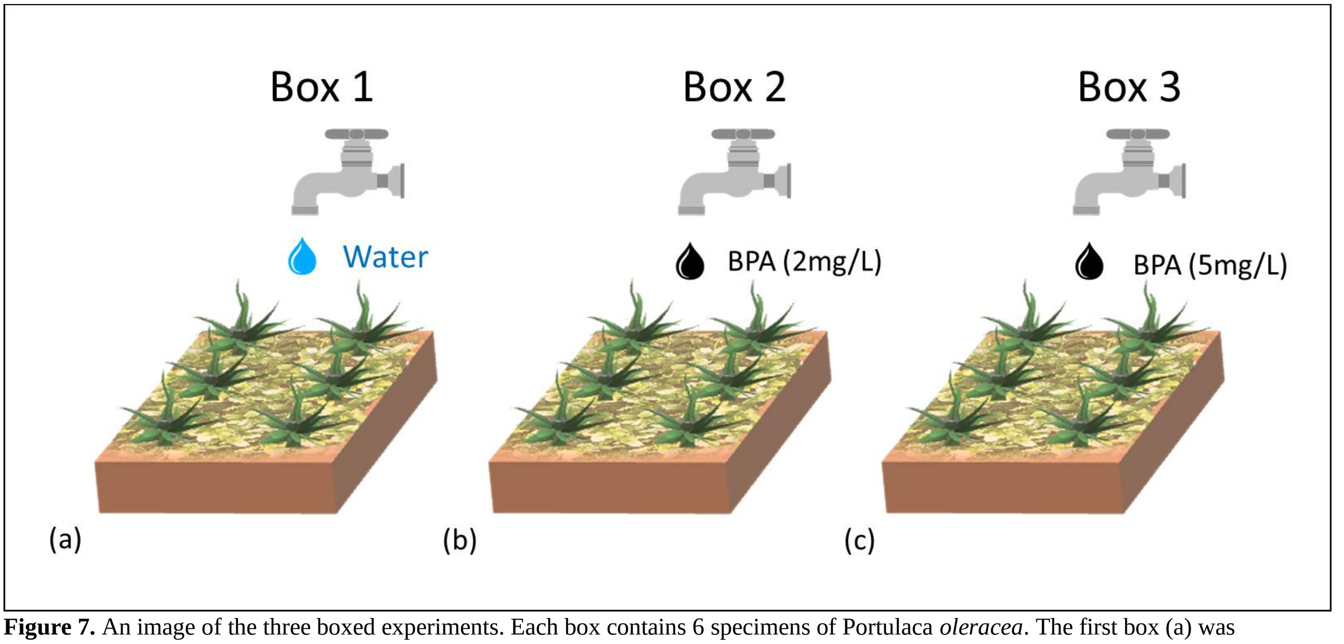


Figure 7. An image of the three boxed experiments. Each box contains 6 specimens of Portulaca *oleracea*. The first box (a) was injected with pure water. The second box (b) was injected with a BPA concentration of 2mg/L. The third box (c) was injected with a BPA concentration of 5mg/L.

As seen in Figure 7, the first box (a) was exposed to pure water, the second (b) to a BPA concentration of 2mg/L and the third (c) to a BPA concentration of 5mg/L. The water injection was implemented as a baseline to determine the plant stress factor; the box irrigated with water acted as a non-polluted control. The boxes irrigated with BPA were compared to Box 1 to determine if the plants were under stress with either the 2mg/L or 5mg/L concentrations of BPA. If the LWP results of Box 2 and Box 3 are similar to Box 1, it is assumed that plants are under minimal to no stress under conditions of BPA injection, and remediation of BPA does not affect the hydrological performance of P. *oleracea*. If the results of Box 2 and Box 3 are noticeably more negative than Box 1, then BPA does affect the hydrological performance of P. *oleracea*, and this plant can therefore not be used to remediate BPA on green roofs without reducing water management capabilities. To determine the hydrological performance of each plant specimen, leaf water potential using the WP4C was measured throughout the day and compared to climate chamber trends including pressure head, soil water content, evaporation and outflow.

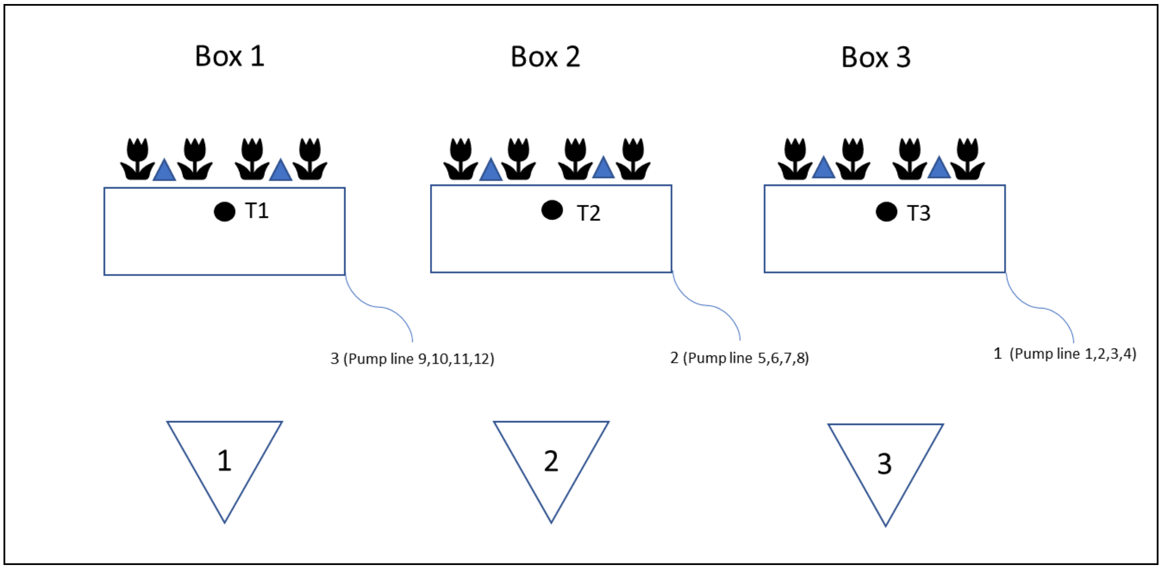


Figure 8. A diagram of the setup of the boxes containing P. *oleracea*. The black circles represent tensiometers that were placed in the root zone to measure soil moisture content. Each box contained two irrigation cells (blue filled triangles) which injected solution into each box at a rate of 10 mL/min. Each irrigation cell was connected to two irrigation lines of a pump. In other words, each box was connected to four pump lines. The amount of discharge was collected by funnels underlying each box (numbered triangles) and measured with tipping buckets.

Figure 8 shows the setup of the project, which consisted of three boxes containing six specimens of P. *oleracea* in each. Two irrigation cells per box provided solution at a rate of 10 ml/min (5ml/min per irrigation cell). Soil moisture and pressure head were measured via tensiometers in the root zone to eliminate possibilities of stress reactions due to drought conditions. By restricting drought responses, any stress observed in leaf water potentials would therefore be a response to BPA toxicity. Beneath each box was a solution outlet leading to a tipping bucket where solution output volumes were measured. Tipping buckets measured data with 1 wipe correlating to 0.2mm of rainfall. For example, 10 wipes would then be equal to 2 mm of rainfall. Through these means, pressure head, soil water content, evaporation and outflow were quantified throughout the study.

RESULTS

Initial Testing Outcomes

Helleborus niger and Sedum telephium

Before using Portulaca *olercea*, the WP4C was first tested using two different plant species: Helleborus *niger* and Sedum *telephium* in Figure 9. In October and November of 2019, the leaf water potential of both plants was measured on three separate days. The purpose of this initial testing period was to verify the accurate representation of leaf water potential trends using the WP4C.

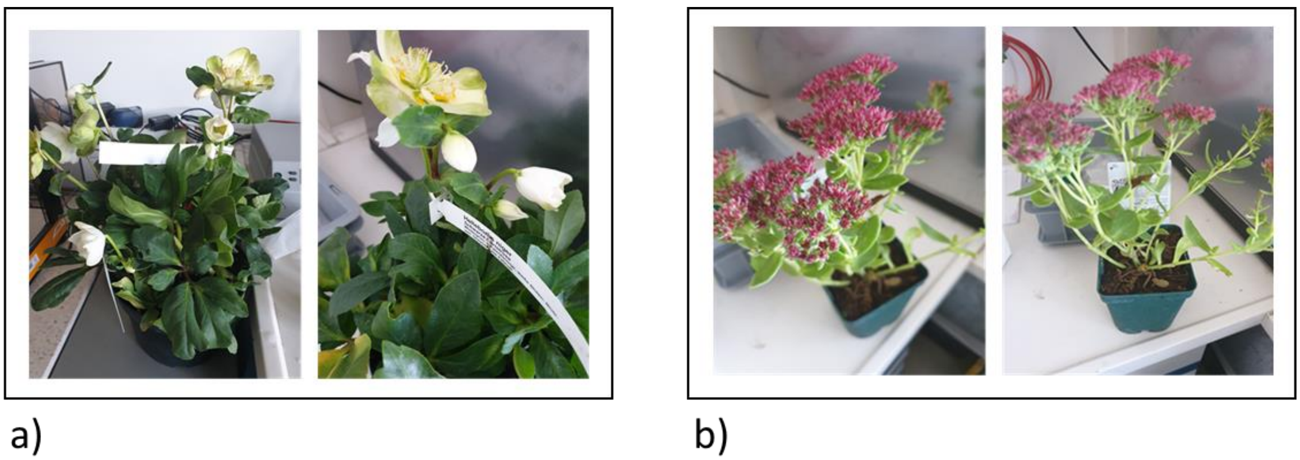


Figure 9. Pictures of (a) Helleborus *niger* and (b) Sedum *telephium* at 10:00am on October 24, 2019.

Figure 10 shows the leaf water potential coming to equilibrium over a period of time on all measurement days for Helleborus *niger*. LWPs were taken at different times on October 24, October 30 and November 5. As can be seen in the graph, equilibrium curves all tend to follow a similar pattern. Likewise, in Figure 11, Sedum *telephium* LWPs are also shown coming to equilibrium. Unlike Helleborus *niger*, the trend in the Sedum *telephium* LWPs contain more variation. These differences can in part be attributed to the CAM photosynthetic nature of Sedum plants.

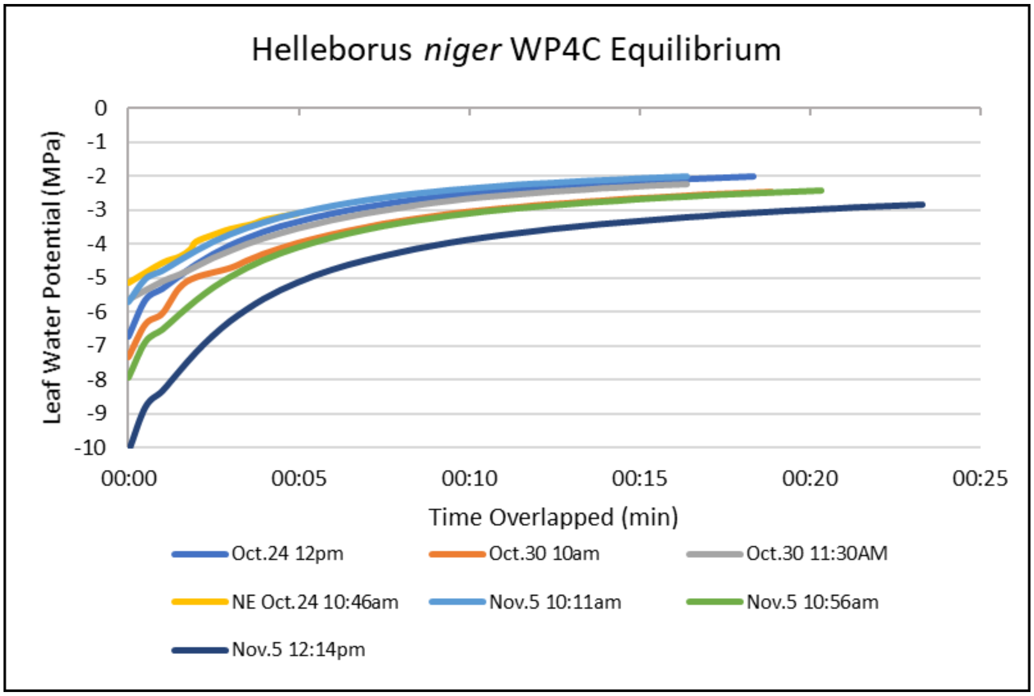


Figure 10. A graph depicting leaf water potential (MPa) equilibrium curves for Helloborus *niger* on October 24th, October 30th and November 5th over time.

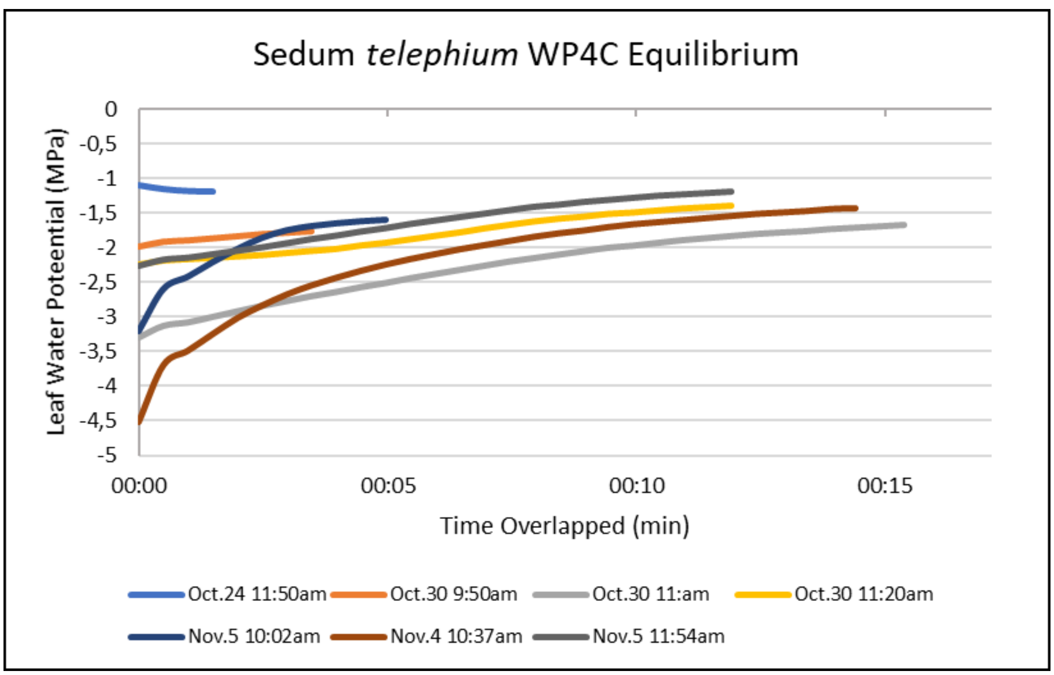


Figure 11. A graph depicting leaf water potential (MPa) equilibrium curves for Sedum *telephium* on October 24th, October 30th and November 5th over time.

