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Vegetation based assessment and monitoring tools for landfill leachate treatment and fugitive plumes

Rahul Sukharia

VEGETATION BASED ASSESSMENT AND MONITORING TOOLS FOR

LANDFILL LEACHATE TREATMENT AND FUGITIVE PLUMES

by

RAHUL SUKHARIA

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Approved by

Joel G. Burken, Advisor

Mark W. Fitch

Joe Guggenberger

David Westenberg

ABSTRACT

Solid waste and leachate generation from solid waste landfills has a legacy of detrimental and toxic impacts on the environment. Disposal practices are expensive, failure prone and have not been able to keep up with the pace of disposal of toxic compounds. In general, a landfill acts as a "bathtub" with infiltration of water through the landfill cover into the landfill, reacting with the waste and transferring toxic components into the leachate. Irrigating the evapotranspiration (ET) covers with leachate collected from the landfill has been developed and applied. Such methods can keep the leached pollutants in a loop, which reduces the risk of leachate contamination of nearby aquifers. Utilizing trees and grasses on ET covers as a means of phytoremediation and stabilization of pollutants, while controlling erosion, is a step towards an efficient and sustainable remediation of landfill systems. Assessment of plant health and stress is critical for optimizing these systems and to avoid mortality of plants and total failure of phytotechnologies and phytoremediation systems. Leachate application rates should provide better treatment efficiency, but not cause toxicity.

Hyperspectral measurements for monitoring plant health and stress were included in this study. Hyperspectral results revealed that plant stress can be sensed remotely, which correlates with destructive testing methods such as biomass measurements. This study provides multiple findings of importance in assessing plant stress while maintaining effective treatment, with low labor costs and the ability to cover large areas rapidly. This study also suggests that remote sensing can be applied to detect plant stress caused by fugitive leachate plumes, thereby mitigating the potential threat to human health and ecological damages from these plumes that would often go unnoticed.

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1. INTRODUCTION

1.1. BACKGROUND

Landfills evolved to mitigate the multitude of problems of solid waste management and disposal, but the evolution has resulted in great expense. Solid waste management initially dealt with food refuse and other aspects of initial urban development that caused public health impacts from vectors of disease, such as rats and the plague. Slowly they evolved from disposal areas away from population centers to the current, complex, and expensive landfill design. Currently, landfills are struggling with an ever-changing waste stream and are posing a long-term waste liability. The modern lifestyle calls for commercial and industrial development in countries around the globe, which results in increased generation and diversity of municipal and industrial waste products. The waste stream includes a wide array of chemical products, electronics with higher metals content, and increasing pharmaceuticals and personal care products. Solid waste generation in the United States of America has increased around three times over the past 50 years (USEPA, 2016) as shown in Figure 1.1. One of the most common solid waste disposal alternatives for many countries is placement in sanitary landfills.

Landfilling also offers decomposition of the waste under controlled conditions, until the waste transmutes into a stabilized and fairly inert matrix (Renou et al., 2008). However, landfills generate tremendous gas volumes and produce a leachate containing inorganic, organic and xenobiotic compounds. A key issue with landfills is leachate production can occur for decades after being capped and can be of concern for environmental and public safety if released unrestrained. Many pre-RCRA landfills were closed with no liners and the leachate produced is not collected or mitigated. Many such landfills have generated considerable plumes that have gone undetected for decades (National Research Council, 2007; Burken, 2015). Even in current landfill design, the collection, storage, and treatment systems can undergo failures and resulting fugitive leachate projects a threat to surface and groundwater contamination.

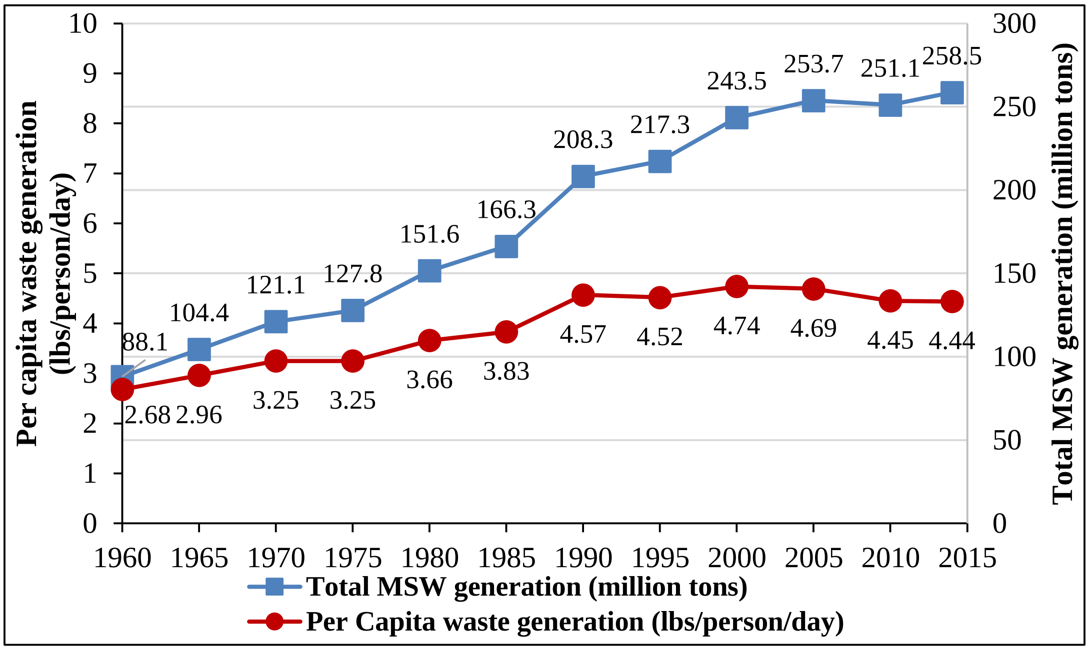


Figure 1.1: Trend of solid waste generation in the USA over past 50 years (adapted from Advancing Sustainable Materials Management: Facts and Figures Report, 2016)

1.2. COMPOSITION OF LANDFILL LEACHATE

The aqueous effluent resulting from the intrinsic moisture content of the waste, rainwater percolating through the waste, and the biochemical reactions occurring within the landfill is referred to as landfill leachate. Leachate composition varies from site to site but can also fluctuate in a single site over time (Steiner et al., 1979). Moreover, the composition of the leachate is also governed by the nature of the waste and the biological, chemical, and physical reactions occurring in the landfill.

Nature of waste has changed over time with society. The leachate may contain organic compounds, inorganic salts, metals, and pathogens. Depending on what is dumped in the landfill, the leachate may contain a complex mixture of organic pollutants, heavy metals, salinity, ammonia, chemical oxygen demand (COD), biochemical oxygen demand (BOD), etc. Heavy metals many times remain relatively insoluble at the higher pH typical of many leachate plumes. Moreover, reducing conditions prevail inside the landfills and these conditions may change the ionic states and increase the solubility of metals like arsenic and chromium (Halim et al., 2004). Once the waste is dumped in the landfill and capped, a series of stages occur. Initially, the rapid utilization of confined oxygen and water results in acetogenic fermentation and a leachate is generated with high BOD, COD, and NH_3-N.

Landfill leachate can be categorized into four primary pollutant groups (Christenson et al., 2001):

a) Heavy metals in leachate- Cadmium (Cd), Copper (Cu), Zinc (Zn), Lead (Pb), etc.
b) Organic matter (dissolved) in leachate- total organic carbon, fatty acids (Christenson et al., 1989), humic and fulvic compounds.
c) Xenobiotic organic compounds in leachate- phenols, chlorinated aliphatic compounds, aromatic hydrocarbons, and pesticides.
d) Inorganic compounds- Sodium, potassium, calcium, magnesium, ammonium, iron, sulfate, manganese, and chlorides (Kjeldsen et al., 2002).

1.2.1. Heavy Metals in Leachate. Heavy metals like copper, zinc, cadmium, arsenic and lead are naturally present in the earth’s crust but usually are widely distributed in the environment due to their anthropogenic use in domestic and industrial applications (Tchounwou et al., 2012). A wide range of heavy metals found in leachate is shown in Table 1.1. These heavy metals are posing a threat to human health and environment due to their toxic properties. Toxicity of such heavy metals is reliant on the gender, age, chemical species, route of exposure, and dosage (Tchounwou et al., 2012). When in soil, heavy metals tend to stay bound to the soil components or are present as precipitates and therefore not readily bio-available to the plants (Raskin et al., 1994). Bioavailability of the heavy metals is dependent on physical (temperature and adsorption) and chemical (kinetics, solubility, partitioning coefficients, equilibrium, pH etc.) factors (Hamelink et al., 1994). Despite the toxic properties possessed by heavy metals, in landfills, heavy metals in the leachate is usually not of a concern because of their relatively low concentrations (Kjeldsen et al., 2001).

Table 1.1: Range of heavy metals in landfill leachate (Kjeldsen et al., 2002)

Compounds	**Range (mg/L)**
Arsenic	0.01 to 1
Cadmium	0.0001 to 0.4
Cobalt	0.005 to 1.5
Lead	0.001 to 5
Chromium	0.02 to 1.5
Copper	0.005 to 10
Mercury	0.00005 to 0.16
Zinc	0.03 to 1000
Nickel	0.015 to 13

1.2.2. Organic (Dissolved) Compounds in Leachate. Organic compounds are ubiquitously present in leachate in varying concentrations. The origin of such compounds includes natural, commercial or industrial sources. Various degradation products such as volatile fulvic and humic compounds are present in the leachate as dissolved organic compounds. Organic compounds in leachate can contaminate the soil and later enter the food chain, eventually causing a potential threat to human health and the environment (Chian et al., 1977). Leachate seepage into groundwater carrying organic compounds can deteriorate aquatic life. Table 1.2 shows a range of dissolved organic compounds in the leachate. Many landfills either pump or transport leachate to wastewater treatment plants. High COD and BOD in the leachate can create a treatment burden on the treatment plants. However, recirculating landfill leachate has demonstrated a decrease in COD and BOD concentrations (Chugh et al., 1998).

Table 1.2: Range of organic matter in leachate (Kjeldsen et al., 2002)

Constituent	**Range (mg/l)**
Total Organic Carbon (TOC)	30 to 29000
Chemical Oxygen Demand (COD)	140 to 152000
Biological Oxygen Demand (BOD)	20 to 57000
BOD_5: COD	1: 4

Higher levels of organic matter in leachate typically result in higher BOD levels that can directly impact groundwater and surface waters and well as have indirect impacts on redox potential and other aspects related to biogeochemistry (Lee et al., 2014; Abd El-Salam et al., 2015). When leachate seepages with higher BOD levels meet surface and groundwater aquifers, it considerably depletes the dissolved oxygen levels of the water body, which may eventually make the aquifer anoxic.

1.2.3. Xenobiotic Compounds in Leachate. Xenobiotic compounds are known to exhibit beneficial and harmful effects. Xenobiotic compounds like phenols, phthalates, pesticides and other aliphatic and aromatic compounds (BTEX and chlorinated hydrocarbons) can be found in MSW landfills (Paxeus, 2002). Mostly, previous studies were focused on xenobiotics such as BTEX, PAHs, halogenated hydrocarbons (Öman et al., 1998; Christensen et al., 2001). These compounds usually degrade and volatilize over time and thus the concentrations in the leachate decrease gradually. Some of the xenobiotic compounds found in leachate are enlisted in Table 1.3.

Table 1.3: Levels of xenobiotic organic compounds detected in landfill leachate (Kjeldsen et al., 2002)

Compounds	**Typical Range (µg/L)**
Benzene	0.2 to 1,630
Toluene	1 to 12,300
Ethylbenzene	0.2 to 2,329
Xylenes	0.8 to 3,500
Trimethylbenzene	0.3 to 250
Naphthalene	0.1 to 260

Many such xenobiotic compounds are enlisted as priority pollutants in Code of Federal Regulations (40 CFR Appendix-A). Pesticides, therapeutic drugs, PCBs, PAHs are known to be harmful to humans, aquatic wildlife, and environment (Dickerson et al., 1994; Luster et al., 1993). Pesticides like phenoxy acids are recalcitrant and are potentially a hazard for human health and environment (Buss et al., 2006). Petroleum derivatives (BTEX) are known to be degraded by microbes when available as a sole source of carbon (Weelink et al., 2010). Benzene of all petroleum hydrocarbons is most recalcitrant (Bjerg et al., 2011). Therefore, appropriate containment of landfill leachate is of prime importance else xenobiotics and other organic compounds could create hazardous conditions for human health and the environment.

1.2.4. Other Inorganic Compounds in Leachate. Landfill leachate often possesses significant levels of inorganic compounds including various cations and anions, as shown in Table 1.4. In lower concentrations, these compounds may undergo ion-exchange, precipitations and redox reactions. At higher concentrations, these ions can form various complexes and subsequently enhance solubility and mobility (Christensen et al., 2001). Seepage of leachate carrying inorganic constituents can contaminate nearby aquifers (Gobler et al., 2003). The presence of nitrogen, phosphorus, and potassium in aquifers can result in algal blooms, which causes depletion of dissolved oxygen in the aquifers (Elser, 2012). Higher nutrient loading into the aquifer causes eutrophication and creates a potential risk to human health and environment (Smith et al., 1999). Moreover, ammoniacal nitrogen in a landfill is due to decomposing protein molecules. Leachate concentrations do not show a significant decrease in ammonia over time (Kruempelbeck et al., 1999). Most of the inorganic compounds present in landfill leachate are utilized by plants as nutrients (Li et al., 2003). Utilizing these nutrients by recirculating leachate back on landfill covers for fertigation of plants can reduce the concentrations significantly.

Table 1.4: Range of inorganic compounds in leachate (Kjeldsen et al., 2002)

Compounds	**Range (mg/L)**
Chloride	15 to 4500
Phosphorus	0.1 to 23
Sulfate	8 to 7750
Calcium	10 to 7200
Magnesium	30 to 15000
Sodium	70 to 7200
Potassium	50 to 3700
Iron	3 to 5500
Manganese	0.03 to 1400
Ammoniacal Nitrogen	50 to 2200

1.3. SUMMARY OF TRADITIONAL LEACHATE TREATMENT

Some of the conventional strategies for treatment of landfill leachate are briefly described as following.

1.3.1. Treatment Along With Wastewater. Customarily, pumping and haulage of landfill leachate to off-site wastewater treatment plants was considered for treatment of leachate (Jones, 2015; Ahn et al., 2002). Wastewater treatment of landfill leachate can be done by biological and/or physicochemical processes. However, transportation of landfill leachate for treatment is often expensive and a debate is persistent in the literature about leachate containing inhibitory compounds such as heavy metals and organic pollutants and that could adversely affect the efficiency of the treatment process, resulting in decreased treatment efficiency and increased effluent concentrations of many wastewater effluent constituents (Cecen et al., 2004).

In order to avoid off-site treatment of leachate, the option of *in situ* treatment strategies can be considered. Nonetheless, on-site treatment of landfill leachate also has its disadvantages such as requirements of capital cost for establishment and maintenance of treatment plant, additional space for construction of new treatment plant, electricity, chemicals (coagulants), sludge disposal and effluent discharge liabilities, valid permits for operating in compliance with environmental authorities such as USEPA.

1.3.1.1 Biological treatment. Biological treatment can be either aerobic or anaerobic. In a typical biological wastewater, treatment microbes undergo degradation of organic compounds to CO_2 and sludge in the presence of oxygen and to biogas in anaerobic conditions (Lema et al., 1988). Moreover, biological treatment exploits biodegradation for its reliability and high cost effectiveness. Nevertheless, aerobic biological wastewater treatment requires considerable infrastructure investment on site, energy intensive pumps, equipment for aeration and temperature control, and trained personnel for operation and maintenance. Anaerobic processes generally require longer retention times and are also not reliable for unmanned systems. Therefore, making biological treatment an expensive and inconvenient choice of treatment of landfill leachate.