Figure 12 depicts the trends in final leaf water potential equilibrium points between both plant species measured at different times throughout the day. The data used in the graph ignores differences connected to separate measurement days, and instead focuses strictly on the time-of-day measurements were taken. LWPs for Sedum *telephium* show a positive trend, indicating increased turgor pressure in the leaf, or decreased transpiration, throughout the day. The opposite is found for Helleborus *niger*, which shows an increasing negative trend of LWP values throughout the day. The difference in trends is due to the carbon fixation methods of each plant; Sedum *telephium* closes its stomata during the day while Helleborus *niger* performs oppositely.

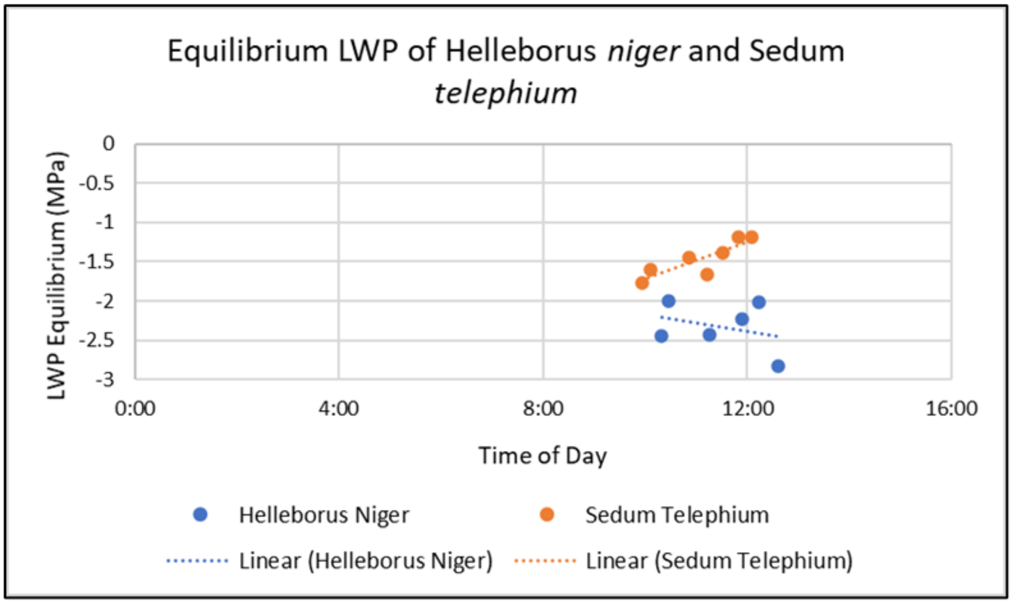


Figure 12. Equilibrium LWP values measured by the WP4C for Helleborus *niger* and Sedum *telephium*.

Sedum *telephium* LWPs, as shown in Figure 12, tend to follow a much more fitted regression than that of Helleborus *niger*. One possible reason for the pattern is the sensitivity of each plant to irrigation. As Sedum *telephium* utilizes drought resistance mechanisms, measured LWPs may not be as subject to change with changes in watering regime as species that do not contain these mechanisms. This would explain the larger variances in observed data for Helleborus *niger* during this period of inconsistent irrigation. This would indicate that, based on these observations and the results of the initial testing period, the WP4C does signify differences in LWPs throughout the day as would be expected of different plant species. It is therefore concluded that the WP4C Dewpoint Potentiameter is a relevant method for observing LWPs.

Portulaca oleracea *pre-BPA*

Before irrigation with BPA, LWPs of the three boxes containing P. *oleracea* were measured to obtain baseline potentials to analyze variances between the boxes, as well as act as a future indicator of changes caused by BPA induced stress. The plants were incubated under same conditions from December 2019 to January 2020. At this point, the seedlings were transferred to three separate boxes in a climate chamber. Until March, the plants were exposed to same conditions and irrigated every Monday and Thursday. The first leaf water potential measurements were taken on March 12, 2020. Due to the occurrence of Covid-19, the project was put on hold for two months after collection of these measurements. During this time, plants remained in the climate chamber, but were only irrigated approximately once every two weeks by building staff. Therefore, as seen in Figure 13, the results from the second measurements taken on May 12, 2020 show more negative LWPs as compared to the first measurements taken in March.

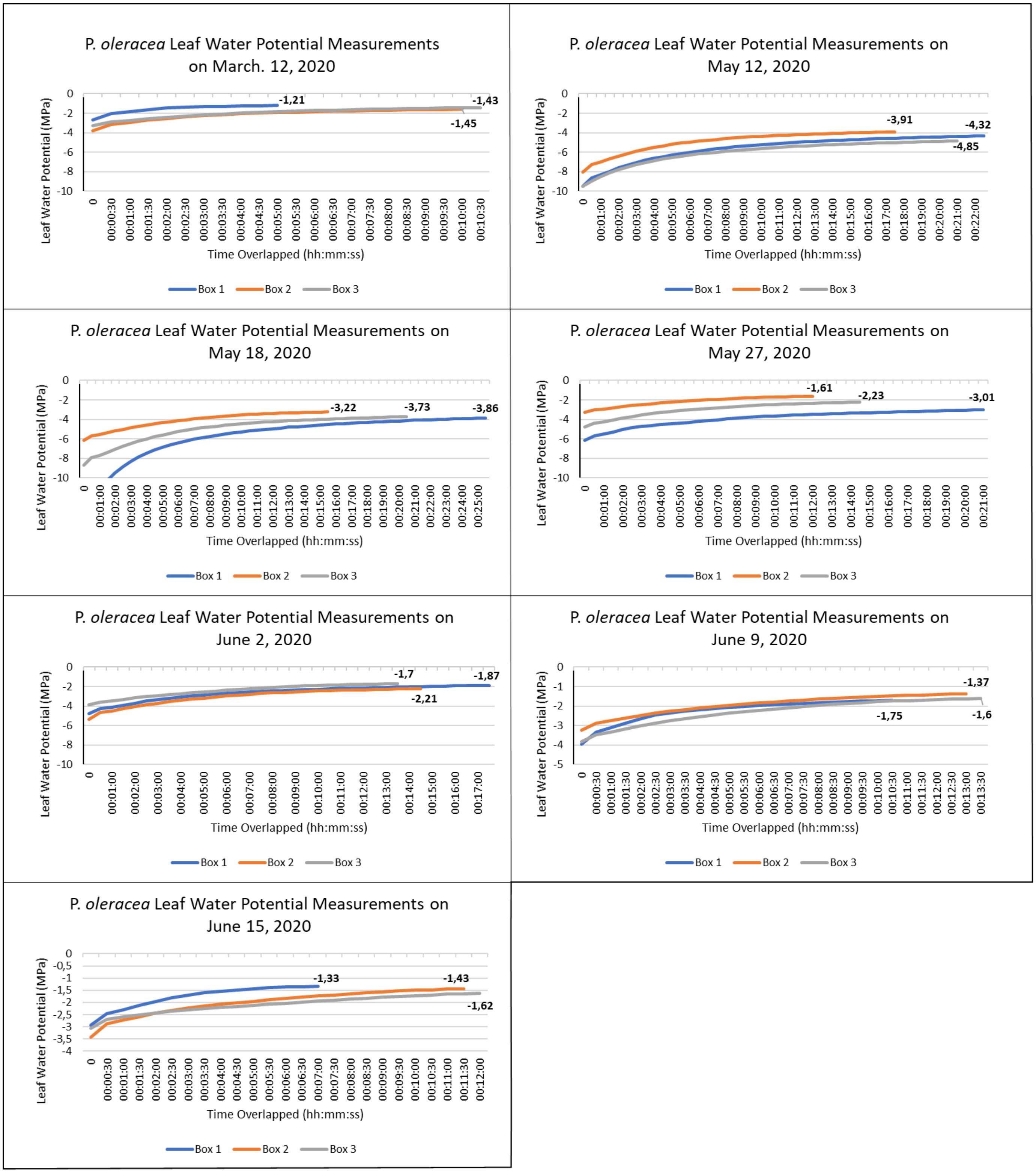


Figure 13. Graphs depicting leaf samples from Box 1, 2 and 3 on March 12, May 12, May 18, May 27, June 2, June 9 and June 15 2020. All measurements were taken between 10:30 am and 12:00 pm.

This difference in results is enunciated in Table 2 which shows the leaf water potentials at equilibrium on each day measurements were taken. There is a clear difference between the March 12 and May 12 results. The more negative LWPs observed on May 12 can be attributed to changes in period of growth and watering regime. After May 12, irrigation took place more frequently and leaf water potentials became less negative over time. Despite the differences that occurred during this period, for each day measurements were taken, values did not exceed a difference of 1.5 MPa between boxes. Based on this observation, leaf water potentials exceeding this variance between each box during BPA injection should be concluded as change resulting form factors outside of natural differences between plants.

Table 2. A summary of the leaf water potentials (MPa) measured for Box 1, Box 2 and Box 3.

	March 12	**May 12**	**May 18**	**May 27**	**June 2**	**June 9**	**June 15**
Box 1	-1.21	-4.32	-3.86	-3.01	-1.87	-175	-1.33
Box 2	-1.59	-3.91	-3.22	-1.61	-2.21	-1.37	-1.43
Box 3	-1.43	-4.85	-3.73	-2.23	-1.70	-1.6	-1.62

As BPA concentrations were introduced to boxes 2 and 3 shortly after May 12, it is more reasonable that values are compared with these measurements rather than those taken in March. In Figure 14, noticeable differences can be observed between the plants on March 12 and those on May 12. Some of these changes include reduced leaf size, increased plant height, production of flowers, increased number of dead leaves and fewer turgid leaves. As the frequency of irrigation increased throughout the months of May and June, leaf water potentials also become less negative.

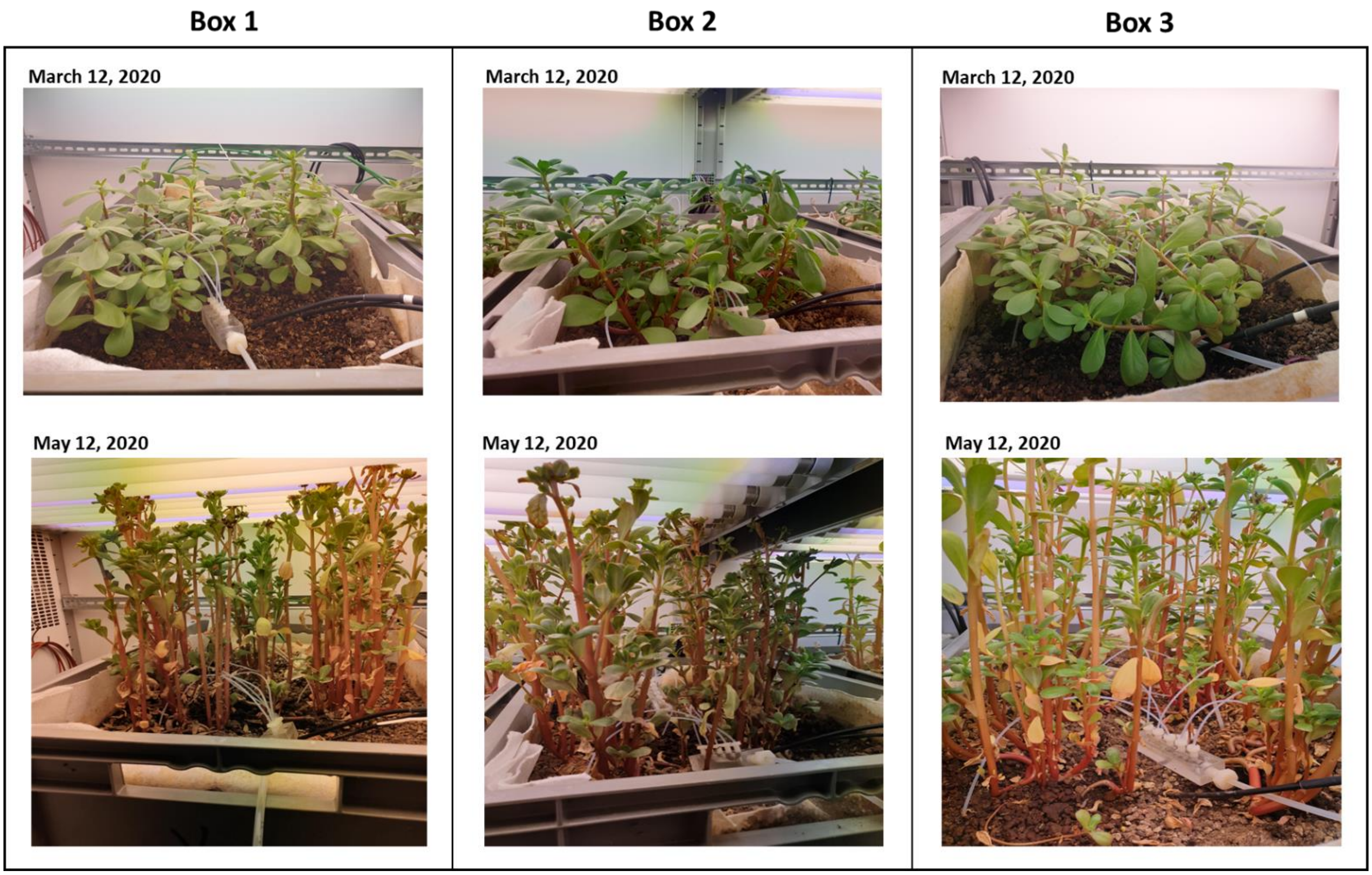


Figure 14. Images showing the changes in physical features of the plants used during this study between March and May 2020.

An important factor to note is the change in physical features of the P. *oleracea* plants between March 12 and May 12. While LWPs were not correlated to plant physiological features, it must be recognized that these changes were a result of drought stress and cannot be used as an indicator of BPA induced stress. However, during regular irrigation, furthering of these physical features may indicate stress originating from another source and would require monitoring.

Variations in Leaf Water Potentials and Physical Features of Box 1, Box 2 & Box 3

During the experimental campaign, LWP measurements were taken for approximately a period of 6-8 hours in a single day. The data was compiled and analyzed using a linear regression. It was expected that LWP trends would show an increasing negativity throughout the day, as is typical of vegetation exhibiting a C3 or C4 carbon fixation method. However, as can be seen in Figure 15, this trend was not always observed. For the most part, however, LWP's did show an increasing negative pattern on most days' measurements were taken.

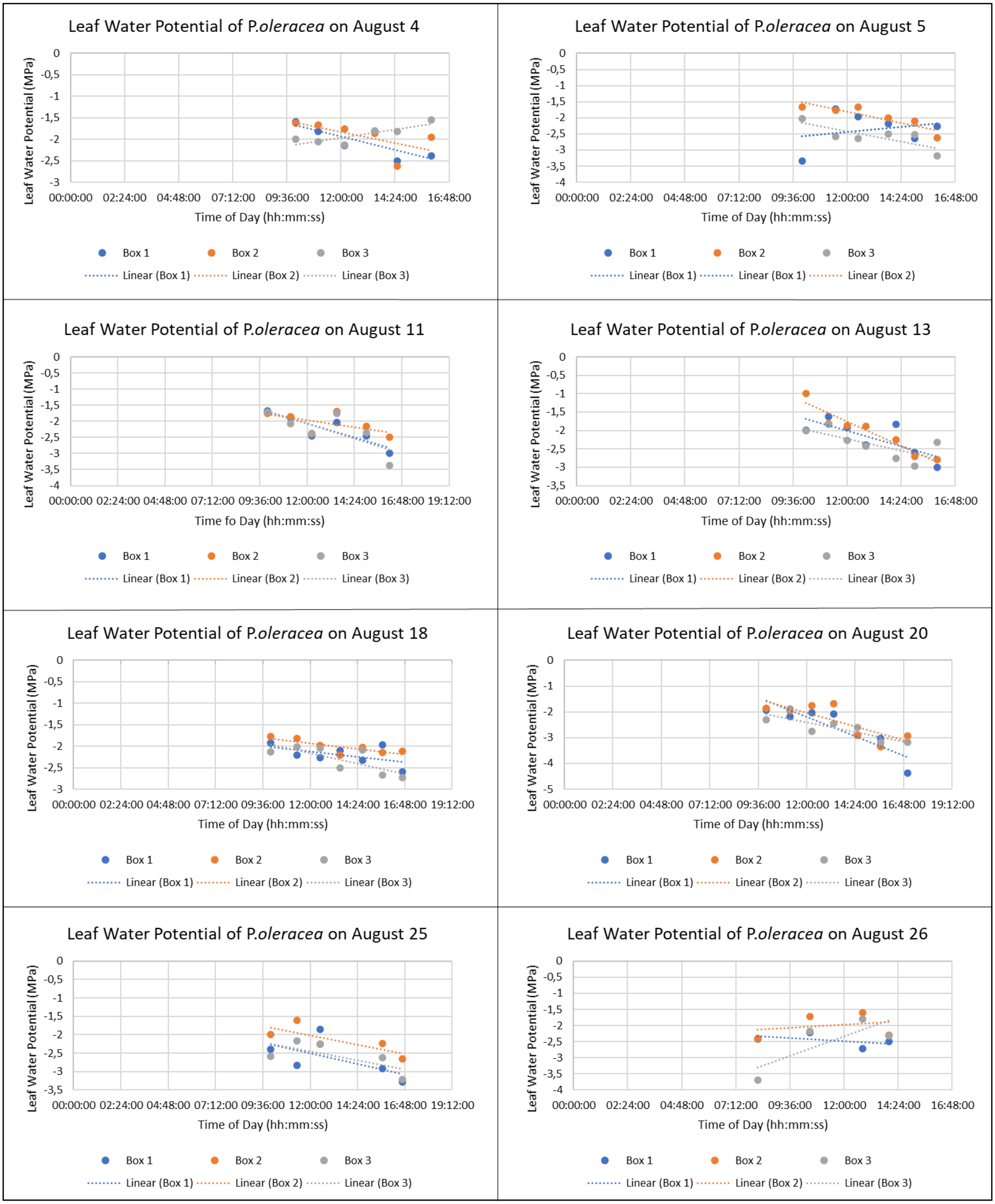


Figure 15. Linear regressions for Box 1, Box 2 and Box 3 on all measurement days.

Any discrepancies for the expected increasing negative LWP trend were not observed solely in a single box, and therefore is most likely not a result of a BPA toxicity effect. While there were no significant differences between each box on any individual day, Box 2 (2mg/L) tended to show slightly more positive LWP's than the other two boxes. Looking solely at the measured LWPs of each box, all show a decreasing trend typical of plants exhibiting a C3/C4 carbon fixation method. In Figure 16, the LWPs for each box are plotted based on the time of day measurements were taken and ignore variable differences between individual days.

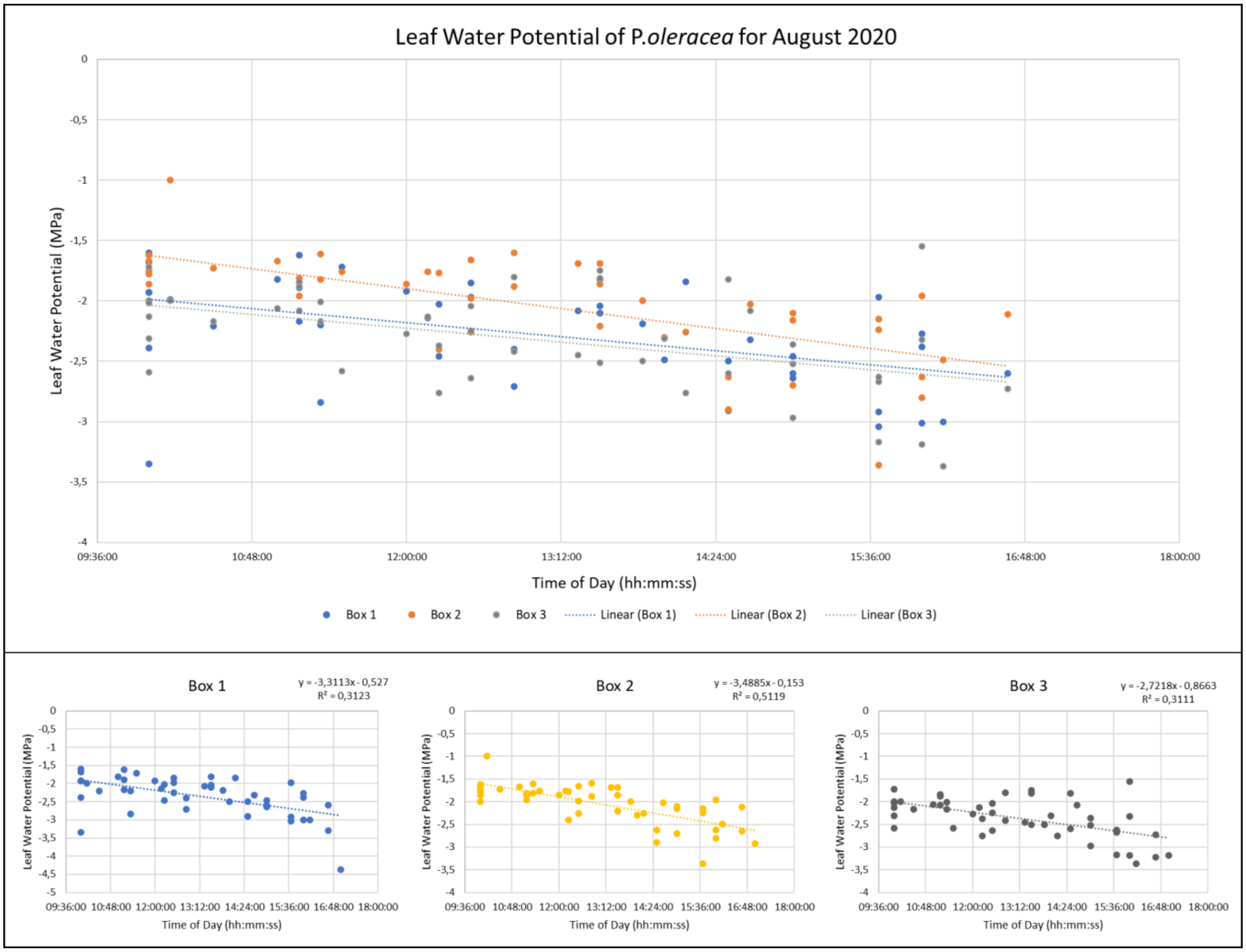
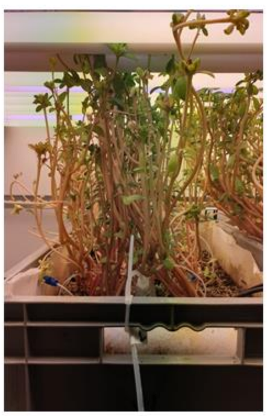


Figure 16. Overall trends in leaf water potentials of P. *oleracea* over August 2020 for Box 1, Box 2 and Box 3.

Based on the LWPs for each box, there are not any noticeable defining trends or variations. If anything, Box 2 seems to have a slightly lower variance than the other two boxes, while Box 3 seems to have a larger variation at later times of the day than Box 1 or Box 2. Despite the minimal differences, Box 3 did express a potential stress response through a progressive decrease in foliage density as compared to Box 1 or Box 2. In Figure 17, this progression can be seen through pictures taken of all three boxes on each measurement day.

	Box 1	Box 2	Box 3
Aug. 4			
Aug. 5			
Aug. 11			
Aug. 13			

Aug. 18

Aug. 20

Aug. 24

Aug. 25

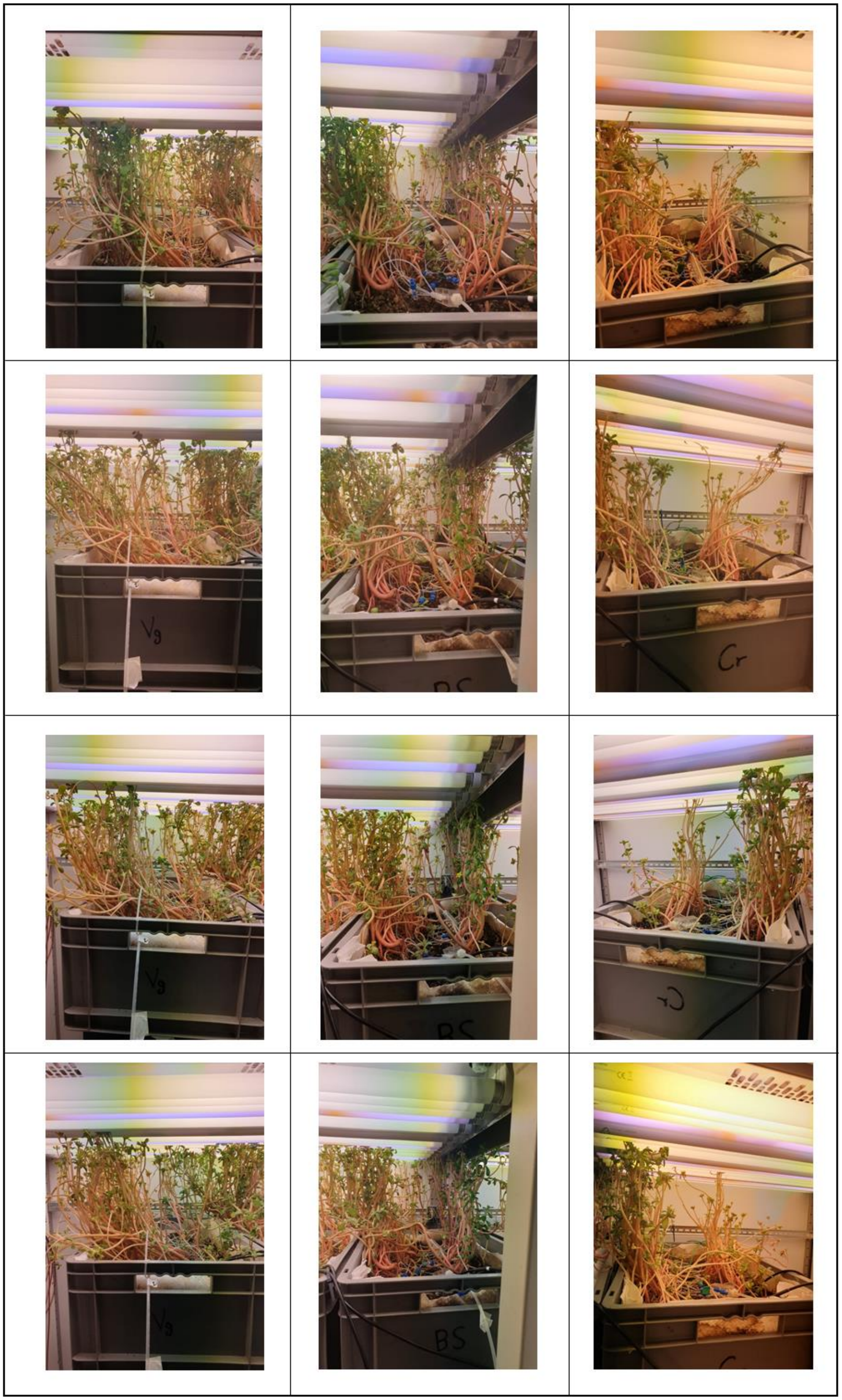


Figure 17. Pictures showing the physical changes between the start of data collection on August 4, 2020 and the final day on August 25, 2020.

Between pictures taken on August 4 and August 26, a drastic difference can be seen in the physical features of the plants in Box 3. The foliage in Box 3 is also much more sparse as compared to the other two boxes. Despite these differences, when compared against pressure head, water content and evaporation, none of the boxes showed a noticeable trend or reaction in response to differences in irrigated BPA concentration.