1.3.1.2 Physicochemical treatment. Physicochemical treatment processes include coagulation, flocculation, floatation, chemical oxidation, and adsorption (Kurniawan et al., 2006). Physicochemical treatment technology can remove suspended solids, colloids, metal ions, and color. Suspended solids in leachate undergo coagulation, followed by flocculation processes to settle the colloidal particles to form sludge (Shammas, 2005; Semerjian et al., 2003). Metals present in the leachate such as cadmium, manganese, and zinc are usually precipitated by using lime (Wang et al., 2005). Typically, physicochemical treatment is coupled with biological treatment for a complete treatment.

High operational cost and high requirements of chemicals for physicochemical treatment are some of the major drawbacks of physicochemical treatment of landfill leachate (Kurniawan et al., 2006). Moreover, a large amount of sludge is produced and sludge disposal creates an environmental threat in long term and is not inexpensive (Kurniawan et al., 2006).

1.3.2. Inference from Traditional Treatments. The existing literature suggests that the idea of traditional leachate treatment could be inconvenient, expensive and pose a threat to the environment and human health. Currently, discharge standards are becoming stringent and a greater number of aged landfills are in need of leachate treatment. Wastewater treatment plant owners are becoming reluctant of receiving landfill leachate for off-site treatment. Therefore, development of new and innovative technologies is required, which creates a possibility of using landfill sites not only for waste disposal but also for the treatment of landfill leachate.

Landfill covers can be used as a treatment component of the landfill site. Compared to traditional leachate treatment technologies, using plants for treatment is much cheaper due to relatively less external power requirements. Functional capabilities of plants for landfill applications are shown in Figure 1.2.

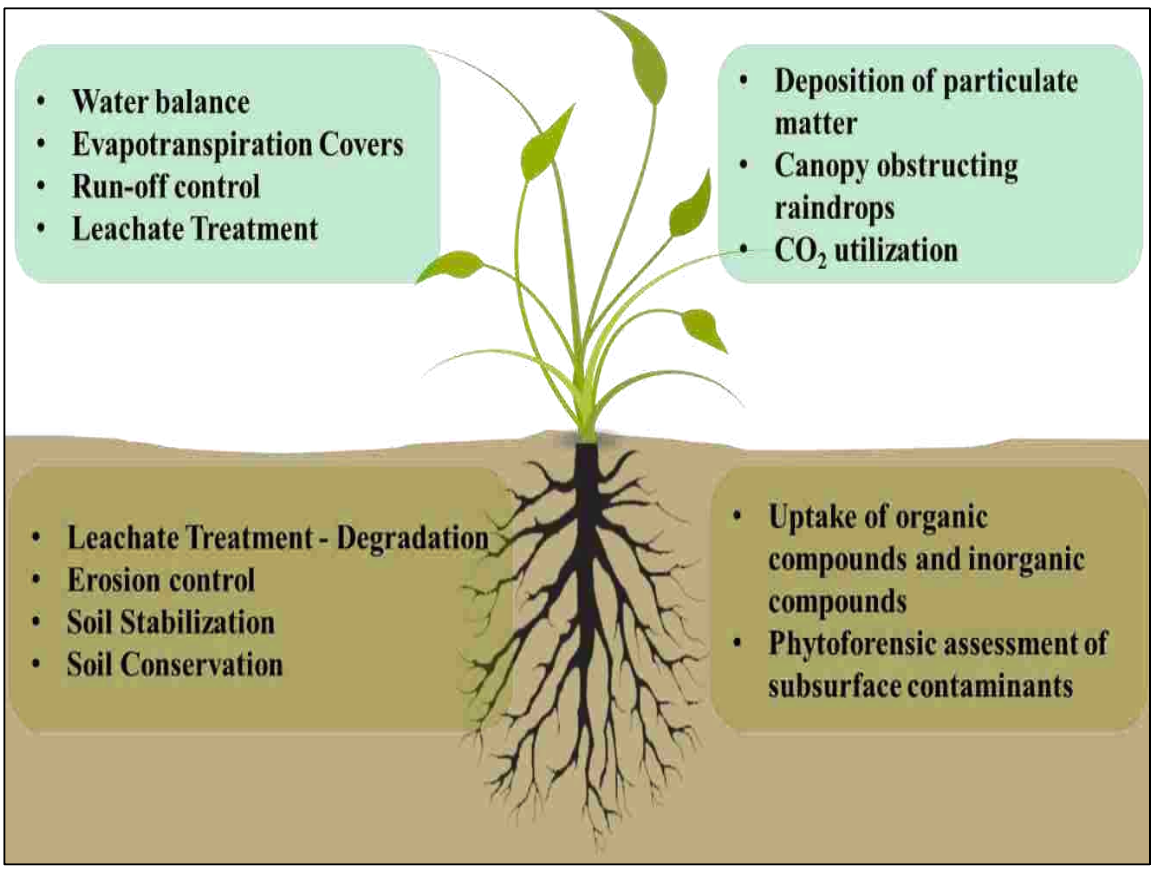


Figure 1.2: Several functional capabilities of vegetation for landfill applications (adapted from Burken et al., 2011)

Using the treatment potential of vegetation present on landfill covers can prevent risks and expenses associated with leachate transportation and traditional treatment. Moreover, plants use solar energy and water for growth, with low maintenance and low complexity. Aesthetically pleasant and functionally effective vegetation on landfill covers is often broadly accepted by the public.

1.4. LANDFILL COVERS

Novel approaches to improve waste disposal are needed with the ever-growing generation of solid waste around the world, including mining wastes, municipal wastes or industrial wastes (Hauser et al., 2004). These increasing waste volumes also have an increasing complexity and toxicity and have great potential to contaminate the environment. Therefore, improved methods of sequestering and managing these wastes are needed, including a need to contain these wastes into landfills and procedures to cover those landfills, is one target area for improving waste disposal and treatment approaches. The landfill covers serve three main purposes (Innovative Technology Summary Report- 2000; Hauser et al., 2004):

1. Waste isolation: These covers isolate the wastes from the surroundings and mitigate transport vectors. Controlling the movement of wastes by wind or water and potential attraction of biological vectors, such as rodents and birds, are necessary.
2. Control of landfill gases: The landfill covers are needed to control transport and release of toxic or explosive gases in the landfill, thereby preventing a fire hazard.
3. Minimization of infiltration: The covers also helps to manage and reduce the

infiltration rate of precipitation into the wastes contained in the landfill. Therefore, leachate formation is limited and management of leachate volumes is decreased.

Nevertheless, by keeping the waste isolated and dry, the waste stabilization period gets extended to several decades (Ham, 1993), thereby preserving the risk of contamination for future generations. The concept of “dry tomb” explains how encapsulated dry landfills can involve extended maintenance and monitoring periods after landfill closure (Lee and Jones, 1996). The increase in stabilization periods of landfills also creates several operational, developmental and economic obstacles. Slow waste stabilization would require more post-closure maintenance and monitoring time than the USEPA specified 30 years’ period (Lee and Jones, 1996). Aerobic and anaerobic microorganisms present in the landfill require moisture to decompose the waste. Balanced moisture content is an essential factor, which enhances waste decomposition (Manzur et al., 2016). Less moisture may decrease microbial activity, whereas excessive moisture content could lead to anaerobic conditions in landfills.

Several problems are associated with the traditional landfill covers, which are used nationwide. Landfill covers are expensive and difficult to construct. Landfill covers are also quite susceptible to failures (Lee and Jones, 1996), particularly in the arid and semi-arid regions. Landfill design failures can occur due to several reasons such as cracks in clay layers and HDPE liners, clogging of leachate collection system, soil erosion, and landfill slope failures. Landfill design failure can lead to seepage of leachate into the underlying and surrounding aquifers. However, landfill hazards can be mitigated by using a well-thought landfill cover design, which considers local environmental conditions and ensures dependability and functionality (Innovative Technology Summary Report- 2000).

1.4.1. Conventional Landfill Covers. Conventional landfill covers are typically more permeable than the base liner system. The purpose of conventional landfill covers is to control percolation of water into the landfill, reduce erosion, prevent exposure to the waste in the landfill, check gas emissions, and provide aesthetic value. Typical layers present in a conventional landfill cover are illustrated in Figure 1.3.

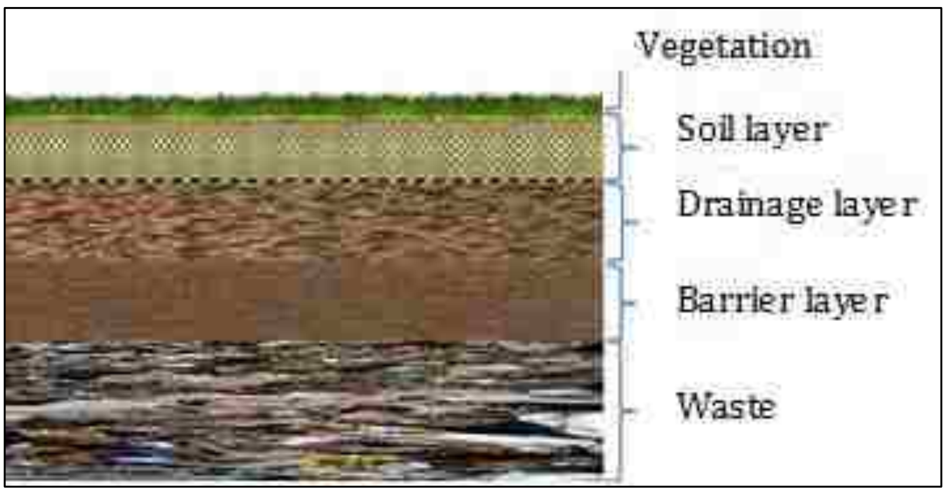


Figure 1.3: Conventionally used vegetation cover for landfills

Conventional landfill covers are designed to reduce percolation by incorporating low permeability barriers such as clay and geomembrane layers (Rock et al., 2012). Soil barriers need more compactive effort to reach the required density and therefore the cost of constructing the barrier rises. Still, multiple failure mechanisms can cause many conventional landfill covers to fail. Clay barrier layers in landfill covers are prone to cracks (Innovative Technology Summary Report, 2000; Bass et al., 1985; Melchoir et al., 1997; Albright et al., 2006). Clay layers have been known to become permeable when reacting with organic and inorganic compounds (Alther, 1987). Failures in clay layers also occur due to moisture deficient conditions (Holzlohner and Ziegler, 1995) in landfills. Furthermore, synthetic polymer (HDPE, PVC, etc.) liners are susceptible to embrittlement when in prolonged contact with leachate containing organic compounds (Surmann et al., 1995). Occurrences of cracking because of temperature changes in the landfills (Thomas et al., 1995) and stress (Rollin et al., 1991) are some other mechanisms of failure of synthetic liners. The inclusion of several layers to contain the waste in a landfill makes conventional covers an expensive option. As mentioned earlier, several design components of conventional landfill covers are flawed and may cause leakage issues over time. Overall, conventional covers are relatively expensive to build (Dwyer, 1998; Hauser et al., 2001; Abhichou et al., 2012), maintain and may need to be replaced in the future. Self-renewing evapotranspiration (ET) covers can solve many of the above-mentioned drawbacks associated with convention landfill covers. A typical schematic of layers in landfill design and how failures in these layers can contaminate the groundwater table is shown in Figure 1.4.

1.4.2. Evapotranspiration Covers. Many conventional covers are commonly used irrespective of regional environmental conditions and ultimately fail. ET cover, unlike conventional landfill cover, does not require a barrier layer. ET covers utilize water balance approach to limit percolation. ET covers involve soil properties such as porosity, water holding capacity, soil texture, and organic matter content, until the water is transpired by the vegetation and evaporated from the soil surface in the ET cover.

ET covers could be either monolithic ET covers or capillary barrier ET covers. The difference between the two is the addition of a coarse-grained material (i.e. sand or gravel) under the monolithic fine-grained layer to form a capillary barrier as shown in Figure 1.5 and Figure 1.6. Water is held in fine-grained layer by capillary forces, in unsaturated conditions. Water moves through the coarse-grained layer into the waste, when saturation occurs in the fine-grained layer.

Moreover, ET covers are self-repairing i.e. presence of vegetation controls soil erosion and unstratified soil fills up the gaps created by seismic activities and settlement of waste (Kulakow et al., 2010). ET covers are estimated to be more economical than conventional landfill covers (Hauser et al., 2001).

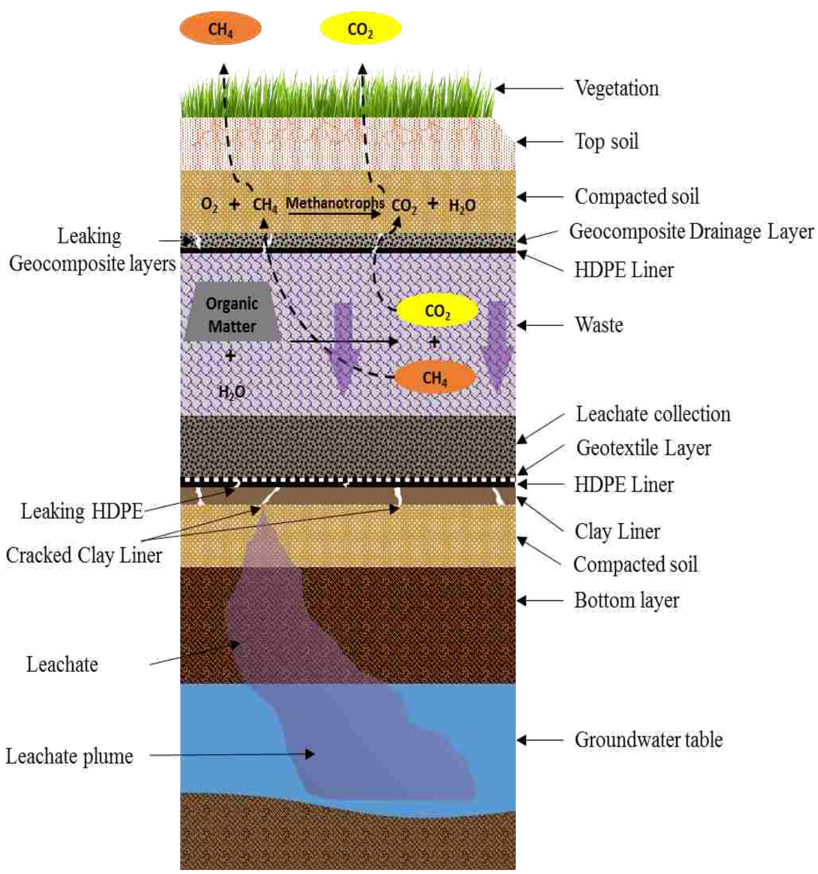


Figure 1.4: Landfill layers (on the right) and associated failures (on the left) in a conventional vegetation cover design (adapted from www.eugris.info and www.randrcontainersmarietta.com)

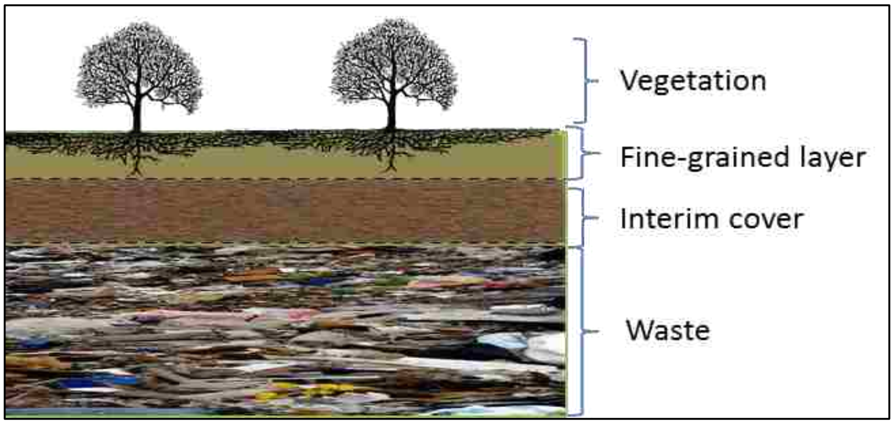


Figure 1.5: Monolithic evapotranspiration cover (adapted from EPA fact sheet, 2011)

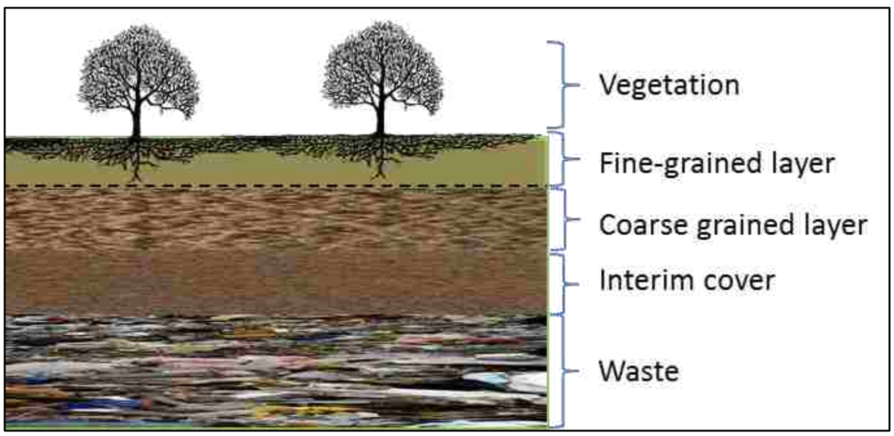


Figure 1.6: Capillary barrier evapotranspiration cover (adapted from EPA fact sheet, 2011)

The ET cover design consists of the following requirements:

i. The vegetation should be stable over a long period and can undergo evapotranspiration.

ii. Local soil should preferably be used to estimate future performance from natural equivalent data.

iii. The soil layer must be fine-grained (i.e. clay or silt).