Pressure Head and Leaf Water Potential

During the study period, the soil matric potential was measured in the root zone of each box to determine any potential changes or stress factors as a result of differences in irrigated BPA concentrations.

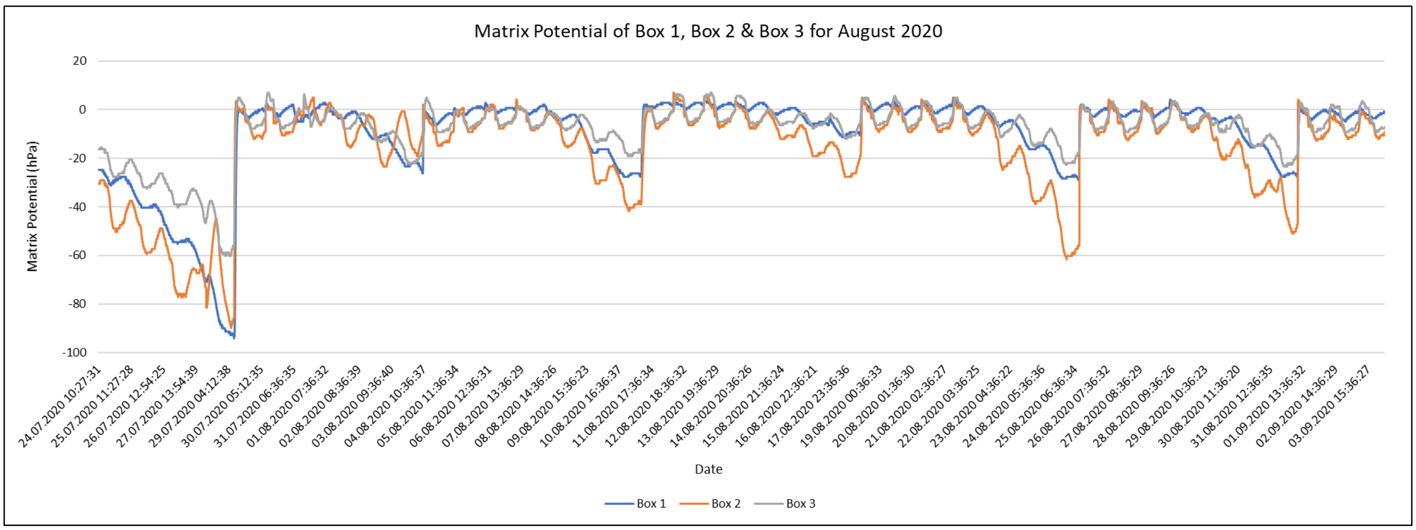


Figure 18. The pressure head (MPa) of Box 1, Box 2 and Box 3 over the month of August 2020.

The graph in Figure 18 shows the pressure head of Box 1, Box 2 and Box 3 over the study period. Although Box 2 tends to show more negative matric potentials in some instances in the graph, this occurred between irrigation days, and therefore is not represented in the results found on measurement days. During the days of the week that measurements took place (4^{th}, 5^{th}, 11^{th}, 13^{th},18^{th}, 20^{th}, 25^{th} & 26^{th} of August), the matric potentials of each box follow a similar trend. While the data in the current figure shows continuous changes over the month of August 2020, it was also necessary to analyze the pressure head of each box on a smaller time scale. For this reasoning, Figure 19 was created to examine pressure head changes solely during periods of time that LWP measurements and irrigation took place.

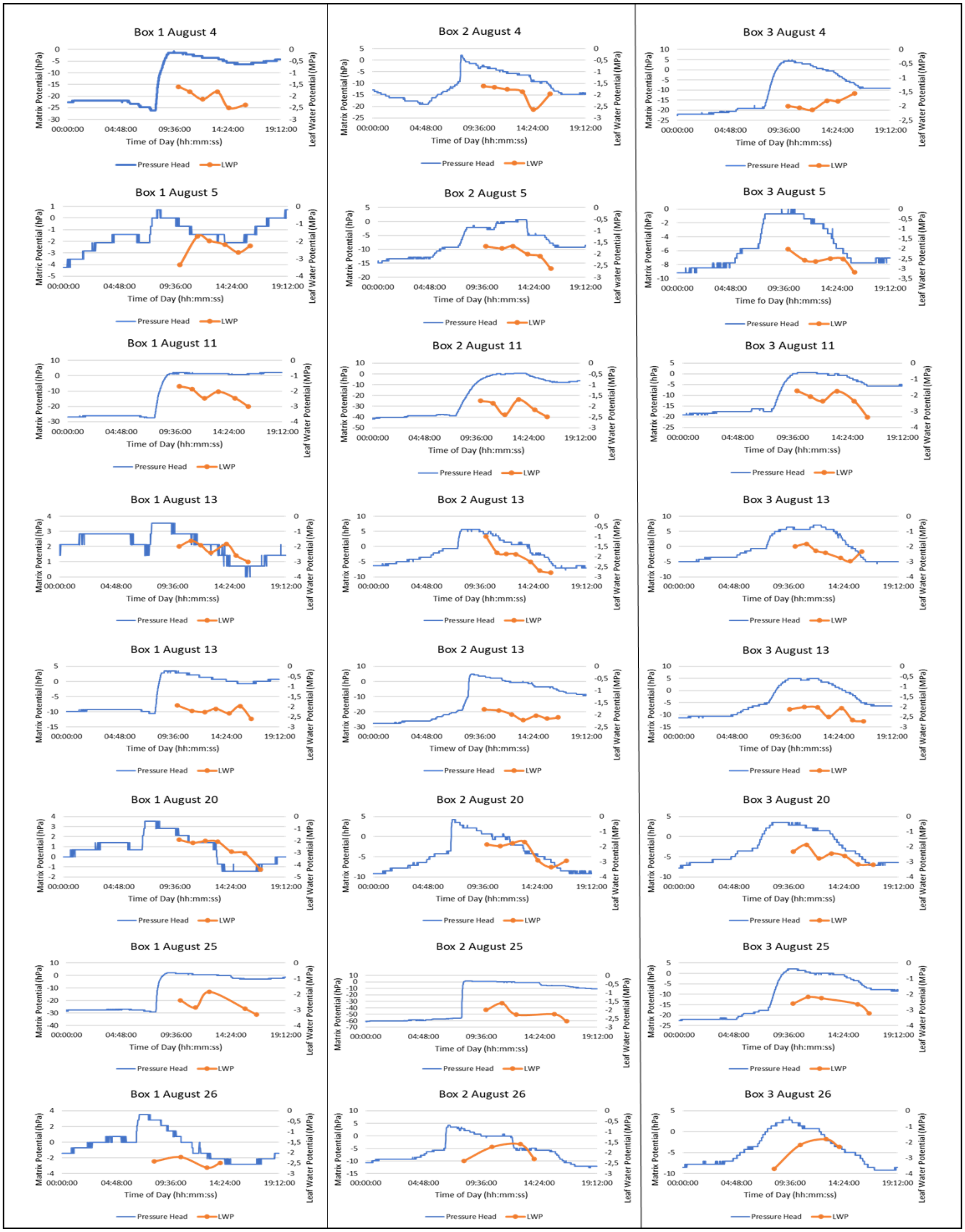


Figure 19. This image depicts the pressure head of Box 1, Box 2 and Box 3 on each individual measurement day. The leaf water potential measurements for each box are compared to the pressure head in these graphs.

Each column inf Figure 19 is used to compare differences between measurement days of each box, while the rows compare differences between Box 1, Box 2 and Box 3 on each individual measurement day. These graphs were used to analyze any trend or pattern pertaining to a relationship between changes in pressure head and variations in leaf water potential. While LWPs do seem to be somewhat affected by pressure head, it is not a sole controlling variable in LWP adaptations. It is also important to analyze the similarities in pressure head trends between the three boxes. Although not the same, the pressure head in each box tend to follow a similar trend as the other boxes on the same day. This indicates that, for soil matric potentials, all boxes performed similarly, and any changes observed in the LWPs of each box occurred independent of pressure head.

Soil Water Content and Leaf Water Potential

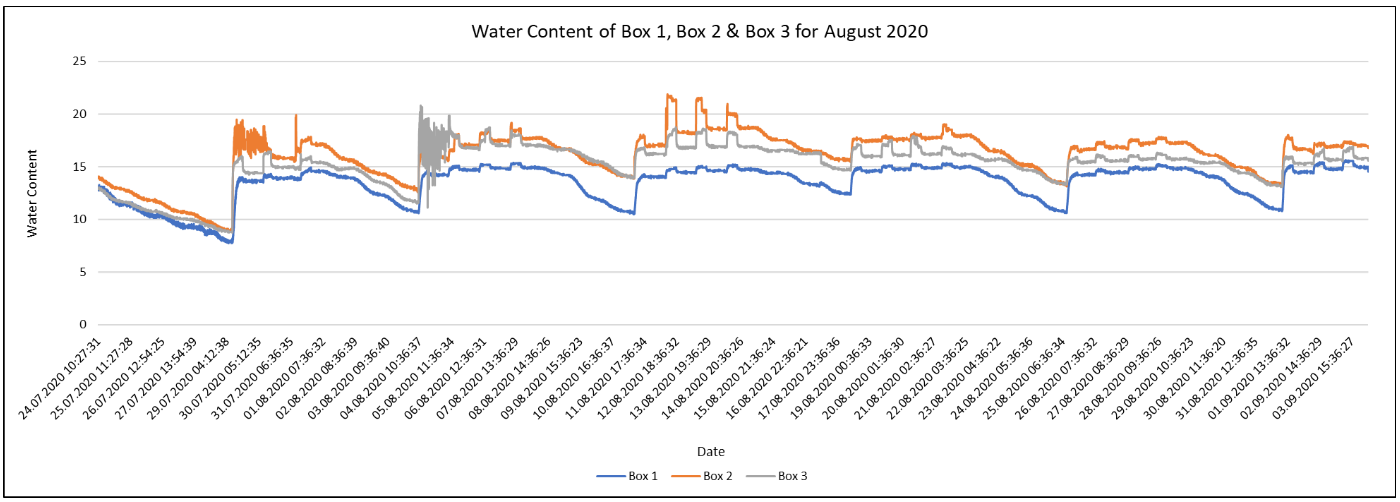


Figure 20. This graph depicts the changes in soil water content between Box 1, Box 2 and Box 3 over the period of August 2020.

Similar to Figure 18, which measured continuous pressure head over duration of the study period, Figure 20 does the same with water content. While it appears Box 1 tends to have a slightly lower water content over the month of August, it follows the same general trend as Box 2 & Box 3. This indicates that any changes in LWP patterns would not be a result of changes in trends of soil water content for the current study. In order to further analyze the data, the water content data was plotted on a time period relating to irrigation and measurement days, as previously seen with the pressure head data.

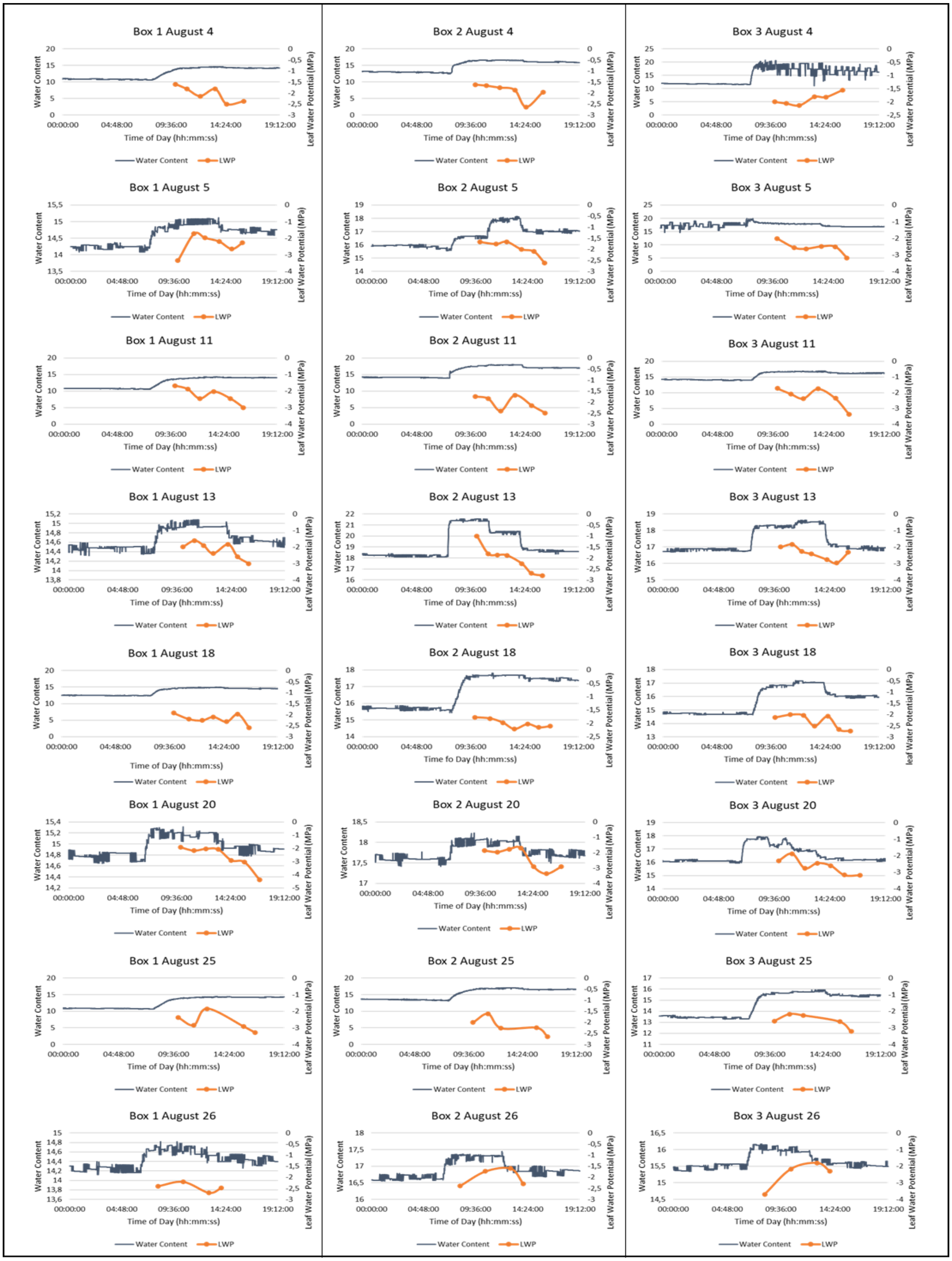


Figure 21. Water content is plotted alongside LWP measurements in the above graphs for Box 1, Box 2 & Box 3 over the period of August 2020.