ET cover design can also be customized to satisfy landfill requirements. The absence of a barrier layer in the design of ET covers creates an option of installation of a gas collection system during or after construction. ET covers are naturally self-renewing relative to typical RCRA Subtitles C liners, and thus have longer service periods with lower failure and maintenance. Although several advantages exist for ET covers, these covers are highly site-specific due to regional weather, soil, and plant types (USEPA, 2003).

1.4.3. Leachate Recirculation. Leachate plumes from leaking landfills can be thousands of meters long. Leachate carrying high concentrations of organic and inorganic pollutants can contaminate groundwater and act as a threat to human health and the environment (Christensen et al., 2001). The concentration, toxicity, and mobility of a plume are naturally countered by a passive remediation mechanism called natural attenuation. Natural attenuation is a gradual process where the pollutants in a plume undergo dispersion, sorption, volatilization, and degradation (USEPA, 1999).

The major challenge with natural attenuation is that high toxicity of pollutants can reduce the rate of degradation, thus making the attenuation process longer. However, several organic compounds attenuate near the origin of the plume, where methanogenic conditions dominate. The presence of microorganisms in and around the plume are known to reduce the plume size over relatively longer periods. Natural attenuation and phytoremediation can be combined to stabilize the leachate near the rhizosphere with improved attenuation and degradation of leachate pollutants.

ET covers can be coupled with recirculation of leachate to provide irrigation to plants, as shown in Figure 1.7. Recirculating the leachate back through the landfill cover is an innovative way to reduce the leachate burden on existing leachate treatment systems or transport. In addition, recirculating the leachate is also an economic way to manage appropriate moisture inside the landfill distributing the microorganisms and nutrients around the waste (Bae et al., 1998). Provided the recycling rate is controlled, the stabilization time could greatly decrease (San and Onay, 2001). Leachate recirculation can create a nutrient loop and provide treatment and stabilization of leachate. Leachate recirculation has also shown higher yields in methane production and better stabilization of waste for further degradation (Mali et al., 2012). Furthermore, leachate recirculation can accelerate the degradation of the waste and enhance waste stabilization over time (Berthe et al., 2005). Prior findings infer that recirculating leachate back on the landfill in a controlled way can provide many benefits such as avoiding off-site leachate treatment expenses and reduction in waste stabilization periods by making a landfill also a treatment site.

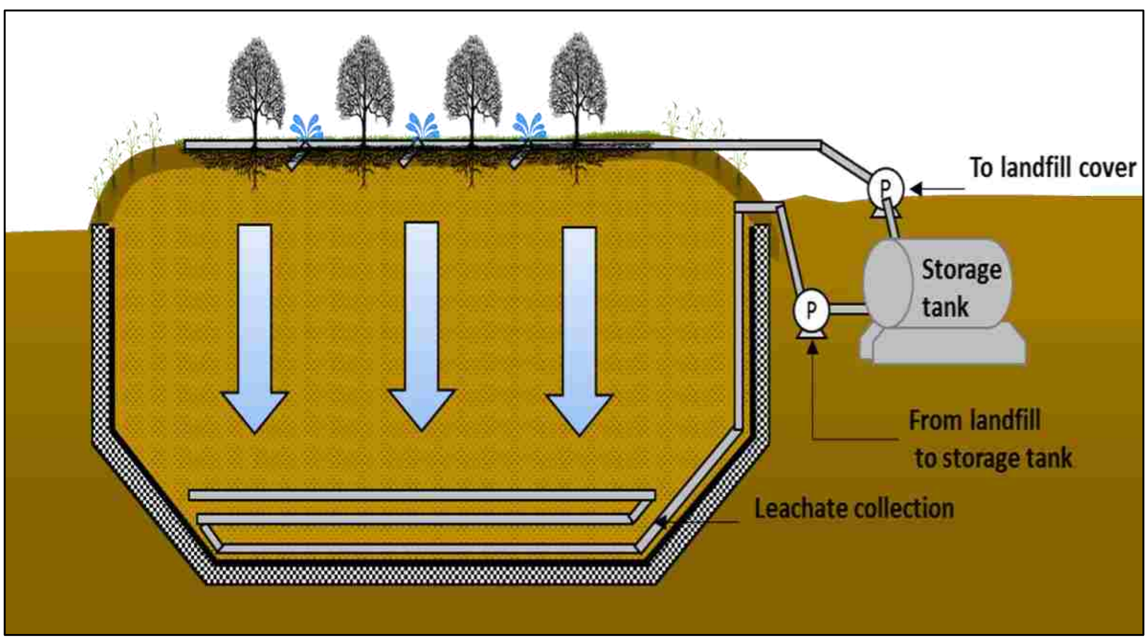


Figure 1.7: Recirculation of landfill leachate on ET cover

1.5. SCOPE OF PHYTOREMEDIATION OF LANDFILL LEACHATE

In the past, contaminated sites in the U.S. such as abandoned mines, dumps, landfills, etc., were a notable environmental liability with no beneficial value. Many sites are now being reclaimed for a range of sustainable and recreational uses such as golf courses, retail buildings, parks, etc. (Jain et al., 2013; Nelson, 1995). Increased demand for land for urban development creates a need for efficient post-closure uses of landfills. Several cleanup technologies are deployed on these sites in order to remediate the pollutants from the matrix. One such cleanup technology for a variety of pollutants is phytoremediation, which provides concurrent remediation of pollutants along with ecological and social value associated with ecosystem services (Holzman, 2012).

Phytoremediation is the utilization of plants to reduce, remove, or restrain environmental pollutants in a media through naturally occurring chemical, biological, and/or physical processes and phenomena in and around the plants. Plants are remarkable organisms, which have developed significant metabolic and pollutant sequestration capabilities. Plants possess transport mechanisms that can remove some pollutants from the growth matrix (soil or water). Pollutant fate in plants is a critical aspect of food safety (Mench et al., 2009). Plant survival is another primary concern for these living systems (Glick, 2003).

Depending on the nature of contaminant and applicability, many processes are possible in phytoremediation. Plants can stabilize, contain, and destroy organic pollutants using various processes such as phytoextraction, phytostabilization, phytodegradation, rhizofiltration, rhizodegradation, or phytovolatilization (USEPA, 1999). Accumulation of contaminants in the harvestable biomass (wood and leaves) is referred to as phytoextraction or phytoaccumulation (Kumar et al., 1995). Phytostabilization limits the movement of the contaminants (stabilizes) near the root system (Vangronsveld et al., 1995). Phytodegradation of xenobiotic compounds is carried out by various enzymes inside plant cells (Burken and Schnoor, 1997). Contaminants stored by plants in their tissues from water bodies is known as phytofiltration (Dushenkov et al., 1995). Phytovolatilization is the transformation of contaminants to a volatile state, which is released in the atmosphere (Burken and Schnoor, 1999). Therefore, using plants for phytoremediation is a cost-effective application of numerous metabolic processes to remove contaminants from media (soil or water). Exploiting the above-mentioned phytoremediation processes for landfill remediation can effectively reduce the threat to human health and environment.

1.6. PLANTS AND LANDFILL LEACHATE

Irrigation of untreated or partially treated leachate on vegetated land is not only a promising remediation option but also creates a closed loop for nutrients while producing effluent with suitable quality (Haarstad and Maehlum, 1999). In essence, a phytoremediation system incorporates a combination of under the ground and over the ground processes. Foliar uptake of volatile organics and soluble nutrients, transpiration, and evaporation occur above the ground surface. Underground processes such as water uptake from the soil drives the leachate on the surface to move towards the root system of the plants. Water uptake by the roots reduces: the quantity and downward movement of the leachate; uptake of nutrient and organics by the roots; sequestration and transport of metals; utilization and degradation of organics; and rhizodegradation (Jones et al., 2006). Moreover, fixation, sorption, complexation, and precipitation mechanisms occur in the soil matrix. The inherent composition of the soil influences these processes extensively and enhancement of the soil structure could increase the efficacy of phytoremediation (Jones et al., 2006).

1.7. PLANT USE ON LANDFILLS

Plant-soil systems are dominated by processes involving microbial degradation of organic compounds (Glick, 2010; Lin et al., 2008). The primary function of plants involved in ET cover is to maintain hydrologic balance and drawing water from the underlying soil, thus preventing infiltration of water into the waste. Also, plants control soil erosion (Watson et al., 1999; Phillips et al., 1993), stabilize shallow landslides (Marden and Rowan, 2015) and provide aesthetic value to the landfill site. Similar to numerous *in-situ* phytoremediation scenarios, migration of contaminants is controlled by plants by drawing water. Therefore, plants on a landfill can attenuate the infiltration of water and stabilize subsurface contaminants present in the soil layers of landfills.

Microorganisms are present ubiquitously in solid waste treatment and soils, even in the leachate. Microbial communities present in leachate is dependent on the age of leachate (Senior, 1995). As shown in Figure 1.8, plant roots can release certain nutrients on which the bacterial communities thrive and therefore increase biological activity in the media (Schnoor, 2002). The presence of roots affects nutrient, water status, and microbial activity in the surrounding soil (Smit et al., 2000; Atkinson et al., 2000). Root structure will also be affected by the presence of contaminants in the media. Roots act as a sensory organ and growth of the root tips exploring through the soil matrix is dependent on numerous environmental signals such as light, gravity, nutrients and contaminants (Balasubramaniyam, 2012).

Several functions of roots are listed as following:

a. Production of root exudates and carbonaceous matter to attract microbial populations and providing a habitat for beneficial microbes (Cheng et al., 2009; Marchand et al., 2010),

b. Alteration in soil properties by changing the pH of the soil and adding organic matter to the soil,

c. Providing a sensory network of roots for regulating plant growth in congruence with plant hormones (Wu et al., 2007) and sugar-like substances (Bolouri-Moghaddam et al., 2010),

d. Utilizing resources available in the soil such as water and nutrients for plant growth, while undergoing hydraulic processes to reach these resources, and

e. Binding to the soil and providing mechanical support to the plant structure while improving the soil quality by the addition of organic matter and stabilizing the soil (preventing erosion).

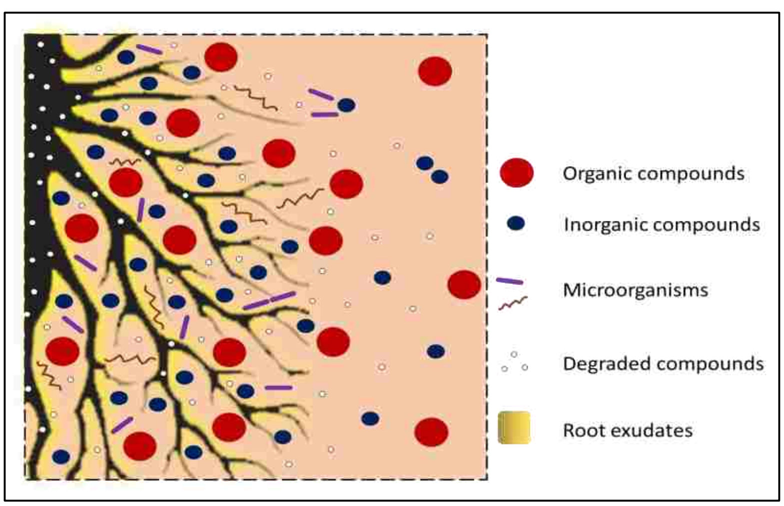


Figure 1.8: Microbial activity in the rhizosphere in presence of organic and inorganic compounds

1.8. REMOTE SENSING TECHNOLOGIES FOR VEGETATIVE ASSESSMENT

Remote sensing technologies can be utilized for vegetation assessments and monitoring plant health and stress due to contaminants.

1.8.1. Introduction to Remote Sensing. Human beings can sense things in the environment with the aid of vision, smell and hearing from a distance. Having these abilities makes us living remote sensors. Remote sensing is the practice of acquiring data from a distance to the object of interest (Lillesand et al., 2014). Various remote sensors are mounted on certain platforms like aircraft, balloons, unmanned aerial vehicles (UAVs) or drones, and spaceborne satellites (Lillesand et al., 2014). Selection of the proper platform depends on the type of sensor and the region to be examined (Graham, 1999). The information that arrived at the sensor is then processed to generate an image which represents the details observed. This phenomenon is similar to what we as humans experience when we see an object and determine its shape, size, color and motion (Eastman, 2010). Various molecules (gases and moisture) are present in the medium (atmosphere) through which the carrier (electromagnetic radiation) travels with the information about the object (Mather, 2005). These molecules have their own specific set of absorption bands in the electromagnetic spectrum and as a result, these molecules absorb and scatter different wavelengths. Therefore, only the wavelength regions outside the absorption bands of these molecules could be used for remote sensing (Principles of Remote Sensing-CRISP). Scattering due to these molecules affects image quality obtained by the sensor (particularly in the visible and near infrared wavelengths) and results in “hazy” images exclusively in the “blue” end of the visible spectrum. Every object has its own reflectance region, which can be detected by remote sensing sensors based on their spectral signature (Xie et al., 2008). Vegetation also has a characteristic spectral signature, i.e. lower visible reflectance and a high near infrared reflectance. Such distinctive spectral signature, is easily notable from other types of objects on the land surface (Principles of Remote Sensing- CRISP). Furthermore, the chlorophyll content in the vegetation can be identified by lower reflectance in red and blue regions of the visible spectrum, whereas the reflectance in the near infrared region is much higher when compared to the visible spectrum (Principles of Remote Sensing- CRISP). Table 1.5 shows various waves and their respective wavelength present in the electromagnetic spectrum.

Table 1.5: Range of different waves and their wavelengths in the electromagnetic spectrum (Principles of Remote Sensing-CRISP)

Type		**Wavelength**
Microwaves		0.001 to 1 m
Infrared	Near Infrared (NIR)	700 to 1500 nm
	Short Wavelength Infrared (SWIR)	1500 to 3000 nm
	Mid-Wavelength Infrared (MWIR)	3000 to 8000 nm
	Long Wavelength Infrared (LWIR)	8000 to 15000 nm
	Far Infrared (FIR)	>15000 nm
Visible light	Red	610 to 700 nm
	Orange	590 to 610 nm
	Yellow	570 to 590 nm
	Green	500 to 570 nm
	Blue	450 to 500 nm
	Indigo	430 to 450 nm
	Violet	400 to 430 nm
Ultraviolet		3 to 400 nm
X-rays		0.01 to 10 nm

1.8.2. Types of Sensors. There are two main types of sensors in remote sensing. Passive sensors can obtain information by natural carriers such as visible or infrared wavelengths of the sunlight reflected by the object of interest. These types of sensors can obtain information only when the natural energy source is available (Mai, 2015). However, active sensors count on their particular source of electromagnetic radiation to gather the information about the objects. Figure 1.9 shows the graphical representation of active and passive sensors. Emission of EM radiation which hits the object and the reflected energy is captured to process the information about the object (Mai, 2015). A camera is a classic example of passive remote sensing.

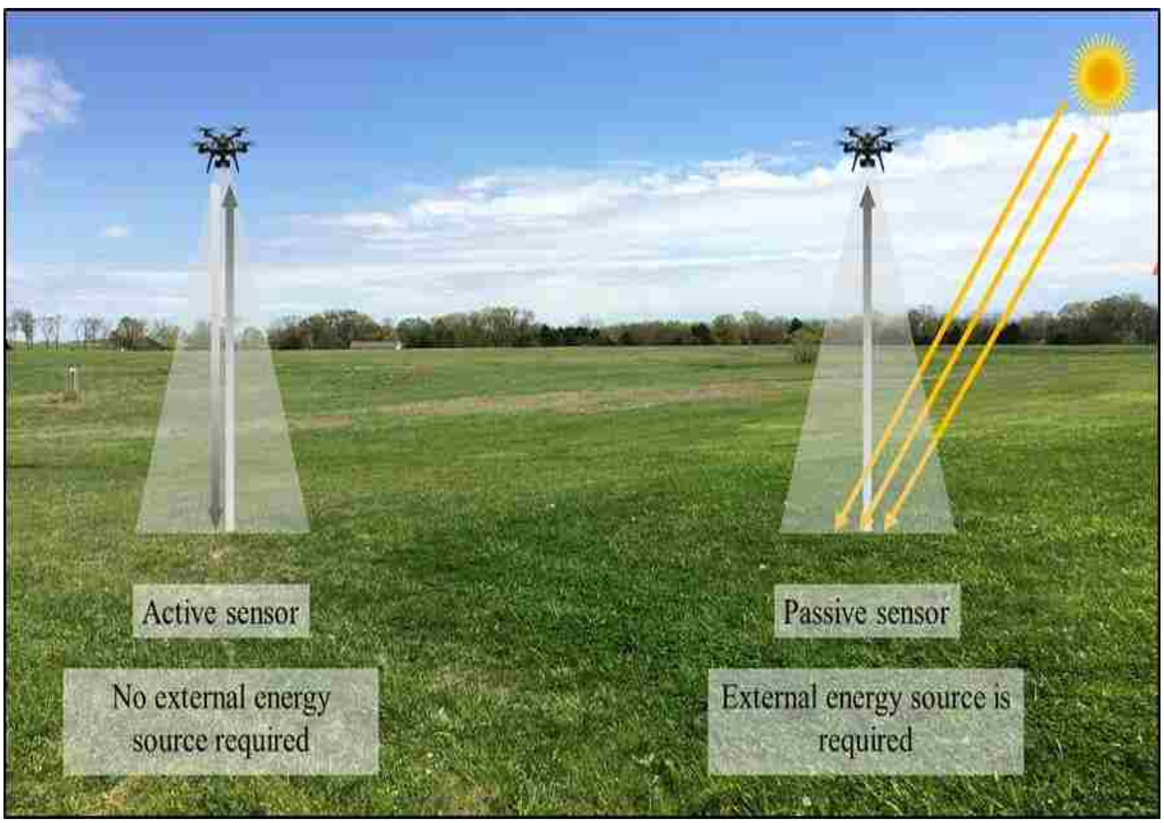


Figure 1.9: Graphical representation of active and passive sensing (UAV image sourced from www.news.3dr.com)

1.8.3. Hyperspectral Imaging System. The deficiencies of conventional multispectral imaging systems, such as to detect specific variables of the various materials, are satisfied by hyperspectral imaging. Hyperspectral imaging has hundreds of adjacent spectral bands which offers abundant spectral information distinguishing different materials. The resultant image is much more precise and loaded with information which is unique to hyperspectral images. The adjoining wavelength bands make a complete spectrum for every single pixel which creates a whole image (Shippert, 2003). The spectrum for each pixel looks similar to that of the spectrum measured using laboratory spectroscopy. The concept of hyperspectral imaging is graphically shown in Figure 1.10.

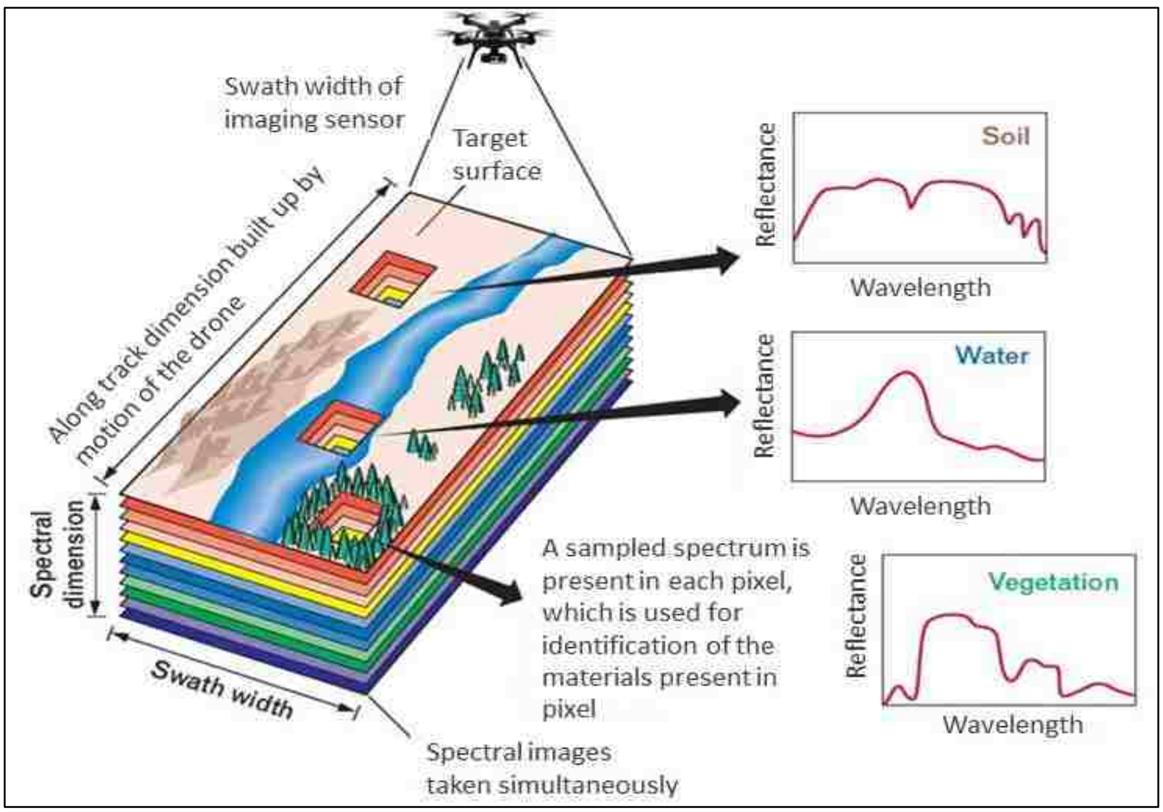


Figure 1.10: Graphical illustration of hyperspectral imaging system (adapted from www.markelowitz.com)

Hyperspectral imaging has been used for mineral mapping and identification of plant species (Clark et al., 1995), to study the chemistry associated with plant canopies, to study plant stress (Merton and Huntington, 1999) and to detect soil properties such as moisture, soil organic matter content, salinity (Ben-Dor et al., 2009). The reflectance spectrum of green poplar leaves: visible, near infrared and middle infrared spectrum along with the red edge is shown in Figure 1.11.

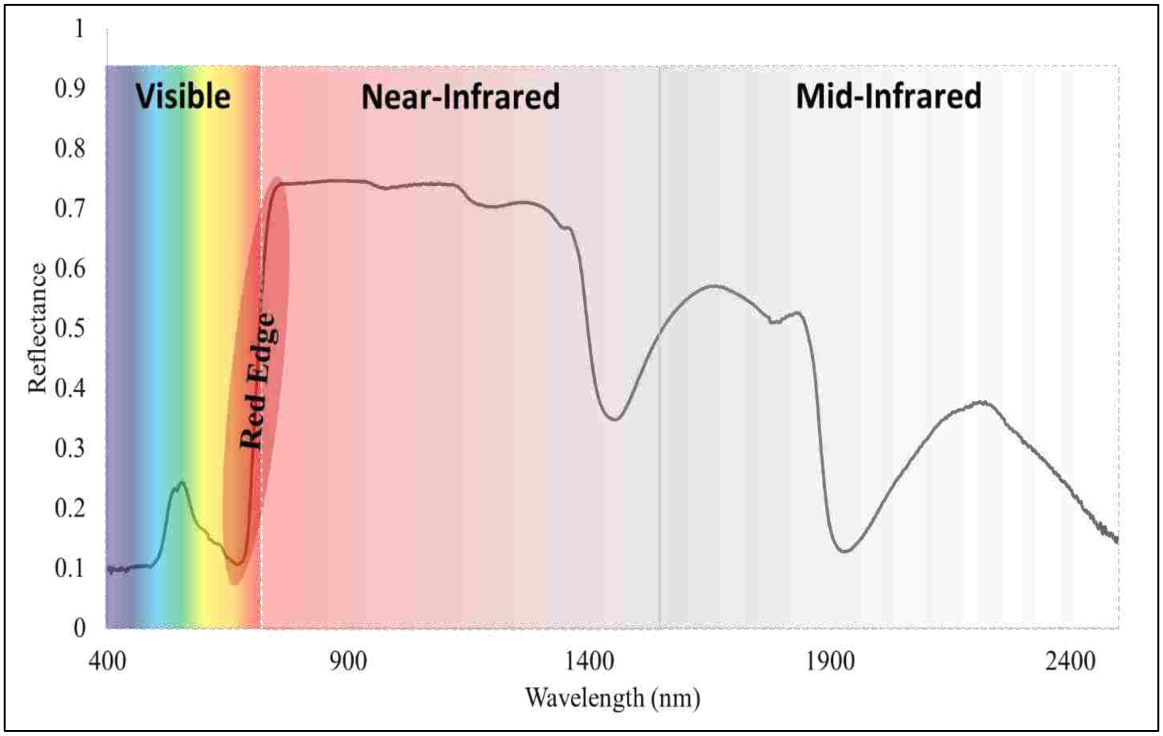


Figure 1.11: Reflectance spectrum of poplar leaves subdivided into 4 optical properties- visible, near IR, middle IR, and red-edge

1.8.4. Hyperspectral Imaging for Landfills. Remote sensing technologies can aid in preventing any hazardous conditions, detecting landfill anomalies such as landfill fires, and monitoring landfill status (Lega and Napoli, 2008). There have been many applications of remote sensing to distinguish and investigate waste disposal sites and landfills (Erb et al., 1981). Airborne hyperspectral imaging systems can be considered as an upgraded form of a lab spectrometer because of its ability to capture 2-D images with each pixel having the spectral information. Therefore, hyperspectral imaging can obtain spectral information along with geospatial information about the target location. The presence of pollutants in the growth media (soil or water) can induce physiological (Gomes et al., 2011; Hayat et al., 2012; Sharma and Dubey, 2005) and spectral changes in plants (Sridhar et al., 2007; Rosso et al., 2005; Su et al., 2007). The presence of contaminants in soil can be detected by hyperspectral imagery (Folkard et al., 1998; Jago et al., 1999). Hyperspectral imaging is used for detection of methane emissions and leachate outflow by quantifying stress in plants on landfill sites (Jones and Elgy, 1994). Hyperspectral information about stressed vegetation can effectively aid in the study of contaminants present in the media of growth. Changes in spectral reflectance can be detected and used to assess plant health by calculating vegetation indices.

Vegetation indices are developed to highlight unique characteristics of vegetation (Fiorani et al., 2013) such as chlorophyll content, water stress, biomass etc. Vegetation indices are combinations of spectral bands, which are obtained by subtraction, addition, ratio, normalization (Jackson et al., 1991). Variations in vegetation indices can occur due to several factors such as seasonal changes in leaves, nutrient and environmental stress, infections, plant senescence, etc. Such changes can be detected and monitored using hyperspectral remote sensing.

2. OBJECTIVES

As phytotechnologies have been applied for landfill applications, particularly leachate treatment, the interactions of plants and leachate are of interest and should be better understood for technological advancement of these phytotechnologies. The primary goal of the study was to assess the leachate - plant interactions, particularly health and stress using direct and also indirect methods during the exposure and treatment period. In order to achieve this goal a set of experiments were conducted with the following objectives:

Objective 1. Quantify plant response, both positive and negative, via biomass production with respect to leachate exposure at various concentrations.

Hypothesis: Leachate dose will affect plant growth (beneficial and harmful).

Objective 2. Assess impacts of leachate exposure on root development by associating root biomass with root traits using novel root imaging technology.

Hypothesis: Leachate exposure will induce changes in root development, related to overall plant health and stress.

Objective 3. Evaluate remote sensing methodologies as indicative tools to assess plant health as impacted by leachate exposure for a variety of species.

Hypothesis: Plants will display spectral changes with respect to leachate exposure.

Objective 4. Evaluate established vegetation indices as indicators of plant health and stress impacts of leachate exposure.

Hypothesis: Specific vegetation indices can indicate plant health and stress impacts from leachate.

Successful completion of these objectives will aid in the development of new approaches for assessment of deployable phytotechnologies for landfill leachate treatment and other phytoremediation applications.

3. MATERIALS AND METHODS

3.1. BIOASSAY OF FOUR PLANT SPECIES

A total of fourteen 6-liter plastic containers were designed as exposure reactors. Leachate was obtained from the leachate storage unit of the Prairie Valley Landfill, Cuba, MO. Leachate was freshly collected from the leachate collection system. The leachate analysis report is shown in Table 3.1. The collected leachate was stored in HDPE containers at room temperature. The containers were filled with 2 liters of landfill leachate with varied concentrations (20%, 40%, 60%, 80%, and 100% of leachate). As a negative control, de-ionized water was used and as a positive control, Hoagland's nutrient solution (Hoagland and Arnon, 1950) was used. The lids of these containers were drilled with 8 holes (~0.3 m diameter) for the conical plastic tubes/Deepots^{TM} in which the plants were to be planted in sand. The tubes were filled with silica sand (< 0.00125 m particle diameter) which was sifted using a #16 sieve. The sand was then soaked and washed using distilled water several times followed by air drying at room temperature for 48 hrs. Each tube was planted with one plant. As shown in Figure 3.1, four plant species were involved in this experiment; 2 dicots (tree cuttings of hybrid *Populus* (DN5) and *Salix* (laurel leaf)) and 2 monocots (*Vetiveria* slips and *Festuca* seeds). The bioassay experiment was carried out in a greenhouse located on the roof of Butler-Carlton Hall (Missouri S & T).