Again, like Figure 19, the columns between the graphs in Figure 21 show differences between different measurement days in each box, while rows define differences between Box 1, Box 2 and Box 3 on each individual measurement day. Water content trends between each box tend to follow a similar pattern on each measurement day as each box was irrigated with the same quantity of solution over the same period of time. From this data, no discrepancies are apparent in the LWPs that could be correlated to soil water content.

Outflow

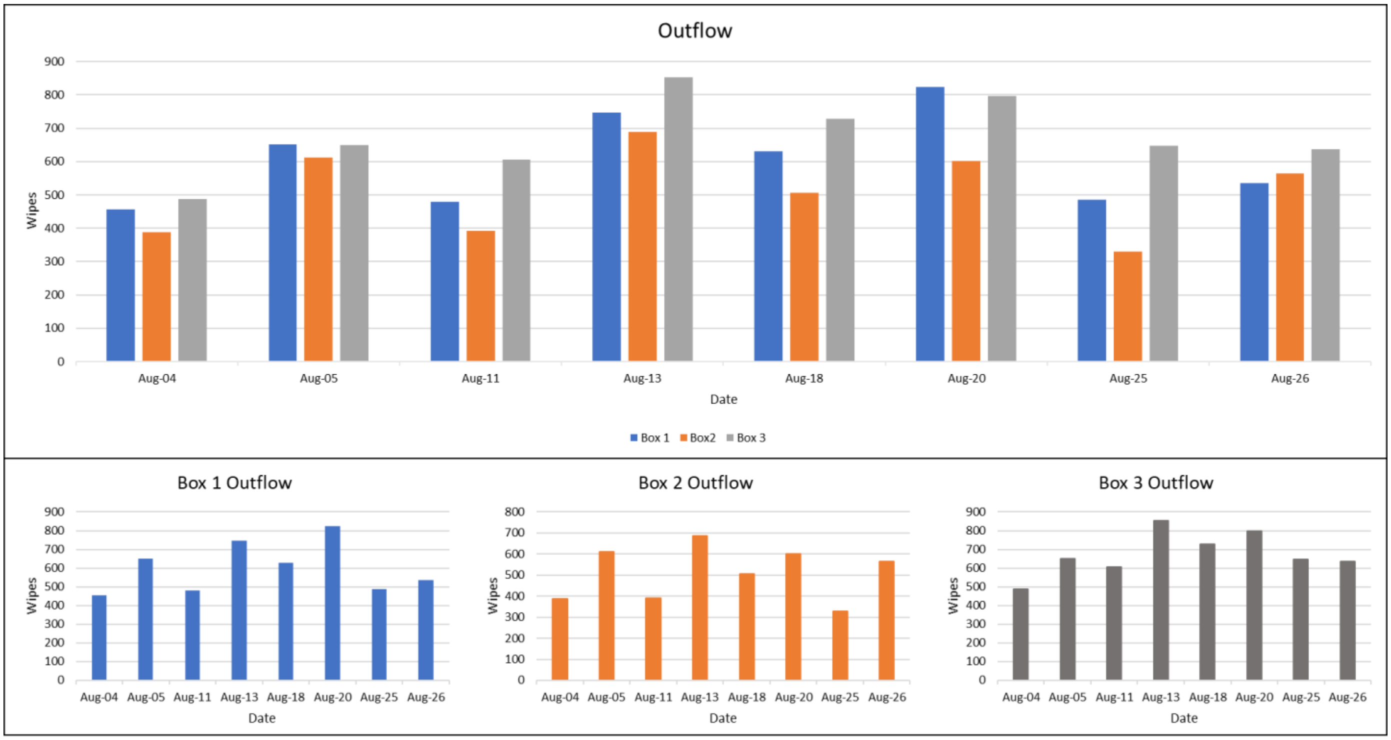


Figure 22. The outflow of Box 1, Box 2 and Box 3 represented in a graph format over the study period.

In Figure 22, the outflow of Box 1, Box 2 and Box are plotted both alongside each other and individually. Where all boxes' outflow data is plotted together, Box 3 tends to have a slightly higher outflow than the other two boxes on most days. This trend is also enunciated in Table 3, which quantifies the overall outflow of each box over the study period. In this table, Box 3 had the highest amount of overall outflow while Box 2 has the lowest. This trend is also observed in Table 4, which summarizes the outflow of each box on each individual measurement day.

Table 3. The sum outflow of each individual box (Box 1, Box 2 and Box 3) over the study period.

Box #	Outflow (wipes)	Outflow (mm rainfall)
Box 1	4810	962
Box 2	4081	816.2
Box 3	5405	1 081

Looking again at Figure 22, when examining the individual outflow data, there is a noticeable difference between every second column. This is due to the pattern in watering regime. Each week, boxes were irrigated for an 8-hour period on four consecutive days. On the first day of irrigation, each box had undergone a 3-day drying period in which no solution was irrigated. The boxes therefore had a higher retention capacity at this point of time and less outflow is observed on these days. For measurements after the first day of irrigation, the soil would have been more saturated and irrigated solutions were released more frequently as outflow. This trend is again seen in Table 4, where outflow quantities vary based on this pattern for each box.

Table 4. The outflow of Box 1, Box 2 and Box 3 on each measurement day.

Date	Box 1 (wipes)	Box 2 (wipes)	Box 3 (wipes)
Aug 4	456	388	488
Aug 5	651	612	649
Aug 11	480	392	606
Aug 13	747	688	582
Aug 18	630	507	728
Aug 20	824	601	797
Aug 25	486	329	648
Aug 26	536	564	637

Despite these patterns, all three boxes do show a general trend of releasing more outflow in later study dates. The final week of the study did break this trend, so further analysis and a longer study period would be required to verify if each box would continue to increase in outflow quantity under this intensity of irrigation. Although Box 3 did show a higher outflow, all three boxes followed a similar pattern regarding individual outflow each week. In other words, no box exhibited an entirely unique pattern that would separate it from the other boxes. Therefore, it is possible that the increases in outflow observed with time could be related to over-irrigation. This aspect also requires further analysis to determine if irrigation amount created or enhanced a stress factor in the plants, and what the limitations of irrigation would be for P. *oleracea* in a green roof environment.

Evaporation and Leaf Water Potential

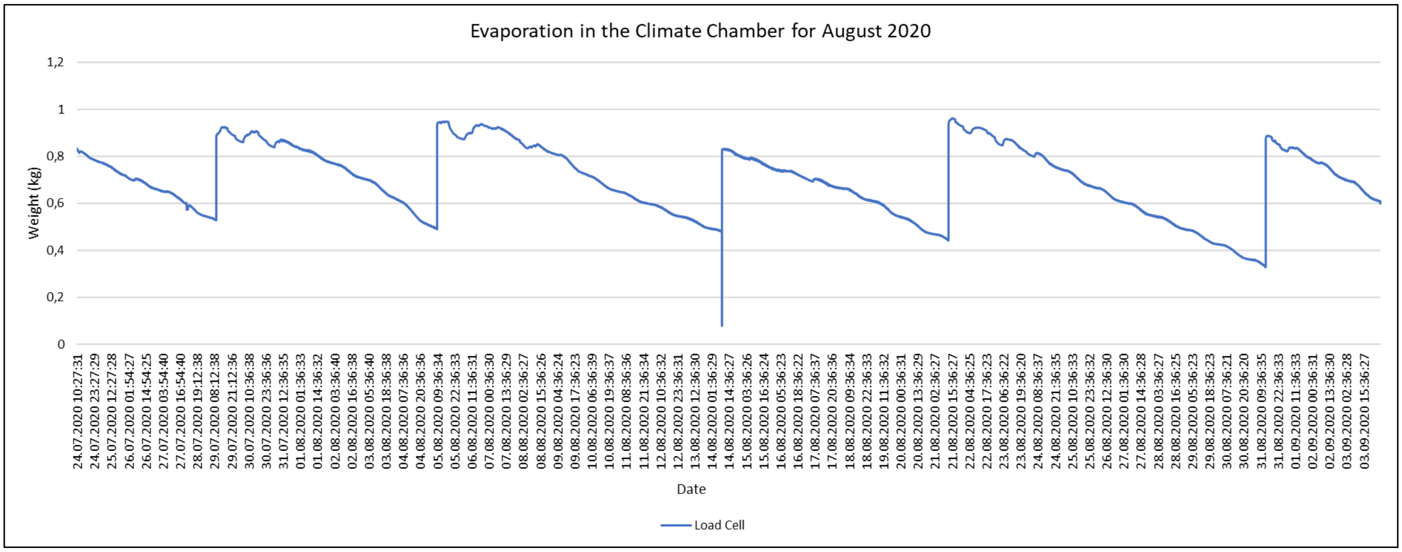


Figure 23. Evaporation trends over the study period measured with an evaporation plate.

Figure 23 shows the evaporation data gathered over the study period. Evaporation was measured via an evaporation plate, which recorded the difference in mass (kg) as water evaporated from the holder. Although it is not specific to any single box, the rate of evaporation can be seen, and trends can be identified using this data. The sharp increases in the data indicate instances where water was added to fill the evaporation plate, while the declining slopes are indicative of rate of evaporation in the climate chamber. For the most part, rate of evaporation tends to follow a similar trend. As would be expected, with increasing temperatures in the climate chamber, evaporation rates should also increase which could account for the variations seen in each evaporation rate slope after refilling of the evaporation plate.

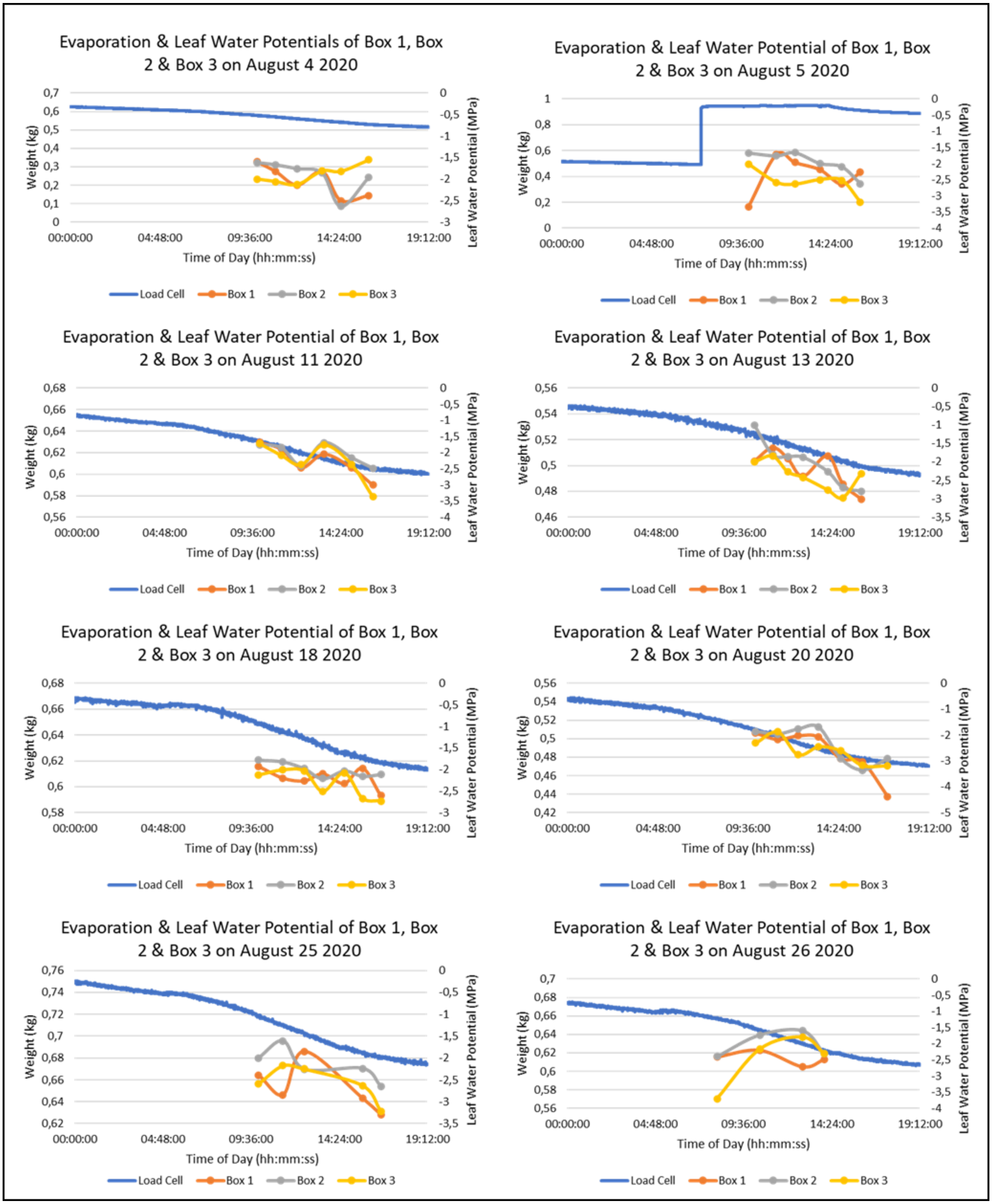


Figure 24. Box 1, Box 2 and Box 3 LWP data plotted alongside evaporation data as measured by an evaporation plate on each individual measurement day.

The data from Figure 23 was broken down into individual measurement days. In the graphs in Figure 24, the evaporation of each day is plotted alongside the LWP measurements of Box 1, Box 2 and Box 3. From this data, there were no obvious trends or patterns that correlate evaporation and LWP, or that differentiated the boxes from one another. For the most part, on any given day, the rate of evaporation remains almost constant with few fluctuations, not including the drastic change in evaporation on August 5 caused by refilling the evaporation plate. With temperature variations, evaporation and transpiration should be affected similarly. Resulting in changes in LWPs. In Figure 4, however, LWPs do not seem to be correlated solely to any variations in rate of evaporation. It may be beneficial in future developments to continuously monitor LWPs in order to compare with evaporation variations.

DISCUSSION

After examining the results of each box, for this study, BPA concentrations up to 5mg/L do not seem to pose a problem for P. *oleracea* in terms of combined phytoremediation and water management purposes in an urban green roof environment. Although Box 2 had the lowest outflow quantity and Box 3 had the highest outflow quantity, further analysis is required to determine if this was indeed a toxicity response to BPA or a result of another variable. It is possible that this response was due to the morphological changes observed in each box. Although these physical changes cannot be determined as a result of irrigated BPA concentrations solely with this study. Further examination of whether these plants would exhibit the same reaction in a repeated or similar experimental setup is advised. It is also necessary to verify whether these changes, if present in a similar study, would worsen over time. Despite these observations, there was no observable trend or distinct changes in pressure head, soil water content, evaporation and LWP measurements. This indicates that P. *oleracea* performed similarly hydrologically under all three solutions of irrigated BPA solution (0 mg/L, 2 mg/L 5m g/L). As a typical green roof has the potential to remove 50% of annual rainfall from a roof through retention and evapotranspiration (Berghage et al., 2009), green roof vegetation installed for phytoremediation purposes would need to retain this function to remain a practical choice. This being the case, P. *oleracea* is still an optional choice of vegetation for urban green roofs to maximize potential ecological benefits.

Sources of Error

In the current study, there are a few sources that could have led to error in the measurements that were taken. The first to be touched upon is temperature. As the study period took place in August, the elevated temperatures outside correlated to an increase in building room temperature. Unlike the plants that were monitored in a climate chamber, the WP4C that was used for measuring LWPs was in a room of its own. Due to its location, the WP4C was affected by room temperature, and would sometimes provide readings such as ‘sample is too hot’. In this instance, sample cups would be refrigerated until cool enough that LWPs could be measured. This process lengthened the time between the actual harvesting of the leaf samples and the time each leaf’s LWP was obtained, which increased the time for moisture to evaporate from each sample and potentially alter the LWP.

For accurate comparison of the LWPs of each box, samples were harvested from all three boxes at the same time. However, the WP4C was only able to measure one sample at any given time. This restriction resulted in the samples from Box 2 and Box 3 remaining in their transport containers for a longer period of time than Box 1 samples. Although the samples were transported between the climate chamber and WP4C in sealed containers to prevent the release of moisture, this may have had an effect on LWPs, especially during time where samples remained in their containers for periods ranging from half an hour to an hour.

Due to Covid-19, the project was put on hold for a few months while the plants remained in the climate chamber. After the end of the global quarantine, the plants displayed obvious changes in physical features including smaller leaf sizes and elongated, skinny stems. Lower LWPs were also observed immediately when resuming the project (-3 to -4 MPa), although they eventually returned to a range (-1.5 to -3 MPa) observed previous to the closure of the lab. Furthermore, during BPA irrigation, the plants of Box 3 produced fewer and smaller leaves. Due to this development, there were fewer leaf samples available and measurements could only be taken twice every week. Although the development of the plants and their LWPs were still able to be observed, more data over the study period may have shown stronger trends or patterns. Although not possible in the current study due to the sample availability limitations, comparing the LWPs of the plant samples on irrigation days versus non-irrigation days could be used to identify and compare differences in plant functionality under these conditions. Also, there were a few occurrences where the climate chamber itself was found to have shut down. Although this problem was always quickly fixed, although minor, the lower temperatures and lack of UV light may have caused a reaction in some of the plants. If this project were to be repeated or continued in the future, it would be important to establish continuity in the care of the plants to minimize any possibility of a stress reaction in the plant samples. LWPs of Portulaca *oleracea* without the long stress period caused by the quarantine could also be compared to those found in this study to verify whether this plant can still perform optimally while under stress factors outside those induced by BPA irrigation.

Potential Improvements of the Current Study

While the results of this study do indicate that P. *oleracea* is able to remediate concentrations of BPA up to 5 mg/L without detrimental effects to functions of transpiration, there are many ways this study could be improved upon and further analyzed. Due to time constraints affected by Covid-19, the study was only able to take place over the period of a month. In the results, the LWPs for each box demonstrate an irregular pattern on the last day of observed measurements. While this is not particular to any individual box, it could demonstrate stress in P. *oleracea* due to the 8-hour, 4-day watering regime. In terms of water management, it would be relevant to further investigate these findings over both longer time periods and different patterns of irrigation. It is also unknown if any of the boxes would show a response to long-term exposure to BPA concentrations. Regarding the physical features of the plants used in this study, Box 3 demonstrated increasing foliage sparsity. Many studies have also reported noticeable changes in plant organ growth depending on concentration and duration of exposure to irrigated BPA. Low doses of BPA (1.5 mg/L) was observed in soybeans and aboveground growth patterns such as increased plant height, stem fresh/dry weight, and leaf area were reported, and many other studies have suggested that low doses of BPA (< 3mg/L) can be beneficial to plant growth to a certain extent (Terouchi et al., 2004; Pan et al., 2013; Qi et al., 2013; Ali et al., 2016; Jiao et al., 2017; Li et al., 2018). In this study, this pattern was also observed in Box 2, reinforcing the assumption that low dose BPA concentrations can enhance the growth of aboveground plant organs. In contrast, it has also been reported that treatments of higher BPA concentrations (10 mg/L) can result in decreased plant growth, which was observed in lettuce, tomatoes, broad beans, durum wheat and mung beans (Ferrara et al., 2006; Qiu et al, 2013; Jiao et al., 2017; Li et al., 2018; Kim et al., 2018). Although the highest concentration in the current study used was 5mg/L BPA, it is possible that the observed change in physical features of Box 3 are in line with the findings of detrimental effects of BPA despite the phytoremediation capabilities of P. *oleracea*. It would therefore be necessary to verify these findings in future related studies, as well as consider the rate and duration of irrigation both in relation to and independent of BPA concentration. It would also be a worthwhile option for future studies to consider an extended range of BPA concentrations. For example, examining any differences observed in P. *oleracea*, both in physical features and remediation/transpiration performance, between plants exposed to 5 mg/L and 10 mg/L. The real-world relevance of this analyses would lie in the possibility of extending remediation purposes beyond the scope of sheer runoff solely from urban environments.

Future Developments and Modifications

While there are many aspects that this base project can be improved upon, this study also opens the pathway for many future investigations of similar topics. In particular, certain key features and processes can be further examined with the possibility of enhancement. In regards specifically to BPA, which exhibits a moderate soil-sediment adsorption and bioaccumulation (Staples et al., 1998), increasing adsorptive properties of the soil may prove beneficial in green roof environments which are space-limited. The remediation of BPA, which can be rapidly biodegraded in natural waters and usually displays a lifespan and less than 5 days (Staples et al., 1998; Kang et al., 2007), would benefit with an increased adsorptivity and retardation in green roof environments. This would slow the advection and dispersion of the contaminant through the soil matrix while increasing time of exposure to plants exhibiting phytoremediation potential. In a study by Vijayaraghavan et al. (2019), it is suggested that biosorbents, which are inactive or dead organic materials, can be used as a soil additive to increase the sorption of pollutants on green roofs. The organic biosorbents mentioned in this study include seaweed, biochar and a mixture of crab shells. Ahmad et al. (2014) also suggests the use of biochar for soil water contaminant management and highlight its benefits of carbon sequestration, soil fertility improvement and pollutant remediation. In order to maximize green roof benefits, in accordance with the current study, it would therefore be beneficial to analyze the effects of biosorbents on green roof environments and their interactions with individual and combined contaminants, soil-plant interactions and water retention capabilities. While biochar can be added for adsorptive benefits, it can also be used to decrease runoff quantity and increase water retention capacities of green roof environments, improving both runoff quantity and quality (Beck et al., 2011). Other compounds can also be used as soil additives in future studies to increase water holding capacity of green roof substrate. Wang et al. (2017) suggest using dual-substrate layers, as well as using a combination of activated charcoal, pumice, expanded perlite and vermiculite to enhance water retention capacity. The addition of silicate granules and hydrogel to green roof substrate has also been suggested by Farrell et al. (2013) to increase water holding capacity and plant available water. Overall, the introduction of soil additives would be a feasible pathway to furthering the knowledge gained by the current study.

The analysis of different vegetation in a similar setting to the current study would also be a viable consideration. While Portulaca *oleracea* is known for its ability to remediate BPA, there are also other plants with a similar ability. For example, tropical evergreen plant Dracaena *sanderiana* has been found to withstand BPA toxicity levels of 80 uM and was able to remediate BPA concentrations through process of translocation (Saiyood et al., 2010). Juncus *acutus*, a wetland halophyte, has also been effective at remediating BPA contaminated groundwater (Christofilopoulos et al., 2016.; Syranidou et al., 2016). While the current study analyzed the toxicity effect of BPA on Portulaca *oleracea*, it is also possible to use this plant for the remediation of contaminants other than BPA. For example, Hamidov et al. (2007) and Kilic et al. (2008) both found P. *oleracea* was an effective species for the remediation of saline soils. In a study by Elshamy et al. (2019), it was also determined that P. *oleracea* could be used as a phytoremediation method for the decontamination of soils polluted with heavy metals such as Mn, Cu, Zn, Fe and Pb. Although in lower concentrations than normally found in urban runoff, some studies have reported the existence of metals in green roof runoff (Berndtsson et al., 2006), with heavy metals such as Fe, Cu and Al observed in some instances (Vijayaraghavan et al., 2012). P. *oleracea* could therefore potentially be used to remediate a combination of pollutants found on green roofs and further improve runoff quality. Furthermore, other plants in the Portulaca genus have also been found to exhibit remediation capabilities. Tiwari et al. (2008) analyzed P. *tuberosa* alongside P. *oleracea* and found both plants showed high accumulation of metals in all plant parts and were able to hyperaccumulate more than one heavy metal (i.e. Cd, Cr and As). Overall, this area of study has much potential and an overwhelming capacity for growth and development.

SUMMARY

In summary, the present study has shown the potential of Portulaca *oleracea* for phytoremediation on a green roof through the toxicity effect of BPA. While variables such as pressure head, soil water content and LWPs did not exhibit an obvious correlation with differences in irrigated BPA concentration, there were noticeable differences between the three boxes regarding outflow and physical features. Box 2 showed fuller foliage and the lowest amount of outflow, while Box 3 showed thinning foliage with smaller leaves and had the highest outflow. If this response is indeed a result of a BPA toxicity effect, up to 2mg/L BPA could be remediated by P. *oleracea* on green roofs with a potential to increase existing green roof benefits by enhancing plant growth. This would also have the potential to remediate most urban wastewater and runoff which contain BPA concentrations below that of mg/L. However, it is also possible that these changes are not a result of a BPA toxicity effect and would require further investigation. Despite these observations, as there was not a significant difference in LWPs, it suggests that hydrological functions, including evapotranspiration, did not change regardless of BPA exposure. This indicates that the plants still performed optimally and have potential for the phytoremediation of BPA on green roofs.

REFERENCES

Ahmad, M., Rajapaksha, A. U., Lim, J. E., Zhang, M., Bolan, N., Mohan, D., ... & Ok, Y. S. (2014). Biochar as a sorbent for contaminant management in soil and water: a review. *Chemosphere*, *99*, 19-33.

Alam, M. A., Juraimi, A. S., Rafii, M. Y., Hamid, A. A., Uddin, M. K., Alam, M. Z., & Latif, M. A. (2014). Genetic improvement of Purslane (Portulaca oleracea L.) and its future prospects. *Molecular biology reports*, *41*(11), 7395-7411.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, *91*(7), 869-881.

Ali, I., Liu, B., Farooq, M. A., Islam, F., Azizullah, A., Yu, C., ... & Gan, Y. (2016). Toxicological effects of bisphenol A on growth and antioxidant defense system in Oryza sativa as revealed by ultrastructure analysis. *Ecotoxicology and environmental safety*, *124*, 277-284.

Alkorta, I., & Garbisu, C. (2001). Phytoremediation of organic contaminants in soils. *Bioresource technology*, *79*(3), 273-276.

Anwar, M., Rasul, M., & Khan, M. M. K. (2012). Green roof for storm water management: a review.

Beck, D. A., Johnson, G. R., & Spolek, G. A. (2011). Amending greenroof soil with biochar to affect runoff water quantity and quality. *Environmental pollution*, *159*(8-9), 2111-2118.

Belgiorno, V., Rizzo, L., Fatta, D., Della Rocca, C., Lofrano, G., Nikolaou, A., ... & Meric, S. (2007). Review on endocrine disrupting-emerging compounds in urban wastewater: occurrence and removal by photocatalysis and ultrasonic irradiation for wastewater reuse. *Desalination*, *215*(1-3), 166-176.

Berghage, R. D., Beattie, D., Jarrett, A. R., Thuring, C., Razaei, F., & O'Connor, T. P. (2009). Green roofs for stormwater runoff control. Retrieved from *HTTP://NEPIS. EPA. GOV/EXE/ZYPURL. CGI? DOCKEY= P1003704. TXT*.

Berndtsson, J. C., Emilsson, T., & Bengtsson, L. (2006). The influence of extensive vegetated roofs on runoff water quality. *Science of the Total Environment*, *355*(1-3), 48-63.

Bhatnagar, A., & Anastopoulos, I. (2017). Adsorptive removal of bisphenol A (BPA) from aqueous solution: a review. *Chemosphere*, *168*, 885-902.

Boivin, M. A., Lamy, M. P., Gosselin, A., & Dansereau, B. (2001). Effect of artificial substrate depth on freezing injury of six herbaceous perennials grown in a green roof system. *HortTechnology*, *11*(3), 409-412.

Bollag, J. M., Mertz, T., & Otjen, L. (1994). Role of microorganisms in soil bioremediation.

Boyer, J. (1967). Leaf water potentials measured with a pressure chamber. *Plant Physiology*, *42*(1), 133-137.

Boyer, J. S. (1990). *U.S. Patent No. 4,952,071*. Washington, DC: U.S. Patent and Trademark Office.

Boyer, J. S., & Knipling, E. B. (1965). Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. *Proceedings of the National Academy of Sciences of the United States of America*, *54*(4), 1044.

Chan, K., Islam, M. W., Kamil, M., Radhakrishnan, R., Zakaria, M. N. M., Habibullah, M., & Attas, A. (2000). The analgesic and anti-inflammatory effects of Portulaca oleracea L. subsp. sativa (Haw.) Celak. *Journal of ethnopharmacology*, *73*(3), 445-451.

Chen, J., Huang, X., & Lee, D. (2008). Bisphenol A removal by a membrane bioreactor. *Process Biochemistry*, *43*(4), 451-456.