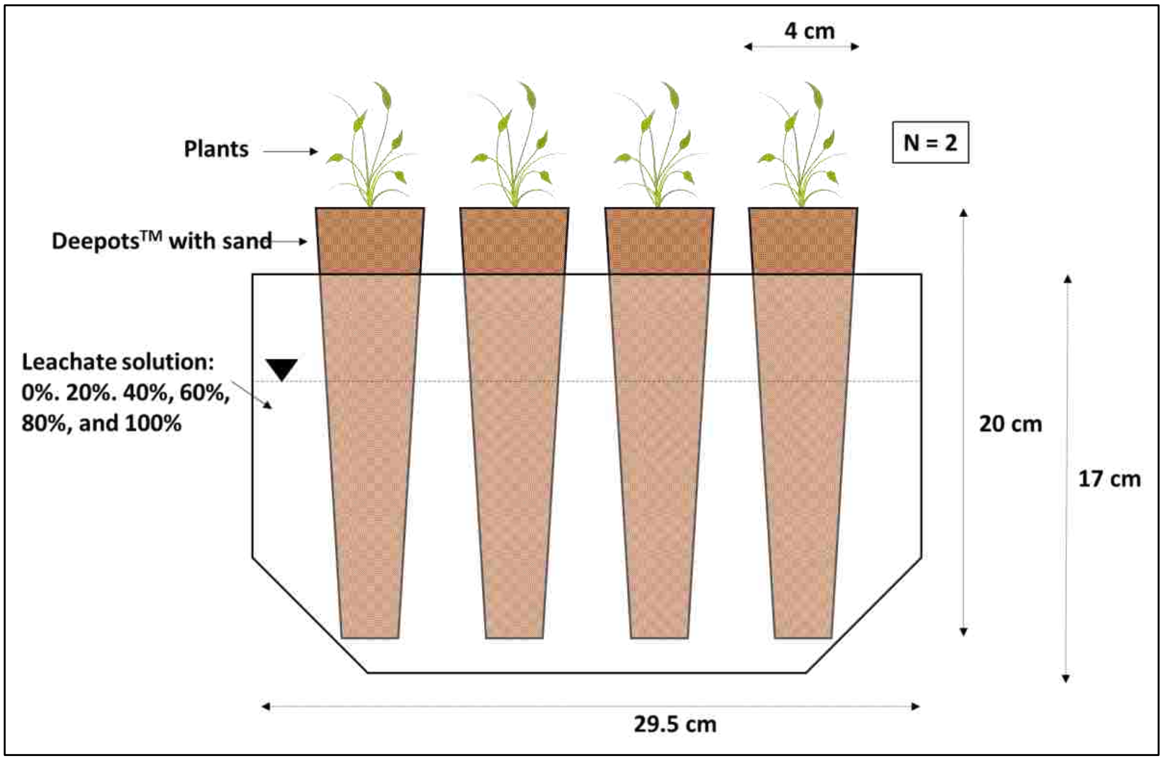


Figure 3.1: Schematic showing setup for bioassay of plants

3.2. RATIONALE FOR CHOOSING FOUR PLANT SPECIES

This experiment was designed to assess the stress of landfill leachate on growth and health of four plant species: Poplar hybrid DN5 (*Populus deltoides* × *Populus nigra* L.), Laurel leaf willow (*Salix pentandra)*, Vetiver grass (*Vetiveria zizanioides*), and Kentucky-31 tall fescue grass (*Festuca arundinacea*).

3.2.1. Hybrid Varieties of *Populus* (Poplar) and *Salix* (Willow). According to Zalesny et al., (2007) looking at the increasing numbers of landfills in North America, there is a rising demand for cost-effective systems for in situ leachate treatment. Poplars (*Populus*) and willows (*Salix*) are rapidly growing trees (Lunáčková et al., 2003) gaining a reputation in the field of phytoremediation over conventional technologies. These are woody, inedible, and short-rotation crops. Moreover, poplars and willows possess the requirements for suitable vegetation cover for landfills such as high ET rates, selective metal uptake, extensive root systems (McLinn et al., 2001), and biomass production. This biomass could be used to generate energy by incineration. Leachate application to *Salix viminalis* and *Salix aquatic* exhibits merits for biomass production and ET, resulting in a reduction of nutrients and volume of leachate (Ettala, 1988). The performance of the plants is dependent on the volume and quality of the leachate, organic content of the soil, and the plant species (Ettala, 1988). Involving *Populus* (poplars) and *Salix* (willows) in the design of the evapotranspiration (ET) caps is one of the most effective technologies in terms of environmental sustainability and economic viewpoint (Ensley, 2000; Glass, 1999; Dickmann et al., 2002). *Populus* and *Salix* are known to provide hydraulic control, alleviate pollutant migration, (Ferro et al., 2001; Vose et al., 2000; Zalesny et al., 2006) and are capable of remediating surface and subsurface contamination (McLinn et al., 2001; Perttu et al., 1994; Perttu et al., 1997) and also erosion control with extensive lateral root system (Wilkinson, 1999). *Populus* and *Salix* can efficiently undergo the phytoremediation processes such as rhizofiltration, rhizodegradation, phytodegradation, phytoextraction, phytovolatilization, and phytostabilization (Banuelos et al., 1999; Sander et al., 1998; Schnoor et al., 1995).

Populus trees are excellent for phytoremediation of leachate with P, K, S, Cu, and Cl with large concentrations of Ca and Mg in the roots and stems; whereas willows are better at remediating B, Zn, Fe, and Al with considerable quantities of Mg and Ca in the roots and stems (Zalesny and Bauer, 2007). In *Populus* and *Salix* genera, 50 to 70 days-old clones display an increase in the number of leaves, height, and diameter of 4-15% when irrigated with leachate compared to water (Zalesny et al., 2009). This increase could be a resultant of the potassium (K), phosphorus (P) and nitrogen (N) content present in the leachate which offers a fertilizer effect (Zalesny et al., 2009). Poplars have the potential for *in-situ* phytoremediation of landfill leachate.

Dilution of leachate with higher toxicity should be considered in order to reduce the adverse effects on plants (Zalesny and Bauer, 2007). Leachate application rates need to be carefully controlled to prevent contaminant transport to the soil and/or groundwater. In *Salix*, phytoextraction of Cd from polluted soil to leaves is observed to be higher in slightly acidic soils with reduction of biomass (Klang et al., 2003). Moreover, in *Salix*, heavy metals such as Cd and Zn can be transported from contaminated soil to above ground biomass, whereas other heavy metals such as Cu, Ni, Pb, and Cr accumulate in and around the roots (Vandecasteele et al., 2005).

3.2.2. *Vetiveria zizanioides* (Vetiver Grass). *Vetiveria zizanioides* (Vetiver) is phylogenetically similar to Sorghum with fragrant grass-like characteristics (Barnard et al., 2013). Originally from Southern India, Vetiver is a non-invasive grass (Wilde et al., 2005) which ranges from 1 to 10 feet in height, with sterile flowers and a root system which is dense and can reach up to 12 feet deep (Truong et al., 2008). Vetiver can tolerate high concentrations of nutrients (N and P), salinity, heavy metals, herbicides (atrazine), and diseases (Truong, 2000). A wide range of pH tolerance and temperature (7° to 130° F) are additional features of *Vetiveria zizanioides* (Truong et al., 2008). Vetivers are also known to resist drought and fires. However, Vetiver requires direct sunlight and can show stunted growth when exposed to shade or completely submerged in water for prolonged times. The sterile variety of *Vetiveria zizanioides* is employed in fields of soil and water remediation due to all the favorable characteristics this perennial grass possesses for phytoremediation. The deep fibrous and dense root system (Gupta et al., 2012) provide a good surface area for absorption of water, nutrients, and contaminants. *Vetiveria* has also demonstrated slope stabilization capabilities (Hengchaovanich et al., 1996), phytoremediation of mine tailings from various mines (gold, coal, platinum etc.) in Australia, purification of landfill leachate (Percy and Truong, 2003; Truong et al., 2008) and removal of nutrients from eutrophic water in China (Zheng et al., 1997).

3.2.3. *Festuca arundinacea* (Tall Fescue). *Festuca arundinacea* is a perennial bunch grass which can grow up to 4 feet tall. *Festuca* has been widely used all over North America for forage and erosion control. *Festuca* has also been used on landfills to create a green cover. *Festuca* is known to be infected widely by endophytic fungi, which can inhibit soil microorganisms such as mycorrhizal fungi. Previous studies have shown capabilities of *Festuca* in slope stabilization (Sleper and Buckner, 1995). Degradation of hydrocarbons was observed to be faster in *Festuca* rhizospheres than in soil without plants (Banks et al., 1997). *Festuca* displayed higher biomass production when dosed with TNT and is known to carry out transformation of TNT (Chekol et al., 2002). Intercropping of *Festuca* with alfalfa can remove PAHs more effectively than monoculture (Sun et al., 2011). *Festuca* has been used for phytoremediation of heavy metal contaminated soils (Zhi et al., 2015).

A total of 8 Deepots^{TM} were placed per container (6L). Two poplars and two willow cuttings (6-inch-long and 6-7 mm thick) were planted 5-inch deep in the sand filled Deepots^{TM}. Two vetiver slips (6-inch long shoots and ~1-inch of roots) were planted in the Deepots^{TM}. Fescue seeds (ten seeds per Deepot^{TM}) were sowed at 1-inch depth in two Deepots^{TM}. Soil was not involved in this experiment, to assess direct effects of landfill leachate on plant health and growth of shoots and the root system. Moreover, the leachate level in the containers was maintained by replacing the remnant whenever the leachate levels were low (< 500 ml). The containers were also wrapped with aluminum foil to prevent algal growth in the translucent containers.

The plants were harvested after 77 days since the first irrigation took place. Plants were carefully removed from the Deepots^{TM}, and sand particles were rinsed off using tap water. Roots were excised from the plants and RGB (red, blue, green) imaging of roots was done for consequent image analysis for root traits. The shoots and roots of each plant were then individually packed in paper bags and placed in drying oven at 80°C for 24 hours. Plant growth was determined by measuring the dry biomass of the harvested plants. The biomass measurements were recorded for shoots and roots of each plant according to the standard operating procedure provided by Environmental Response Team/Scientific Engineering Response & Analytical Services (ERT/SERAS).

3.3. ROOT ANALYSIS USING DIGITAL IMAGING FOR ROOT TRAITS

The plants grown for the bioassay were harvested after 77 days and transported to Donald Danforth Plant Science Center (St. Louis, MO) for plant root imaging. Image analysis of the roots was done by using high throughput computing platform called Digital Imaging for Root Traits (*D.I.R.T.*). Imaging of the roots was done by using a *Canon EOS 50D* 15.1-megapixel camera with image resolution of 3168×4752 pixels. Imaging was done according to the protocol performed by Bucksch et al., 2014. The root architecture computation system accessible through *D.I.R.T.* is automatic and includes estimation more than 75 root traits (Das et al., 2015).

Table 3.1: Composition of leachate obtained from Prairie Valley Landfill (Leachate analysis report is shown in Appendix)

Parameter	**Concentration (mg/l)**	**Method of analysis**
Fluoride	34	EPA 300.0
Biochemical Oxygen Demand	1200	SM 5210B
Total Suspended Solids	39	SM 2540D
Mercury	< 0.0002	EPA 245.1 / SW 7470
Arsenic	< 0.015	EPA 200.7
Beryllium	< 0.001	EPA 200.7
Boron	3.2	EPA 200.7
Cadmium	< 0.002	EPA 200.7
Chromium	0.014	EPA 200.7
Copper	0.0042	EPA 200.7
Lead	< 0.01	EPA 200.7
Molybdenum	< 0.012	EPA 200.7
Nickel	0.045	EPA 200.7
Selenium	0.035	EPA 200.7
Silver	0.0097	EPA 200.7
Zinc	0.019	EPA 200.7
Cyanide	< 0.0025	SM 4500-CN C E
Chromium-VI	< 0.005	SM 3500-Cr B
Oil & Grease	< 5.3	EPA 1664
Phenol	< 0.18	EPA 420.1
Benzene	5.4	EPA 624
Surrogate 1,2-Dichloroethane-d4	83%	EPA 624
Surrogate Toluene-d8	85%	EPA 624
Surrogate Bromofluorobenzene	112%	EPA 624

First, the plants were uprooted from the Deepots™ gently with minimum damage to the roots. Next, the sand particles stuck to the roots were rinsed off by water. The roots were excised from the plants and were placed in a black tub, which was filled with water, and the thin roots float and spread evenly. A scale marker (plain white poker chip) of known diameter (39.0 mm) was kept beside the roots. The scale marker aids in the conversion of pixels to mm or cm. The poker chip should be clearly visible in the image frame of the camera. The images of the roots were taken using *Canon EOS 50D* camera mounted on a tripod as shown in Figure 3.2. Diffused lights were used to get consistent light distribution for all the plants. Furthermore, the black cloth was replaced by a black tub which was filled with water and the plant roots were excised from the stem and were submerged in this tub to keep the thin roots afloat.

Figure 3.2: Plant imaging setup for D.I.R.T. analysis using Canon EOS 50D camera

3.4. SPECTRAL MEASUREMENTS

Spectral reflectance was measured for each plant using a hyperspectral camera and a spectroradiometer.

3.4.1. Hyperspectral Imaging. Hyperspectral images (HSI) of all the plants in the bioassay experiment were taken using a Headwall Nano-Hyperspec® hyperspectral VNIR Imager (Headwall Photonics, Inc.). The Headwall Nano-Hyperspec® hyperspectral VNIR has an integrated sensor with a wavelength range of 400 to 1000nm and 270 spectral bands; it has been used for a variety of applications in areas of food safety (Qin et al., 2017), forestry (Saari et al., 2011), and precision agriculture. Headwall Nano-Hyperspec® hyperspectral VNIR Imager can be easily mounted on UAVs as shown in Figure 3.3.

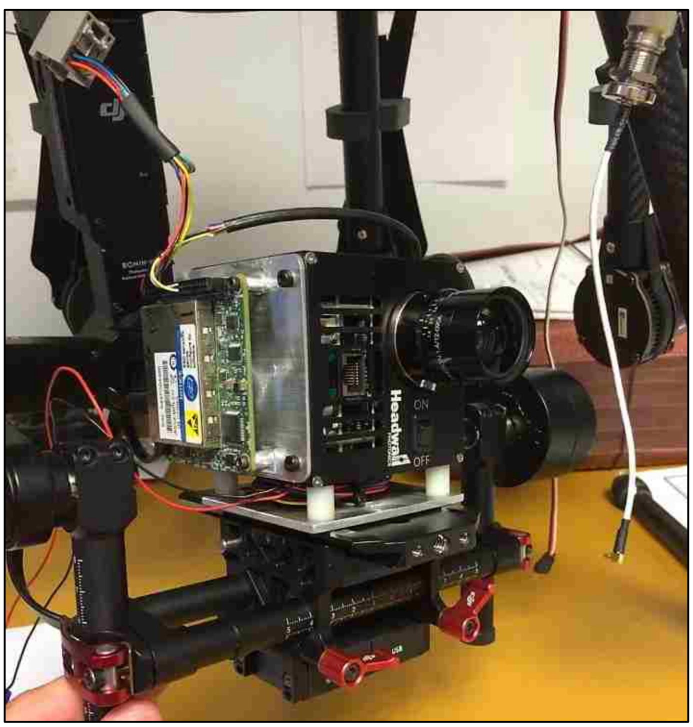


Figure 3.3: Headwall Nano-Hyperspec® hyperspectral VNIR Imager mounted on a UAV

After 53 days of bioassay experiment, a Headwall Nano-Hyperspec® hyperspectral VNIR Imager (Headwall Photonics, Inc.) was used to obtain hyperspectral images. A 1000W halogen lamp was used as a light source (27000 lumens). The vertical distance of the plants from the hyperspectral camera was maintained at ~3 meters. The images were then processed using an image processing software called ENVI (Exelis Visual Information Solutions Inc.). Radiometric correction of the images was done to avoid radiometric errors and distortion. The reflectance data was obtained for each plant at three different locations on the plant which were later averaged to get a representative HSI data set of each plant. The reflectance values were used to calculate various vegetation indices, which combines reflectance at different wavelengths to express a specific property of the target plants. Furthermore, for each plant species, the vegetation indices at different leachate concentrations were calculated to related to leaf counts.