Chouhan, S., Yadav, S. K., Prakash, J., & Singh, S. P. (2014). Effect of Bisphenol A on human health and its degradation by microorganisms: a review. *Annals of microbiology*, *64*(1), 13-21.

Christofilopoulos, S., Syranidou, E., Gkavrou, G., Manousaki, E., & Kalogerakis, N. (2016). The role of halophyte Juncus acutus L. in the remediation of mixed contamination in a hydroponic greenhouse experiment. *Journal of Chemical Technology & Biotechnology*, *91*(6), 1665-1674.

Colin, A., Bach, C., Rosin, C., Munoz, J. F., & Dauchy, X. (2014). Is drinking water a major route of human exposure to alkylphenol and bisphenol contaminants in France?. *Archives of environmental contamination and toxicology*, *66*(1), 86-99.

Cousins, I. T., Staples, C. A., Klečka, G. M., & Mackay, D. (2002). A multimedia assessment of the environmental fate of bisphenol A. *Human and Ecological Risk Assessment*, *8*(5), 1107-1135.

Cushman, J. and Bohnert, H. (1997) Molecular genetics of crassulacean acid metabolism. *Plant Physiol.*113: 667–676.

Danin, A., Baker, I., & Baker, H. G. (1978). Cytogeography and taxonomy of the Portulaca oleracea L. polyploid complex. *Israel Journal of Botany*, *27*(3/4), 177-211.

Dehghani, M. H., Ghadermazi, M., Bhatnagar, A., Sadighara, P., Jahed-Khaniki, G., Heibati, B., & McKay, G. (2016). Adsorptive removal of endocrine disrupting bisphenol A from aqueous solution using chitosan. *Journal of Environmental Chemical Engineering*, *4*(3), 2647-2655.

Denmead, O. T., & Shaw, R. H. (1962). Availability of soil water to plants as affected by soil moisture content and meteorological conditions 1. *Agronomy journal*, *54*(5), 385-390.

Dietz, A. C., & Schnoor, J. L. (2001). Advances in phytoremediation. *Environmental health perspectives*, *109*(suppl 1), 163-168.

Dong, J., Li, X. L., Ruan, T. G., Zhou, S. C., & Lin, L. (2009). Phenol pollution in the sediments of the Pearl River estuary area and its potential risk assessment to the eco-security. *J Safety Environ*, *9*, 113-6.

Drewes, J. E., Bellona, C., Oedekoven, M., Xu, P., Kim, T. U., & Amy, G. (2005). Rejection of wastewater-derived micropollutants in high-pressure membrane applications leading to indirect potable reuse. *Environmental Progress*, *24*(4), 400-409.

Dvorak, B., & Volder, A. (2010). Green roof vegetation for North American ecoregions: a literature review. *Landscape and urban planning*, *96*(4), 197-213.

EFSA. (2015). No consumer health risk from bisphenol A exposure. *European Food and Safety Auhtority*. Retrieved from: https://www.efsa.europa.eu/en/press/news/150121

Farrell, C., Ang, X. Q., & Rayner, J. P. (2013). Water-retention additives increase plant available water in green roof substrates. *Ecological Engineering*, *52*, 112-118.

Ferrara, G., Loffredo, E., & Senesi, N. (2006). Phytotoxic, clastogenic and bioaccumulation effects of the environmental endocrine disruptor bisphenol A in various crops grown hydroponically. *Planta*, *223*(5), 910-916.

Ehleringer, J. R., & Cerling, T. E. (2002). C3 and C4 photosynthesis. *Encyclopedia of global environmental change*, *2*, 186-190.

Ehlig, C. F. (1962). Measurement of energy status of water in plants with a thermocouple psychrometer. *Plant Physiology*, *37*(3), 288.

Elfving, D. C., KAUFMANN, M. R., & HALL, A. E. (1972). Interpreting leaf water potential measurements with a model of the soil-plant-atmosphere continuum. *Physiologia plantarum*, *27*(2), 161-168.

Elshamy, M. M., Heikal, Y. M., & Bonanomi, G. (2019). Phytoremediation efficiency of Portulaca oleracea L. naturally growing in some industrial sites, Dakahlia District, Egypt. *Chemosphere*, *225*, 678-687.

Felton, G. W., & Gatehouse, J. A. (1996). Antinutritive plant defence mechanisms. In *Biology of the insect midgut* (pp. 373-416). Springer, Dordrecht.

Fukazawa, H., Hoshino, K., Shiozawa, T., Matsushita, H., & Terao, Y. (2001). Identification and quantification of chlorinated bisphenol A in wastewater from wastepaper recycling plants. *Chemosphere*, *44*(5), 973-979.

Fürhacker, M., Scharf, S., & Weber, H. (2000). Bisphenol A: emissions from point sources. *Chemosphere*, *41*(5), 751-756.

Garnier, E., Berger, A., & Martin, M. (1988). How to estimate leaf transpiration from water potential measurements?. *Flora*, *181*(1-2), 131-135.

Getter, K. L., & Rowe, D. B. (2006). The role of extensive green roofs in sustainable development. *HortScience*, *41*(5), 1276-1285.

Gonzalez-Casado, A., Navas, N., Del Olmo, M., & Vilchez, J. L. (1998). Determination of bisphenol A in water by micro liquid—liquid extraction followed by silylation and gas chromatography—mass spectrometry analysis. *Journal of chromatographic science*, *36*(11), 565-570.

Gorito, A. M., Ribeiro, A. R., Almeida, C. M. R., & Silva, A. M. (2017). A review on the application of constructed wetlands for the removal of priority substances and contaminants of emerging concern listed in recently launched EU legislation. *Environmental pollution*, *227*, 428-443.

Gould, J. C., Leonard, L. S., Maness, S. C., Wagner, B. L., Conner, K., Zacharewski, T., & Gaido, K. W. (1998). Bisphenol A interacts with the estrogen receptor α in a distinct manner from estradiol. *Molecular and cellular endocrinology*, *142*(1-2), 203-214.

Gubiani, P. I., Reichert, J. M., Campbell, C., Reinert, D. J., & Gelain, N. S. (2013). Assessing Errors and Accuracy in Dew-Point Potentiometer and Pressure Plate Extractor Meaurements. *Soil Science Society of America Journal*, *77*(1), 19-24.

Guerra, P., Kim, M., Teslic, S., Alaee, M., & Smyth, S. A. (2015). Bisphenol-A removal in various wastewater treatment processes: operational conditions, mass balance, and optimization. *Journal of environmental management*, *152*, 192-200.

Guralnick, L. J., Edwards, G., Ku, M. S., Hockema, B., & Franceschi, V. (2002). Photosynthetic and anatomical characteristics in the C4–crassulacean acid metabolism-cycling plant Portulaca grandiflora. *Functional Plant Biology*, *29*(6), 763-773.

Hamada, H., Tomi, R., Asada, Y., & Furuya, T. (2002). Phytoremediation of bisphenol A by cultured suspension cells of Eucalyptus perriniana-regioselective hydroxylation and glycosylation. *Tetrahedron letters*, *43*(22), 4087-4089.

Hamidov, A. (2007). *Salt removing species. Phytoremediation technique for Uzbekistan* (Doctoral dissertation).

Hernando, M. D., Mezcua, M., Gómez, M. J., Malato, O., Agüera, A., & Fernández-Alba, A. R. (2004). Comparative study of analytical methods involving gas chromatography–mass spectrometry after derivatization and gas chromatography–tandem mass spectrometry for the determination of selected endocrine disrupting compounds in wastewaters. *Journal of Chromatography A*, *1047*(1), 129-135.

Hu, Y., Zhu, Q., Yan, X., Liao, C., & Jiang, G. (2019). Occurrence, fate and risk assessment of BPA and its substituents in wastewater treatment plant: A review. *Environmental research*, *178*, 108732.

Huang, Y. Q., Wong, C. K. C., Zheng, J. S., Bouwman, H., Barra, R., Wahlström, B., ... & Wong, M. H. (2012). Bisphenol A (BPA) in China: a review of sources, environmental levels, and potential human health impacts. *Environment international*, *42*, 91-99.

Ignatius, C. M., Francis, E. E., Emeka, E. N., Elvis, N. S., & Ebele, J. I. (2010). BPA and environmental estrogen in potable water sources in Enugu municipality, South-East, Nigeria. *Bulletin of environmental contamination and toxicology*, *85*(5), 534-537.

Imai, S., Shiraishi, A., Gamo, K., Watanabe, I., Okuhata, H., Miyasaka, H., & Hirata, K. (2007). Removal of phenolic endocrine disruptors by Portulaca oleracea. *Journal of bioscience and bioengineering*, *103*(5), 420-426.

Jackson, J., & Sutton, R. (2008). Sources of endocrine-disrupting chemicals in urban wastewater, Oakland, CA. *Science of the total environment*, *405*(1-3), 153-160.

Jarvis, P. G. (1976). The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *273*(927), 593-610.

Jiao, L., Ding, H., Wang, L., Zhou, Q., & Huang, X. (2017). Bisphenol A effects on the chlorophyll contents in soybean at different growth stages. *Environmental Pollution*, *223*, 426-434.

Joss, A., Keller, E., Alder, A. C., Göbel, A., McArdell, C. S., Ternes, T., & Siegrist, H. (2005). Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water research*, *39*(14), 3139-3152.

Kaneda, H., Matsui, T., Tomiyasu, R., Kuroda, Y., Higashimoto, Y., Oda, T., ... & Hirata, K. (2012). Isolation of polyphenol oxidase genes from Portulaca oleracea and evaluation of their ability to metabolize endocrine-disrupting chemicals. *Plant Biotechnology*, 12-0423.

Kanemasu, E. T., & Tanner, C. B. (1969). Stomatal diffusion resistance of snap beans. I. Influence of leaf-water potential. *Plant physiology*, *44*(11), 1547-1552.

Kang, J. H., Katayama, Y., & Kondo, F. (2006). Biodegradation or metabolism of bisphenol A: from microorganisms to mammals. *Toxicology*, *217*(2-3), 81-90.

Karalius, V. P., Harbison, J. E., Plange-Rhule, J., Van Breemen, R. B., Li, G., Huang, K., ... Tuchman, N. C. (2014). Bisphenol A (BPA) found in humans and water in three geographic regions with distinctly different levels of economic development. *Environmental health insights*, *8*, EHI-S13130.

Kiliç, C. C., Kukul, Y. S., & Anaç, D. (2008). Performance of purslane (Portulaca oleracea L.) as a salt-removing crop. *Agricultural water management*, *95*(7), 854-858.

Kim, D., Kwak, J. I., & An, Y. J. (2018). Effects of bisphenol A in soil on growth, photosynthesis activity, and genistein levels in crop plants (Vignaradiata). *Chemosphere*, *209*, 875-882.

Kluge, M., & Ting, I. P. (1978). Crassulacean acid metabolism: an ecological analysis. *Ecological studies series, 30*, 209.

Knipling, E. B. (1967). Measurement of leaf water potential by the dye method. *Ecology*, *48*(6), 1038-1041.

Knipling, E. B., & Kramer, P. J. (1967). Comparison of the dye method with the thermocouple psychrometer for measuring leaf water potentials. *Plant physiology*, *42*(10), 1315-1320.
Koch, K., & Kennedy, R. A. (1980). Characteristics of crassulacean acid metabolism in the succulent C4 dicot, Portulaca oleracea L. *Plant Physiology*, *65*(2), 193-197.
Koch, K. E., & Kennedy, R. A. (1982). Crassulacean acid metabolism in the succulent C4 dicot, Portulaca oleracea L under natural environmental conditions. *Plant Physiology*, *69*(4), 757-761.
Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B., & Buxton, H. T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999− 2000: A national reconnaissance. *Environmental science & technology*, *36*(6), 1202-1211.
Ku, S., Shie, Y., Reger, B. and Black, C.C. (1981) Photosynthetic characteristics of *Portulaca grandiflora*, a succulent C_4 dicot. *Plant Physiol.*68: 1073–1080.
Lang, I. A., Galloway, T. S., Scarlett, A., Henley, W. E., Depledge, M., Wallace, R. B., & Melzer, D. (2008). Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. *Jama*, *300*(11), 1303-1310.
LaPensee, E. W., Tuttle, T. R., Fox, S. R., & Ben-Jonathan, N. (2009). Bisphenol A at low nanomolar doses confers chemoresistance in estrogen receptor-α–positive and–negative breast cancer cells. *Environmental health perspectives*, *117*(2), 175-180.
Lara, M.V., Disante, K.B., Podestá, F.E., Andreo, C.S. and Drincovich, M.F. (2003) Induction of a crassulacean acid like metabolism in the C_4 succulent plant, *Portulaca oleracea* L.: physiological and morphological changes are accompanied by specific modifications in phosphoenolpyruvate carboxylase. *Photosynth. Res.*77: 241–254.
Lee, H. B., & Peart, T. E. (2000a). Determination of bisphenol A in sewage effluent and sludge by solid-phase and supercritical fluid extraction and gas chromatography/mass spectrometry. *Journal of AOAC international*, *83*(2), 290-298.
Lee, H. B., & Peart, T. E. (2000b). Bisphenol A contamination in Canadian municipal and industrial wastewater and sludge samples. *Water Quality Research Journal*, *35*(2), 283-298.
Li, W. C., & Yeung, K. K. A. (2014). A comprehensive study of green roof performance from environmental perspective. *International Journal of Sustainable Built Environment*, *3*(1), 127-134.
Li, X., Wang, L., Wang, S., Yang, Q., Zhou, Q., & Huang, X. (2018). A preliminary analysis of the effects of bisphenol A on the plant root growth via changes in endogenous plant hormones. *Ecotoxicology and environmental safety*, *150*, 152-158.
Li, Y., & Babcock, R. W. (2014). Green roofs against pollution and climate change. A review. *Agronomy for Sustainable Development*, *34*(4), 695-705.
Lintelmann, J., Katayama, A., Kurihara, N., Shore, L., & Wenzel, A. (2003). Endocrine disruptors in the environment (IUPAC Technical Report). *Pure and Applied Chemistry*, *75*(5), 631-681.
Locatelli, L., Mark, O., Mikkelsen, P. S., Arnbjerg-Nielsen, K., Jensen, M. B., & Binning, P. J. (2014). Modelling of green roof hydrological performance for urban drainage applications. *Journal of hydrology*, *519*, 3237-3248.
Lück, H. (1965). Peroxidase. In *Methods of enzymatic analysis* (pp. 895-897). Academic Press.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., ... & Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the total environment*, *473*, 619-641.