3.4.2. Spectroradiometric Measurements. After 75 days, before harvesting the plants from the bioassay experiment, FieldSpec®-Pro (Analytical Spectral Devices (ASD) Inc.) was used to obtain spectral reflectance of each individual plant. Shown in Figure 3.4, FieldSpec®-Pro is a portable spectroradiometer with a broader spectral range of 350 to 2500nm. In existing literature, FieldSpec®-Pro has demonstrated several applications like soil characterization (Brown et al., 2006), mineral analysis (Kruse et al., 2009), pigment analysis (Mihelutti et al., 2010), precision agriculture (Fitzgerald et al., 2006) and remote sensing (Meroni et al., 2009). A 70W quartz-tungsten-halogen light source (Analytical Spectral Devices (ASD) Inc.) was used for illumination. FieldSpec®-Pro was calibrated for existing light conditions, followed by, recording the dark reference and white reference. A bare fiber optic was used for all plants and was oriented directly above at a distance of 0.25 m. Calibration was done according to a National Institute of Standards and Technology (NIST) reflectance standard.

Figure 3.4: FieldSpec-Pro connected with the laptop and ready to use (Source: goo.gl/2TZ08E)

Dark reference was obtained by FieldSpec®-Pro, by closing the shutter. White reference was obtained by using a white reference panel (99% reflectance) Spectralon® (Labsphere®) The reflectance was recorded by using RS^3 Spectral Acquisition Software (Analytical Spectral Devices (ASD) Inc.) at three different locations on the plant and his procedure was repeated for each plant (Manley, 2016). The reflectance data was analyzed using ViewSpec™-Pro (Analytical Spectral Devices (ASD) Inc.). The reflectance values obtained from each plant were averaged and a resultant reflectance spectrum was acquired for individual plants. The reflectance values were used to calculate various vegetation indices related to chlorophyll content, biomass, carotenoids, senescence, vegetation stress etc. List of vegetation indices calculated are shown in Table 3.2.

Table 3.2: The list of vegetation indices calculated from Headwall Nano-Hyperspec® hyperspectral VNIR Imager and FieldSpec®-Pro

Vegetation Index	Equation	Related to	Reference
NDVI750	$\frac{R750 - R705}{R750 + R705}$	Chlorophyll	Sims et al., 2002,
NDVI	$\frac{R800 - R670}{R800 + R670}$	Biomass	Dang et al., 2011
NDVI2	$\frac{R900 - R680}{R900 + R680}$	Biomass	Serrano et al., 2003
WI	$\frac{R900}{R970}$	Water Content	Penuelas, et al., 1997
WBI	$\frac{R970}{R900}$	Water Status	Penuelas et al., 1994
MCARI1	$1.2[2.5(R800 - R670) - 1.3(R800 - R550)]$	Chlorophyll	Haboudane, 2004
MCARI2	$\frac{1.2(2.5(R800 - R670) - 1.3(R800 - R550))}{SQRT((2 * R800 + 1)^2 - (6 * R800 - 5 * SQRT(R670)) - 0.5)}$	Chlorophyll	Haboudane, 2004
PSRI	$\frac{R678 - R500}{R750}$	Plant Senescence	Merzlyak et al., 1999
GM2	$\frac{R750}{R700}$	Chlorophyll	Gitelson et al., 1997
R801/R550	$\frac{R801}{R550}$	Photosynthesis	Daughtry, 2000
R740/R850	$\frac{R740}{R850}$	Stress	Naumann et al., 2010
LIC1	$\frac{R800 - R680}{R800 + R680}$	Stress	Lichtenthaler, 1996
LIC2	$\frac{R440}{R690}$	Stress	Lichtenthaler, 1996
GM1	$\frac{R750}{R550}$	Chlorophyll	Gitelson et al., 1996
VOG1	$\frac{R740}{R720}$	Chlorophyll	Vogelmann et al., 1993
VOG2	$\frac{R734 - R747}{R715 + R726}$	Chlorophyll	Vogelmann et al., 1993
VOG3	$\frac{R734 - R747}{R715 + R720}$	Chlorophyll	Vogelmann et al., 1993
Carter-1	$\frac{R695}{R420}$	Stress	Carter, 1994

4. RESULTS AND DISCUSSION

4.1. BIOASSAY RESULTS

In the bioassay greenhouse study, leaves started to grow from the buds of many poplar and willow cuttings after two days of leachate irrigation. Fescue also started to germinate from the seeds in two days after leachate irrigation. After 15 days of exposure, poplar, willow and vetiver in the containers with Hoagland's solution, 20%, 40% and 60% leachate solution appeared to be healthier than the plants growing in nutrient deficient control solution (0%), and in the 80% and 100% leachate solutions. However, the difference in leachate concentrations did not visibly affect seed germination in fescue seeds. After 42 days, leaves of poplar and willow were noticeably impacted, demonstrating wilting and drying of leaf tips. Also, plants growing in Hoagland's nutrient solution showed chlorosis in vetivers and fertilizer burns in leaves of dicots after 42 days. For the dicots, number of leaves were counted at regular intervals till the plants were harvested, and are shown in Table 4.1 and Table 4.2. In monocots, no considerable changes were observed in number of leaves.

Table 4.1: Mean leaf counts for *Populus* during bioassay experiment

Mean leaf counts *Populus*					
Concentration	**30days**	**40days**	**50days**	**60days**	**75days**
0%	10	10	13	12	12
20%	10	11	12	13	10
40%	10	12	15	17	11
60%	6	9	11	14	7
80%	5	10	11	9	4
100%	8	13	5	5	3

Table 4.2: Mean leaf counts for *Salix* during bioassay experiment

Mean leaf counts in *Salix*					
Concentration	**30days**	**40days**	**50days**	**60days**	**75days**
0%	13	19	14	13	11
20%	15	18	20	23	14
40%	12	17	18	21	11
60%	13	16	18	21	10
80%	10	16	8	9	3
100%	10	13	3	3	0

Over the period of leachate exposure to all the plants, several other observations were recorded. Visual indications of stress such as chlorosis, premature leaf drying and falling incidents along with brown edges and white depositions on the leaves were observed. In poplars and willows, leaves nearest to the ground were showing signs of chlorosis with subsequent leaf fall. Chlorosis was also observed in poplars and willows growing in 0% leachate dose. The rate of wilting leaves and leaf fall was observed to be increased as the leachate dosage changed from 20% to 100%. Dicots growing in 80% and 100% leachate doses, were visually more stressed than others, showing excessive chlorosis, necrosis and leaf curling and leaf fall. However, in monocots, no visual stress factors were observed except for the change in biomass allocation.

Plants are known to exhibit '*functional equilibrium*' in terms of biomass allocation (Iwasa et al., 1984; Thornley, 1972). In other words, plants growing in nutrient deficient media would show increased root biomass, relative to above ground tissues (Brouwer, 1963), whereas plants growing in conditions with low light and carbon dioxide have a tendency to distribute biomass in shoots. Plants exhibit equivalent biomass distribution when the limiting factors above and below the ground are of similar extent (Bloom et al., 1985). Therefore, leachate concentration at which plants are showing higher biomass production can be considered either beneficial or less harmful than plants growing at different leachate concentrations with lower biomass production.

4.1.1. Shoot Biomass Production. The shoot biomass production in the plots demonstrates hormetic pattern (Cedergreen et al., 2007) i.e. stimulating a response at lower leachate concentrations and inhibiting response at higher doses (Calabrese and Blain, 2009). In dicots, poplars displayed stimulating response with an increase in biomass production in plants growing in 20% and 40% leachate solutions and lower biomass production was recorded at 0%, 60%, 80%, and 100% leachate solution. Similar, growth pattern was observed for willows with higher biomass production in plants growing at 20% and 40% and a decline in biomass production from 60% to 100% was observed. Also, dicots and monocots showed highest shoot biomass production in Hoagland's nutrient solution. The overall biomass production for poplars and willows is shown in Figure 4.1 and Figure 4.2, respectively. In monocots, both vetiver and fescue displayed similar growth patterns with relatively higher biomass production at 20 %, 40%, and 60%, and a lower biomass production at 0%, 80%, and 100% leachate exposure. The overall biomass production data for vetiver and fescue is shown in Figure 4.3 and Figure 4.4 respectively.

The increased biomass production for all four species tested; showed that leachate irrigation can promote plant growth, while simultaneously treating the leachate. Previous work has shown the leachate application can have either growth promoting benefits (Nunes et al., 2016; Del Moro et al., 2014; Alaribe et al., 2016) or toxicity (Klauck et al., 2013; Clement et al., 1995; Sang et al., 2004. The actual leachate application or dilution rates that benefit growth without toxicity for a specific landfill will vary along with the leachate constituents and concentrations. The findings indicate that hormetic impacts occurred for all four species tested. For full-scale systems, monitoring plant health would be a key parameter for efficient and effective application of leachate to the vegetative leachate treatment systems. An additional benefit to consider is the value of the biomass generated for biomass crops such as poplar and willows, and the ecological services that could be provided by other species which could be selected in a vegetative leachate treatment system.

4.1.2. Root Biomass Production. Root biomass production shows similar patterns as observed in shoot biomass production. In poplars and willows, growing at 0% and 40% leachate dose, root biomass production was higher than 20%, 60%, 80% and 100% leachate solutions. Higher root biomass at 0% solution is indicative of allocation of resources in roots in search of nutrients. Although not ideal, such process of biomass allocation towards roots growing in nutrient deficient conditions can be useful in field conditions for erosion control and slope stabilization. However, for better leachate treatment and survival of plants involved in phytotechnologies, leachate concentrations must be controlled with respect to plant health. Also, increased root biomass at 0% confirms the "*functional equilibrium*" phenomenon for biomass allocation due to nutrient deficiency. Whereas, in *Vetiveria* the root biomass production was highest in the range of 40% and 60% leachate applications. In fescue, highest biomass production was observed at 40% leachate solution.

Plants growing in nutrient deficient media would show increased root biomass, relative to above ground tissues (Brouwer, 1963). Biomass allocation in shoots is known to be higher than in root system in plants which have better growth conditions such as light, water, and nutrients with relatively less environmental stress. Moreover, photosynthates (carbohydrates) transferred from shoot system to the root systems through phloem tissues and transfer of nutrients such as N and P from roots to shoots through xylem tissues also are considered to be factors affecting root and shoot growth (Dewar, 1993; Thornley, 1972; Hoad et al., 2001). Therefore, the findings indicate that leachate doses which displayed higher root biomass production in plants were experiencing nutrient deficiency stress due to which photosynthates transfer from shoots to roots were higher than the nutrient transfer from roots to shoots. Such results also are suggestive of leachate concentrations which are tolerable for respective plants with better plant survivability while undergoing effective leachate treatment. Statistical significance in the difference of biomass production was determined at 90% confidence intervals using Student's T distribution with α=0.01.

4.1.3. Root to Shoot Ratio (R:S). Root biomass in dicots was observed to be higher than shoot biomass, thus the overall R:S was higher in dicots. Whereas, in monocots, unlike dicots the R:S was lower, showing higher biomass production in shoots than roots. Root to shoot ratios for *Populus* and *Salix* at different leachate concentrations are shown in Figure 4.5 and Figure 4.6, respectively. Root to shoot ratios of Vetiveria and Festuca are shown in Figure 4.7 and Figure 4.8. Root and shoot growth continuously adjust to the availability of primary growth resources (nutrients, light, and water) and the presence of toxic substances, hence biomass distribution in the plant is affected. Root and shoot biomass production is affected significantly with changing above ground and below ground conditions, which leads to changes in R:S. R:S can provide information about plant health conditions. Plants with lower R:S are known to be healthier (Ericsson ,1981).

However, root to shoot ratios are not generally considered as a conclusive measure as R:S changes with plant growth over time.

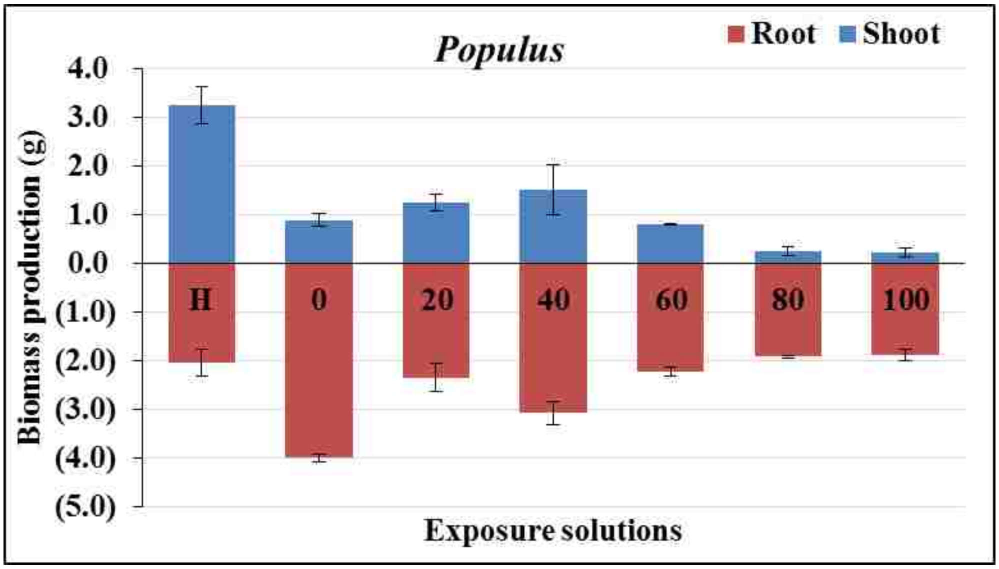


Figure 4.1: Aboveground and belowground biomass production in *Populus* at different solutions. Error bars show 90% confidence intervals (α=0.01)

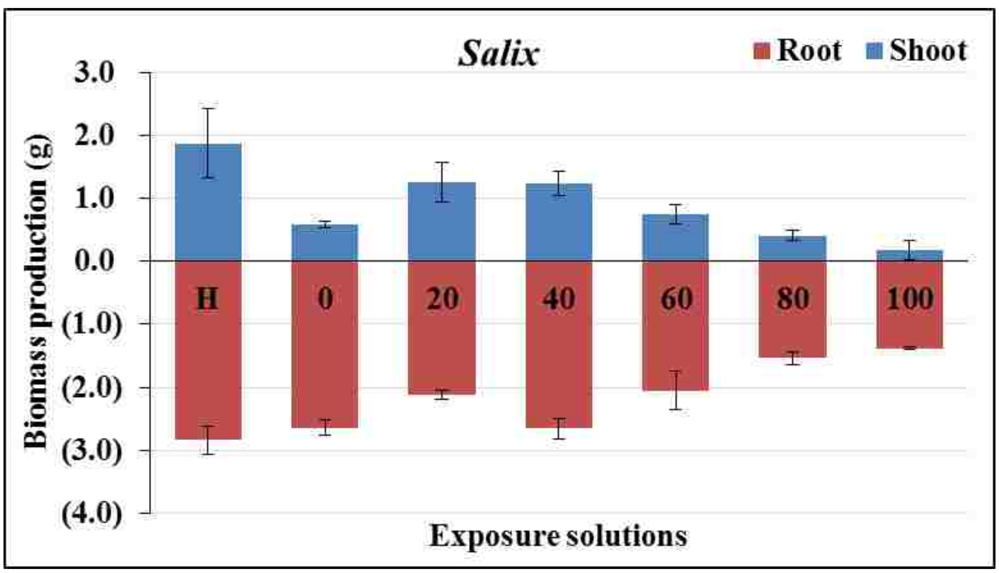


Figure 4.2: Aboveground and belowground biomass production in *Salix* at different solutions. Error bars show 90% confidence intervals (α=0.01)

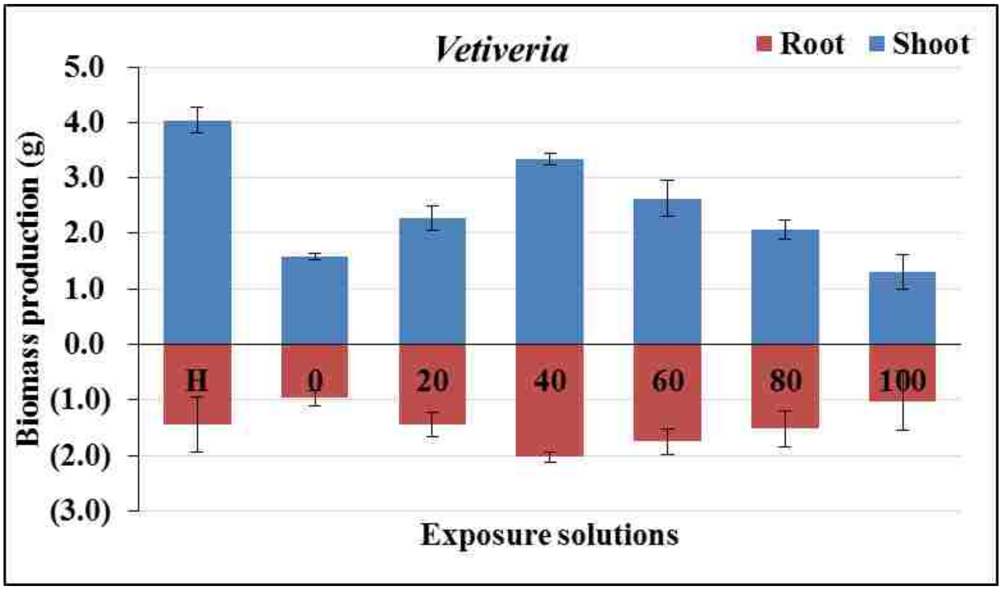


Figure 4.3: Aboveground and belowground biomass production in *Vetiveria* at different solutions. Error bars show 90% confidence intervals (α=0.01)

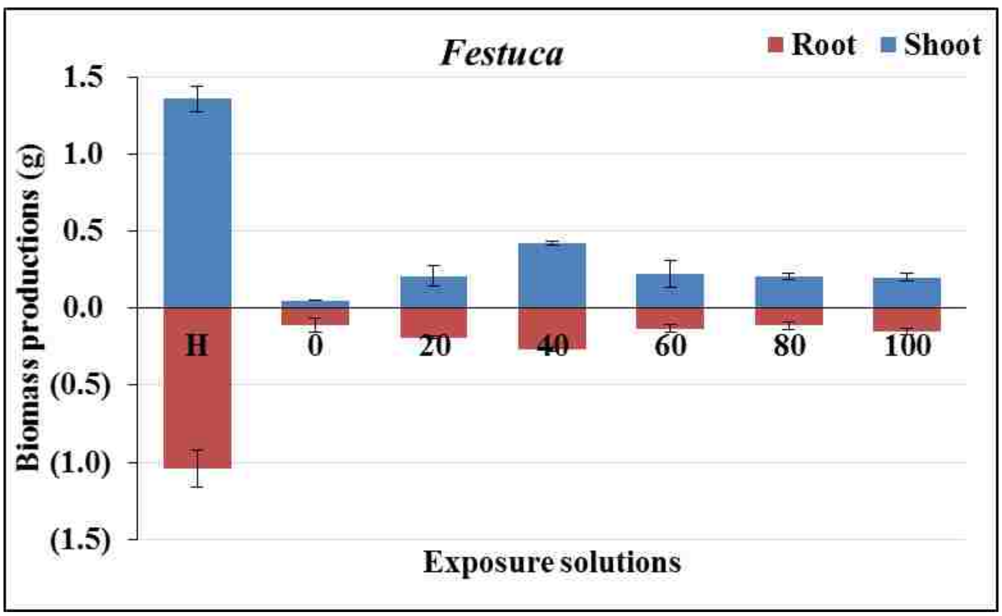


Figure 4.4: Aboveground and belowground biomass production in *Festuca* at different solutions. Error bars show 90% confidence intervals (α=0.01)

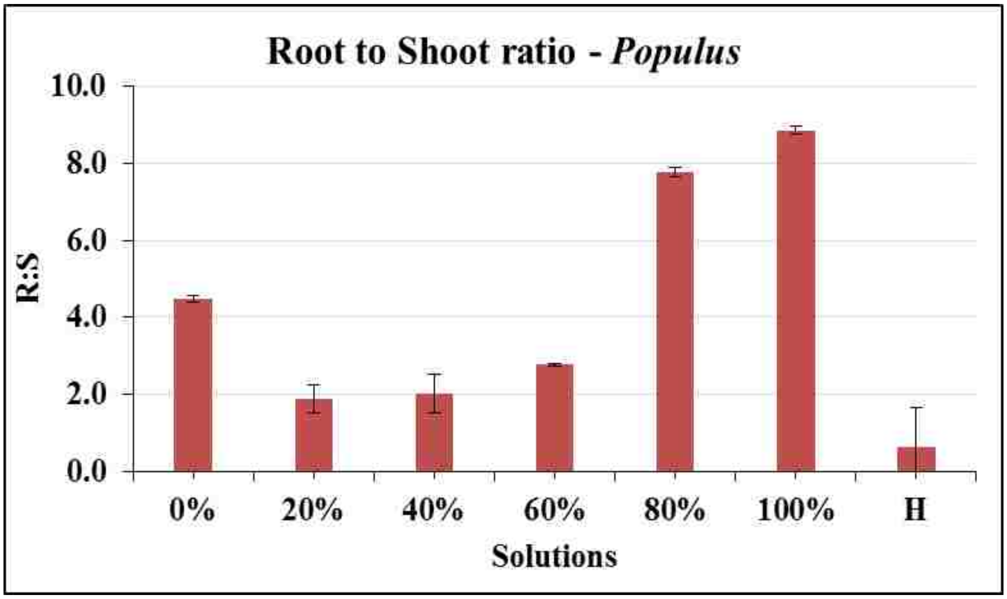


Figure 4.5: Root to shoot ratio for *Populus* at different solutions (Error bars show 90% confidence intervals α=0.01)

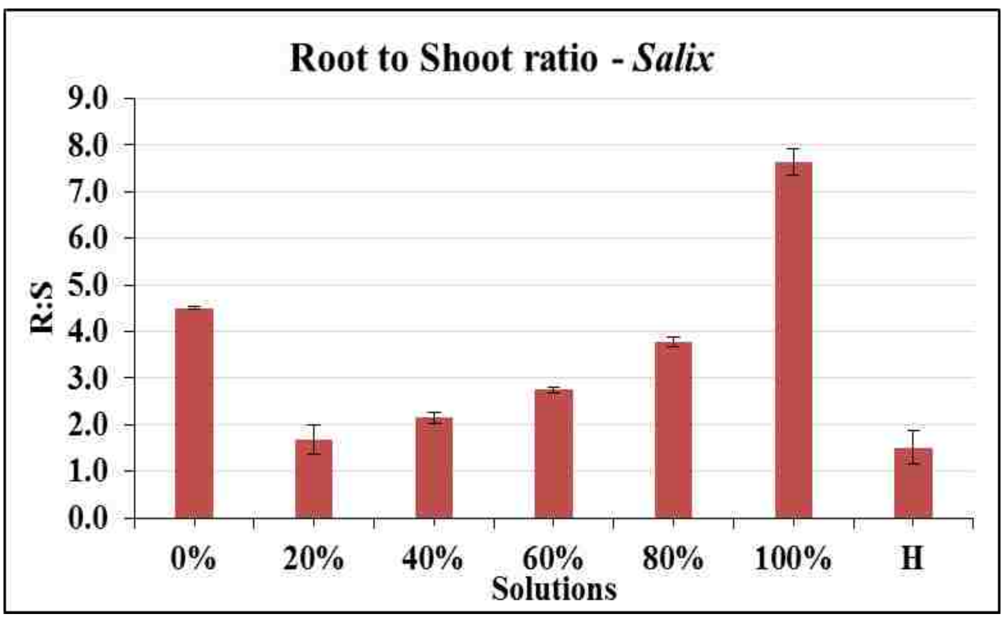


Figure 4.6: Root to shoot ratio for *Salix* at different solutions (Error bars show 90% confidence intervals (α=0.01)

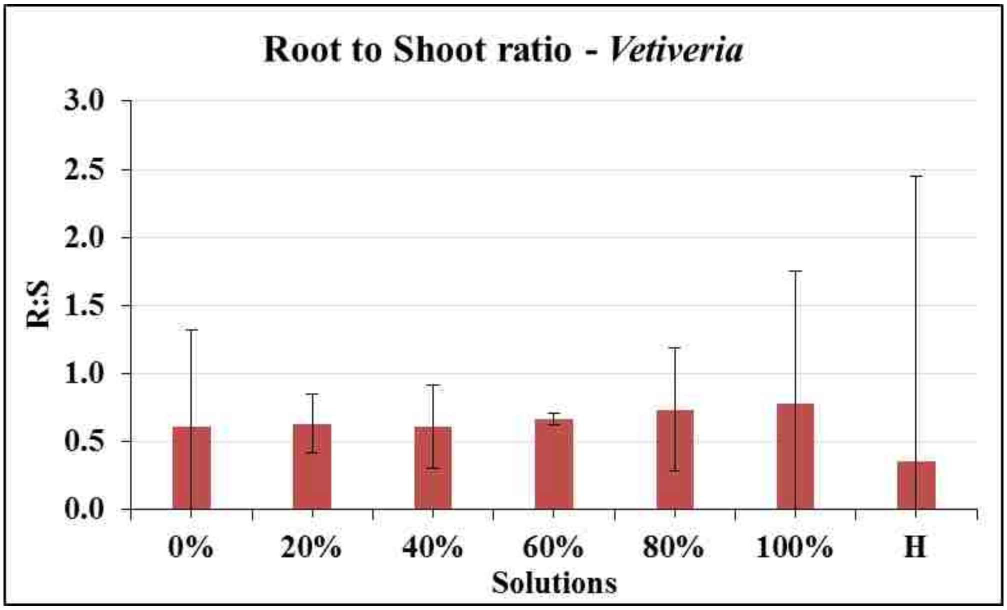


Figure 4.7: Root to shoot ratio for *Vetiveria* at different solutions (Error bars show 90% confidence intervals (α=0.01)

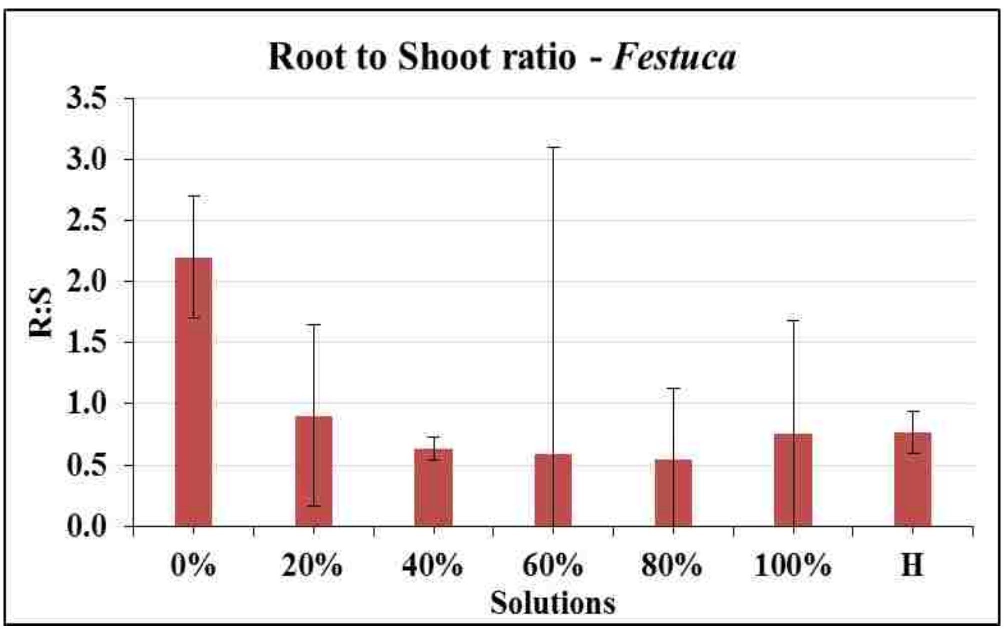


Figure 4.8: Root to shoot ratio for *Festuca* at different solutions (Error bars show 90% confidence intervals (α=0.01)

4.2. ROOT TRAITS ANALYSIS BY DIGITAL IMAGING FOR ROOT TRAITS

Root architecture and development is not typically central to previous plant research (Wright et al., 2004). However, knowledge about plants roots can provide assistance in the development of many root parameters (Smit et al., 2000). The composition of the growth media, the presence of water, nutrients and toxic substances can affect number and length of roots (Malamy, 2005; Finnegan et al., 2003). Branching and dispersal of secondary and tertiary roots from the primary root confirm that roots undergo sensory exploration in pursuit of nutrients (Gasparikova, 2002). Root growth in pursuit of nutrients and water results in allocation of biomass to root structure. The presence of such interlocking roots of the plants mechanically reinforces the soil stability (Preston et al., 1999; Morgan et al., 1995; Waldron et al., 1981). Also, manual study of root growth is a time-consuming process and also usually invasive (Silva et al., 2011; Reubens et al., 2007). Therefore, root imaging and analysis for root traits provides quicker insights about root architecture and is easy to use. Studying root structure and growth behavior can help understand plant's capability to control soil erosion and slope stabilization in various environmental conditions.

After 77 days, the plants grown for the bioassay experiment were uprooted and cleaned for imaging. Several root traits were obtained for dicots and monocots by processing the root images using D.I.R.T. (Digital Imaging for Root Traits). D.I.R.T. directly assesses several root traits such as projected root area, root width, root depth, number of basal roots and adventitious roots etc. The complete list of all the root traits for all 4 plant species is shown in Appendix.

4.2.1. Root Traits Analysis for Dicots. Pearson correlation coefficients (*r*) (Microsoft Excel) and the corresponding *p*-values (Minitab®) between selected root traits and root biomass production in poplars and willows which were grown in 20% to 100% leachate concentrations are shown in Table 4.3. Pearson correlation coefficient (*r*) was determined using the following equation: $= PEARSON(array1, array2)$

Here, $array1$ is root biomass from 20% to 100% and $array2$ is root traits (projected root area, root depth, root width etc.) from 20% to 100%. Corresponding *p*-values for each correlation was determined using correlation functions in Minitab®.

Pearson correlation coefficient can indicate the extent of relationship between root biomass production and several root traits including root area, root depth, number of roots etc. In other words, increase or decrease in the selected root parameters will be reflected by increase or decrease in root biomass production, respectively. In poplars, no significant correlations were observed in terms of the width of the roots and number of adventitious roots. Also in willows, number of adventitious roots did not show a statistically significant correlation with root biomass production. Both the dicots displayed a strong positive correlation with projected root area and number of root tips. In willows, correlation between root biomass production and number of adventitious roots was not statistically significant. This indicates that willows irrigated with leachate concentrations did not have a significant effect on the number of adventitious roots. Therefore, it can be interpreted that changes in biomass production in the root system of dicots caused due to leachate exposure, can also result in variations in the projected root area, width of the roots, number of root tips and adventitious root counts. Also, the number of adventitious roots in willows during the leachate exposure period were relatively unaffected. This outcome suggests that out of the two dicots, willows could have a better adventitious root system than poplars.