MacIvor, J. S., & Lundholm, J. (2011). Performance evaluation of native plants suited to extensive green roof conditions in a maritime climate. *Ecological Engineering*, *37*(3), 407-417.

Matsui, T., Nomura, Y., Takano, M., Imai, S., Nakayama, H., Miyasaka, H., ... & Kato, K. (2011). Molecular cloning and partial characterization of a peroxidase gene expressed in the roots of Portulaca oleracea cv., one potentially useful in the remediation of phenolic pollutants. *Bioscience, biotechnology, and biochemistry*, 1104042436-1104042436.

Matthews, J. F., & Levins, P. A. (1985). The genus Portulaca in the southeastern United States. *Castanea*, 96-104.

McNeill, J. (1974). Synopsis of a revised classification of the Portulacaceae. *Taxon*, *23*(5-6), 725-728.

MeterGroup. (2019). WP4C: Soil Water Potential in the Lab: METER Environment. Retrieved from https://www.,etergroup.com/environment/products/wp4c/

Meunier, B. (2003). 8.11 – Heme-peroxidases. In E. Constable, *Comprehensive coordination chemistry II:* from biology to nanotechnology (pp. 261-280). Newnes.

Nagase, A., & Dunnett, N. (2012). Amount of water runoff from different vegetation types on extensive green roofs: Effects of plant species, diversity and plant structure. *Landscape and urban planning*, *104*(3-4), 356-363.

Nobel, P. S. (1999). *Physicochemical & environmental plant physiology*. Academic Press, Inc., San Diego, CA. 635 pp.

Nyananyo, B. L. (1986). A new combination in Portulaca (Portulacaceae). *Taxon*, *35*(3).

Okuhata, H., Ninagawa, M., Takemoto, N., Ji, H., Miyasaka, H., Iwamoto, A., & Arizono, K. (2013). Phytoremediation of 4, 4′-thiodiphenol (TDP) and other bisphenol derivatives by Portulaca oleracea cv. *Journal of bioscience and bioengineering*, *115*(1), 55-57.

Padaki, N. V., Das, B., & Thirumalesh, R. M. (2015). Enzyme applications in silk processing. In *Advances in Silk Science and Technology* (pp. 111-120). Woodhead Publishing.

Pan, W. J., Xiong, C., Wu, Q. P., Liu, J. X., Liao, H. M., Chen, W., ... & Zheng, L. (2013). Effect of BPA on the germination, root development, seedling growth and leaf differentiation under different light conditions in Arabidopsis thaliana. *Chemosphere*, *93*(10), 2585-2592.

Paz-Alberto, A. M., & Sigua, G. C. (2013). Phytoremediation: a green technology to remove environmental pollutants.

Petrie, B., Lopardo, L., Proctor, K., Youdan, J., Barden, R., & Kasprzyk-Hordern, B. (2019). Assessment of bisphenol-A in the urban water cycle. *Science of the total environment*, *650*, 900-907.

Petrović, M., Gonzalez, S., & Barceló, D. (2003). Analysis and removal of emerging contaminants in wastewater and drinking water. *TrAC Trends in Analytical Chemistry*, *22*(10), 685-696.

Pilon-Smits, E. (2005). Phytoremediation. *Annu. Rev. Plant Biol.*, *56*, 15-39.

Qin, H. P., Li, Z. X., & Fu, G. (2013). The effects of low impact development on urban flooding under different rainfall characteristics. *Journal of environmental management*, *129*, 577-585.

Qiu, Z., Wang, L., & Zhou, Q. (2013). Effects of bisphenol A on growth, photosynthesis and chlorophyll fluorescence in above-ground organs of soybean seedlings. *Chemosphere*, *90*(3), 1274-1280.

Rashed, A. N., Afifi, F. U., & Disi, A. M. (2003). Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L.(growing in Jordan) in Mus musculus JVI-1. *Journal of ethnopharmacology*, *88*(2-3), 131-136.

Rawlins, S. L., & Campbell, G. S. (1986). Water potential: Thermocouple psychrometry. *Methods of Soil Analysis: Part 1 Physical and Mineralogical Methods*, *5*, 597-618.

Rubin, B. S., & Soto, A. M. (2009). Bisphenol A: perinatal exposure and body weight. *Molecular and cellular endocrinology*, *304*(1-2), 55-62.

Ruby, M., & Appleton, B. (2010). Using landscape plants for phytoremediation. In *Low Impact Development 2010: Redefining Water in the City* (pp. 323-332).

Saiyood, S., Vangnai, A. S., Thiravetyan, P., & Inthorn, D. (2010). Bisphenol A removal by the Dracaena plant and the role of plant-associating bacteria. *Journal of hazardous materials*, *178*(1-3), 777-785.

Salt, D. E., Smith, R. D., & Raskin, I. (1998). Phytoremediation. *Annual review of plant biology*, *49*(1), 643-668.

Savage, M. J., Cass, A., & de Jager, J. M. (1982). An accurate temperature correction model for thermocouple hygrometers. *Plant physiology*, *69*(2), 526-530.

Scruggs, C., Hunter, G., Snyder, E., Long, B., & Snyder, S. A. (2005). EDCs in wastewater: what's the next step?. *Water Environment and Technology*, *17*(3), 24-31.

Seachrist, D. D., Bonk, K. W., Ho, S. M., Prins, G. S., Soto, A. M., & Keri, R. A. (2016). A review of the carcinogenic potential of bisphenol A. *Reproductive Toxicology*, *59*, 167-182.

Shafique, M., Kim, R., & Rafiq, M. (2018). Green roof benefits, opportunities and challenges–A review. *Renewable and Sustainable Energy Reviews*, *90*, 757-773.

Shao, X. L., Ma, J., & Wen, G. (2008). Investigation of endocrine disrupting chemicals in a drinking water work located in Songhua River basin. *Huan jing ke xue= Huanjing kexue*, *29*(10), 2723-2728.

Sherman, T. D., Vaughn, K. C., & Duke, S. O. (1991). A limited survey of the phylogenetic distribution of polyphenol oxidase. *Phytochemistry*, *30*(8), 2499-2506.

Sonune, A., & Ghate, R. (2004). Developments in wastewater treatment methods. *Desalination*, *167*, 55-63.

Speak, A. F., Rothwell, J. J., Lindley, S. J., & Smith, C. L. (2014). Metal and nutrient dynamics on an aged intensive green roof. *Environmental pollution*, *184*, 33-43.

Staniszewska, M., Falkowska, L., Grabowski, P., Kwaśniak, J., Mudrak-Cegiołka, S., Reindl, A. R., ... & Zgrundo, A. (2014). Bisphenol A, 4-tert-octylphenol, and 4-nonylphenol in the Gulf of Gdańsk (Southern Baltic). *Archives of environmental contamination and toxicology*, *67*(3), 335-347.

Staples, C. A., Dome, P. B., Klecka, G. M., Oblock, S. T., & Harris, L. R. (1998). A review of the environmental fate, effects, and exposures of bisphenol A. *Chemosphere*, *36*(10), 2149-2173.

Steffens, J. C., Harel, E., & Hunt, M. D. (1994). Polyphenol oxidase. In *Genetic engineering of plant secondary metabolism* (pp. 275-312). Springer, Boston, MA.

Suchkova, N., Tsiripidis, I., Alifragkis, D., Ganoulis, J., Darakas, E., & Sawidis, T. (2014). Assessment of phytoremediation potential of native plants during the reclamation of an area affected by sewage sludge. *Ecological Engineering*, *69*, 160-169.

Sumner, J. B., & Dounce, A. L. (1937). Crystalline catalase. *Science*, *85*(2206), 366-367.

Sun, S., Bao, Z., Li, R., Sun, D., Geng, H., Huang, X., ... & Zhao, X. (2017). Reduction and prediction of N2O emission from an Anoxic/Oxic wastewater treatment plant upon DO control and model simulation. *Bioresource technology*, *244*, 800-809.

Syranidou, E., Christofilopoulos, S., Politi, M., Weyens, N., Venieri, D., Vangronsveld, J., & Kalogerakis, N. (2017b). Bisphenol-A removal by the halophyte Juncus acutus in a phytoremediation pilot: characterization and potential role of the endophytic community. *Journal of hazardous materials*, *323*, 350-358.

Tabei, K., & Sakakibara, Y. (2006). Removal of endocrine disrupting chemicals by phytoremediation. In *Paper M-17, Proceedings of 5th International Conference on Remediation of Chlorinated and Recalcitrant Compounds*. Battelle Press Monterey, CA.

Terouchi, N., Takano, K., Nakamura, Y., Enomoto, K., Hosoya, N., & Nishinari, N. (2004). Bisphenol A stimulates growth and shoot differentiation in plants. *plant Biotechnology*, *21*(4), 307-308.

Ting, I. P. (1985). Crassulacean acid metabolism. *Annual review of plant physiology*, *36*(1), 595-622.

Tiwari, K. K., Dwivedi, S., Mishra, S., Srivastava, S., Tripathi, R. D., Singh, N. K., & Chakraborty, S. (2008). Phytoremediation efficiency of Portulaca tuberosa rox and Portulaca oleracea L. naturally growing in an industrial effluent irrigated area in Vadodra, Gujrat, India. *Environmental monitoring and assessment*, *147*(1), 15-22.

Tsai, W. T., Lai, C. W., & Su, T. Y. (2006). Adsorption of bisphenol-A from aqueous solution onto minerals and carbon adsorbents. *Journal of hazardous materials*, *134*(1-3), 169-175.

Umar, M., Roddick, F., Fan, L., & Aziz, H. A. (2013). Application of ozone for the removal of bisphenol A from water and wastewater–a review. *Chemosphere*, *90*(8), 2197-2207.

United States Environmental Protection Agency. (2009). Green Roofs for Stormwater Runoff Control. *EnviroAtlas*. Retrieved: February, 20, 2020, from www.epa.gov/enviroatlas

VanWoert, N. D., Rowe, D. B., Andresen, J. A., Rugh, C. L., & Xiao, L. (2005). Watering regime and green roof substrate design affect Sedum plant growth. *HortScience*, *40*(3), 659-664.

Vigneswaran, C., Ananthasubramanian, M., & Kandhavadivu, P. (2014). 2-Industrial enzymes. *Bioprocessing of Textiles: Woodhead Publishing India*, 23-52.

Vijayaraghavan, K. (2016). Green roofs: A critical review on the role of components, benefits, limitations and trends. *Renewable and sustainable energy reviews*, *57*, 740-752.

Vijayaraghavan, K., Joshi, U. M., & Balasubramanian, R. (2012). A field study to evaluate runoff quality from green roofs. *Water research*, *46*(4), 1337-1345.

Vijayaraghavan, K., Reddy, D. H. K., & Yun, Y. S. (2019). Improving the quality of runoff from green roofs through synergistic biosorption and phytoremediation techniques: A review. *Sustainable Cities and Society*, *46*, 101381.

Wang, X., Tian, Y., Zhao, X., & Peng, C. (2017). Hydrological performance of dual-substrate-layer green roofs using porous inert substrates with high sorption capacities. *Water Science and Technology*, *75*(12), 2829-2840.

Watanabe, T., Yamamoto, H., Inoue, K., Yamaguchi, A., Yoshimura, Y., Kato, K., ... & Nakashima, K. (2001). Development of sensitive high-performance liquid chromatography with fluorescence detection using 4-(4, 5-diphenyl-1H-imidazol-2-yl) benzoylchloride as a labeling reagent for determination of bisphenol A in plasma samples. *Journal of chromatography B: Biomedical sciences and applications*, *762*(1), 1-7.

Wiltse, C. C., Rooney, W. L., Chen, Z., Schwab, A. P., & Banks, M. K. (1998). Greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes. *Journal of Environmental Quality*, *27*(1), 169-173.

Whitaker, J. R. (1995). Polyphenol oxidase. In *Food Enzymes* (pp. 271-307). Springer, Boston, MA.

Winter, K., & Smith, J. A. C. (1996). Crassulacean acid metabolism: current status and perspectives. In *Crassulacean acid metabolism* (pp. 389-426). Springer, Berlin, Heidelberg.

Wolf, D., & Lundholm, J. T. (2008). Water uptake in green roof microcosms: effects of plant species and water availability. *Ecological Engineering*, *33*(2), 179-186.

Xiang, L., Xing, D., Wang, W., Wang, R., Ding, Y., & Du, L. (2005). Alkaloids from Portulaca oleracea L. *Phytochemistry*, *66*(21), 2595-2601.

Yamamoto, T., Yasuhara, A., Shiraishi, H., & Nakasugi, O. (2001). Bisphenol A in hazardous waste landfill leachates. *Chemosphere*, *42*(4), 415-418.

Yamane, H., Konno, K., Sabelis, M., Takabayashi, J., Sassa, T., & Oikawa, H. (2010). Chemical defence and toxins of plants.

Yang, S., Hai, F. I., Nghiem, L. D., Nguyen, L. N., Roddick, F., & Price, W. E. (2013). Removal of bisphenol A and diclofenac by a novel fungal membrane bioreactor operated under non-sterile conditions. *International Biodeterioration & Biodegradation*, *85*, 483-490.

Zafra, A., del Olmo, M., Suárez, B., Hontoria, E., Navalón, A., & Vílchez, J. L. (2003). Gas chromatographic–mass spectrometric method for the determination of bisphenol A and its chlorinated derivatives in urban wastewater. *Water research*, *37*(4), 735-742.

Zhang, X. J., Ji, Y. B., Qu, Z., Xia, J. C., & Wang, L. (2002). Experimental studies on antibiotic functions of Portulaca oleracea L. in vitro. *Chinese J Microecol*, *14*(5), 277-280.

Zhou, H. D., Huang, X., Wang, X. L., & Wen, X. H. (2009). Evaluation of estrogenicity of sewage samples from Beijing, China. *Huan jing ke xue= Huanjing kexue*, *30*(12), 3590-3595.

Zielińska, M., Bułkowska, K., Cydzik-Kwiatkowska, A., Bernat, K., & Wojnowska-Baryła, I. (2016). Removal of bisphenol A (BPA) from biologically treated wastewater by microfiltration and nanofiltration. *International Journal of Environmental Science and Technology*, *13*(9), 2239-2248.