Root traits and root biomass for dicots, which had similar patterns have stronger correlations than the plots with dissimilar patterns. Figure 4.9 shows plots with root traits (projected root area, skeleton width, number of root tips and number of adventitious roots) and root biomass for *Populus* and *Salix*.

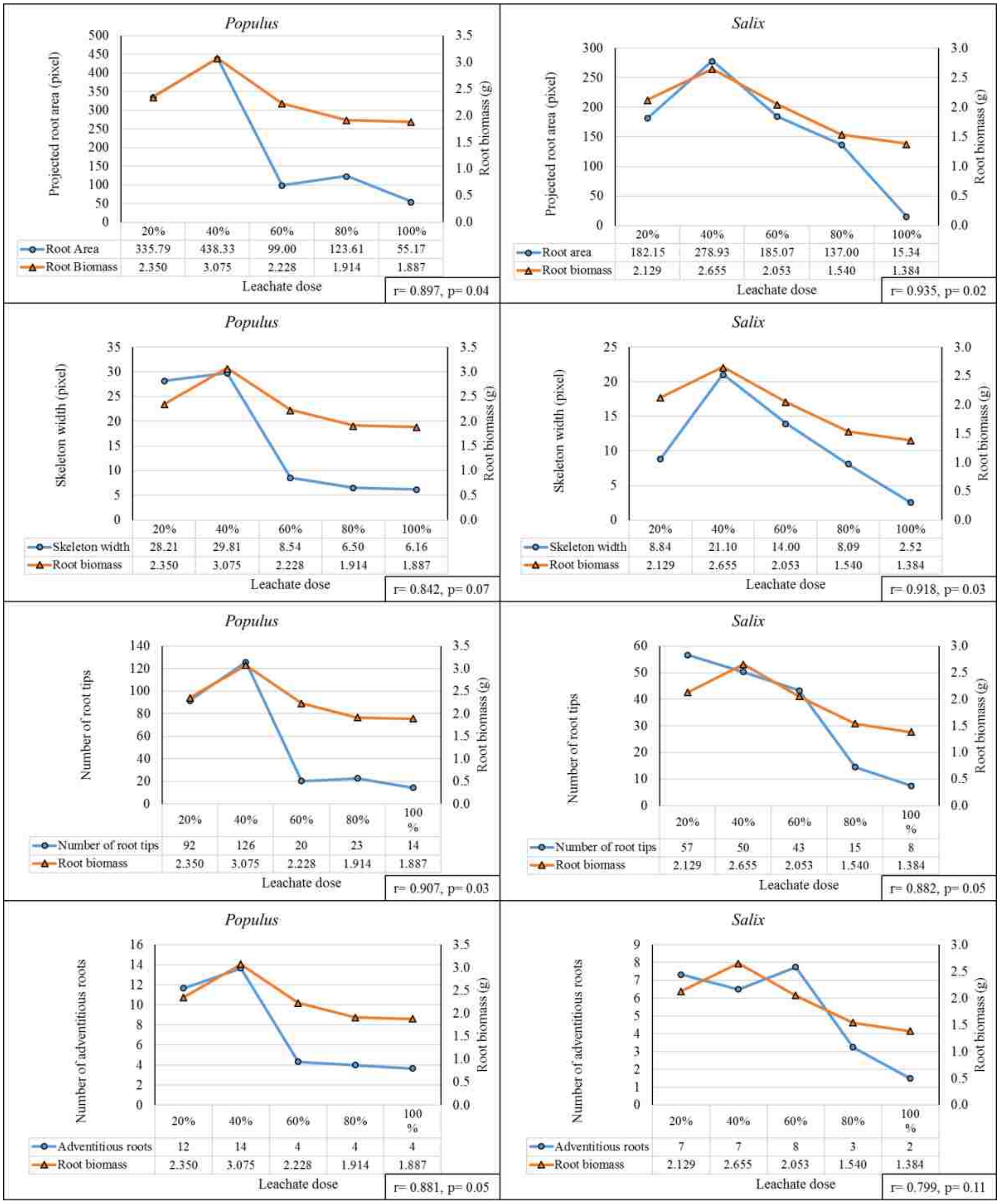
4.2.2. Root Traits Analysis for Monocots. In monocots, the root and shoot biomass production exhibited a similar pattern for respective leachate concentrations. However, root traits in vetiver and fescue differ from each other, as shown in Table 4.4. Unlike fescue, vetiver demonstrated a strong correlation between root biomass production and some of the root traits such as root area, average root density, root width and number of adventitious roots. Whereas, root biomass production was strongly correlated with root traits such as skeletal depth and number of basal roots. Therefore, root traits such as root depth and number of basal roots were not affected by leachate exposure in vetiver. Such finding is consistent with other parameters that indicate vetiver was the least sensitive of the tested species. However, in fescue, the projected root area, average root density, root width and number of adventitious roots were relatively independent of leachate exposure. Even with same environmental conditions both the monocots express several differences with respect to root traits. Differences between vetiver and fescue suggest that both the monocots have a different way of coping with stress caused due to leachate applications.

Table 4.3: Pearson's correlation coefficient (r) and p-value between measured belowground biomass and computed root trait values for dicots (20% to 100) (shaded values shows strong positive correlation and $p \leq 0.05$)

Root Traits	**Description**	***Populus***		***Salix***	
		r	***p***	***r***	***p***
Projected root area	Total number of pixels belonging to the root	**0.897**	**0.04**	**0.935**	**0.02**
Skeleton width	Width calculated from the medial axis	0.842	0.07	**0.918**	**0.03**
Number of Root Tips	Corresponds to the overall number of tips detected in the image	**0.907**	**0.03**	**0.882**	**0.05**
Number of adventitious roots	Number of adventitous roots estimated	**0.881**	**0.05**	0.799	0.11

Number of basal roots and adventitious roots are functionally different and change in the distribution of these roots can describe the root behavior under stressed conditions. The increase in number of adventitious roots often results in stunted growth of basal roots (Walk et al., 2006). Also, basal roots near ground surface compete with adventitious roots but complement adventitious roots with increased depth. Adventitious roots explore near the surface while basal roots tend to propagate deeper (Walk et al., 2006). Vetiver displaying weaker correlation for root depth and number of basal roots indicates that vetiver can be considered a better choice where root depth is required in leachate contaminated sites; whereas, fescue can be considered for root distribution near the surface.

Root traits and root biomass for dicots, which had similar patterns have stronger correlations than the plots with dissimilar patterns. Figure 4.10 shows plots with root traits (projected root area, average root density, skeleton depth, skeleton depth, skeleton width and number of adventitious roots) and root biomass for *Vetiveria* and *Festuca*.



Populus	20%	40%	60%	80%	100%
Root Area	335.79	438.33	99.00	123.61	55.17
Root Biomass	2.350	3.075	2.228	1.914	1.887

Salix	20%	40%	60%	80%	100%
Root area	182.15	278.93	185.07	137.00	15.34
Root biomass	2.129	2.655	2.053	1.540	1.384

Populus	20%	40%	60%	80%	100%
Skeleton width	28.21	29.81	8.54	6.50	6.16
Root biomass	2.350	3.075	2.228	1.914	1.887

Salix	20%	40%	60%	80%	100%
Skeleton width	8.84	21.10	14.00	8.09	2.52
Root biomass	2.129	2.655	2.053	1.540	1.384

Populus	20%	40%	60%	80%	100 %
Number of root tips	92	126	20	23	14
Root biomass	2.350	3.075	2.228	1.914	1.887

Salix	20%	40%	60%	80%	100 %
Number of root tips	57	50	43	15	8
Root biomass	2.129	2.655	2.053	1.540	1.384

Populus	20%	40%	60%	80%	100 %
Adventitious roots	12	14	4	4	4
Root biomass	2.350	3.075	2.228	1.914	1.887

Salix	20%	40%	60%	80%	100 %
Adventitious roots	7	7	8	3	2
Root biomass	2.129	2.655	2.053	1.540	1.384

Figure 4.9: Root traits and root biomass of dicots at 20% to 100% leachate doses (with *r* and *p* values)

Table 4.4: Pearson's correlation coefficient (r) and *p*-value between measured belowground biomass for 20% to 100% leachate dosages and computed root trait values for monocots (shaded values shows strong positive correlation and $p \leq 0.05$)

Root Traits	**Description**	***Vetiveria***		***Festuca***	
		r	***p***	***r***	***p***
Projected root area	Total number of pixels belonging to the root	**0.961**	**0.01**	0.720	0.17
Average root density	Ratio of foreground to background pixels within the root shape	**0.878**	**0.05**	-0.014	0.98
Skeleton depth	Rooting depth calculated from the skeleton	0.386	0.52	**0.884**	**0.05**
Skeleton width	Width calculated from the medial axis	**0.957**	**0.01**	0.401	0.50
Number of basal roots	Number of basal roots estimated	-0.137	0.83	**0.970**	**0.01**

Overall, the rooting structure analysis showed similar patterns to the biomass production and plant health. Plant development can be influenced due to changes in the environment, which changes the root morphology (Itai et al., 1991). *D.I.R.T.* can be a useful tool in monitoring how plant roots respond to leachate concentrations early in the growth or exposure period as an indicator of plant stress or growth promotion in leachate phytotreatment systems. The early stages of root establishment can indicate plant health and stress (Postma et al., 2014). Methods for monitoring plant health and stress are essential for effective and efficient application of phytotechnologies for the treatment of diverse sites and leachate types. Plants with tolerant root traits can be screened using *D.I.R.T.* and also plant breeders can use this technology in breeding new hybrid varieties with root traits which can be beneficial for phytotechnologies. It is important to consider presence of soil in field conditions, as plant roots in soil are bound tighter than in sand. Presence of soil can affect root traits significantly and should be studied with respect to leachate exposure.

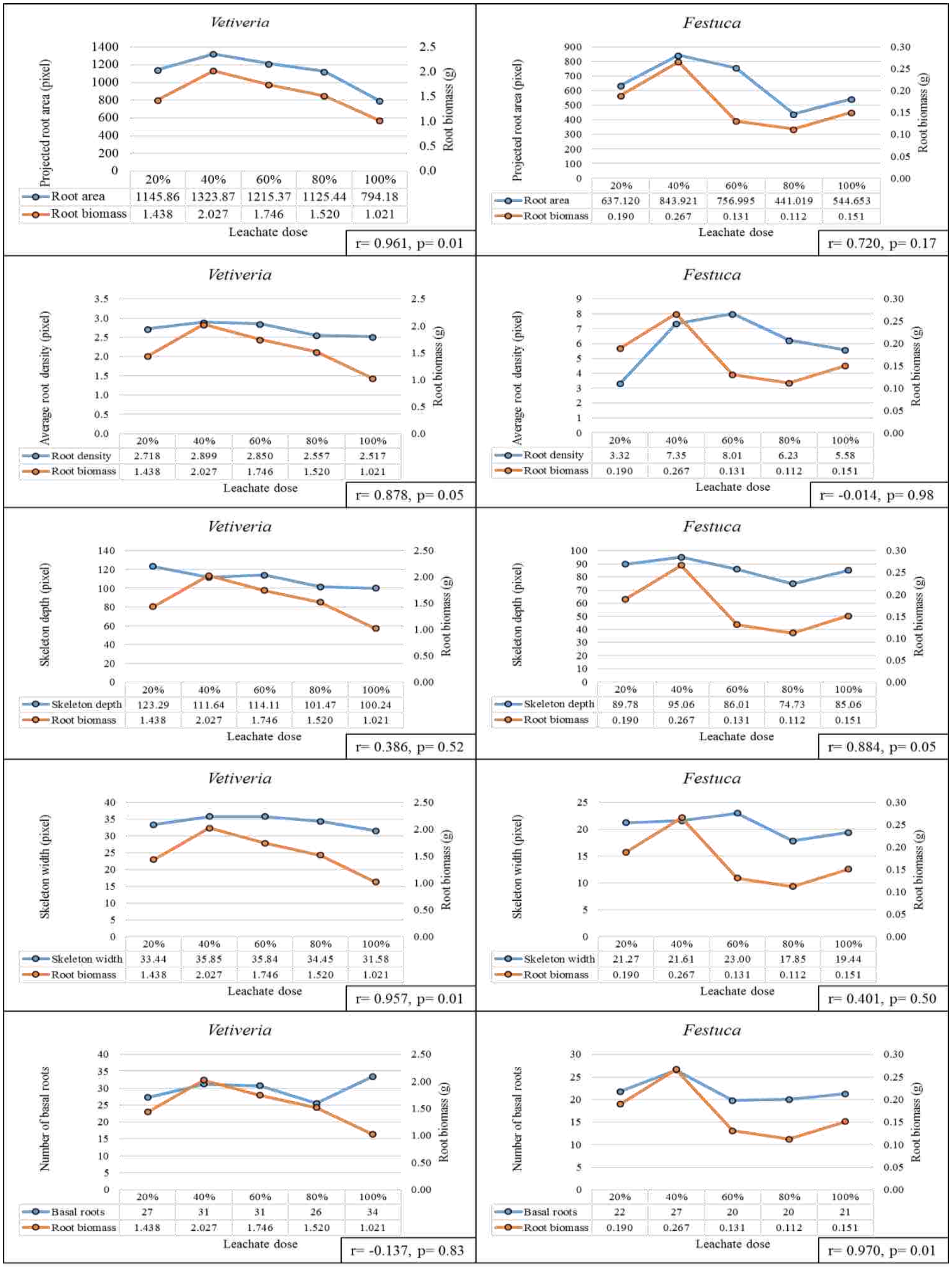


Figure 4.10: Root traits and root biomass for monocots at 20% to 100% leachate doses (with *r* and *p* values)

4.3. HYPERSPECTRAL DATA ANALYSIS AND RESULTS

This section describes the hyperspectral results obtained by the hyperspectral camera and the spectroradiometer.

4.3.1. Headwall Nano-Hyperspec Hyperspectral VNIR Imager Results. After 53 days of leachate exposure, the hyperspectral reflectance for each plant was recorded by using a Headwall Nano-Hyperspec hyperspectral VNIR Imager. Hyperspectral images have been used to identify vegetation stress (Nilsson, 1995). Various vegetation indices were then quantified to assess plant health at different leachate exposures.

As an example, *Populus* images taken by Headwall Nano-Hyperspec hyperspectral VNIR Imager and the respective processed Differential Vegetation Index (DVI) images are shown in Figure 4.11. DVI is a simple vegetation index, which distinguishes vegetation from the background. DVI ignores the differences between radiance and reflectance caused by shadows (Tucker, 1979). DVI is calculated using the expression: DVI $= NIR - Red$. Such images are an excellent way to visualize plant status with respect to environmental stress. However, in HSI, low pixel resolution and low growth density of the monocots (mostly fescue) made detection of monocots growing in stressed conditions, difficult. Hence, reflectance data from three points per plants were averaged as a representative for each individual plant. Same data collection procedure was used to obtain representative reflectance for all plants using FieldSpec-Pro.

The reflectance obtained by both the instruments during the experimental period are strongly correlated with each other (Pearson's correlation coefficient $r = 0.997$, coefficient of determination $r^2 = 0.994$ and calculated probability p-value <0.001). Therefore, hyperspectral reflectance data obtained by either of these instruments can be utilized for calculation of vegetation indices and assess plant health and stress. Reflectance measurements were used to calculate several vegetation indices. To link the above ground biomass production to all vegetation indices a Pearson's correlation coefficient was used with *p*-values to choose vegetation indices showing significant associations with leaf counts (dicots), as shown in Table 4.5.

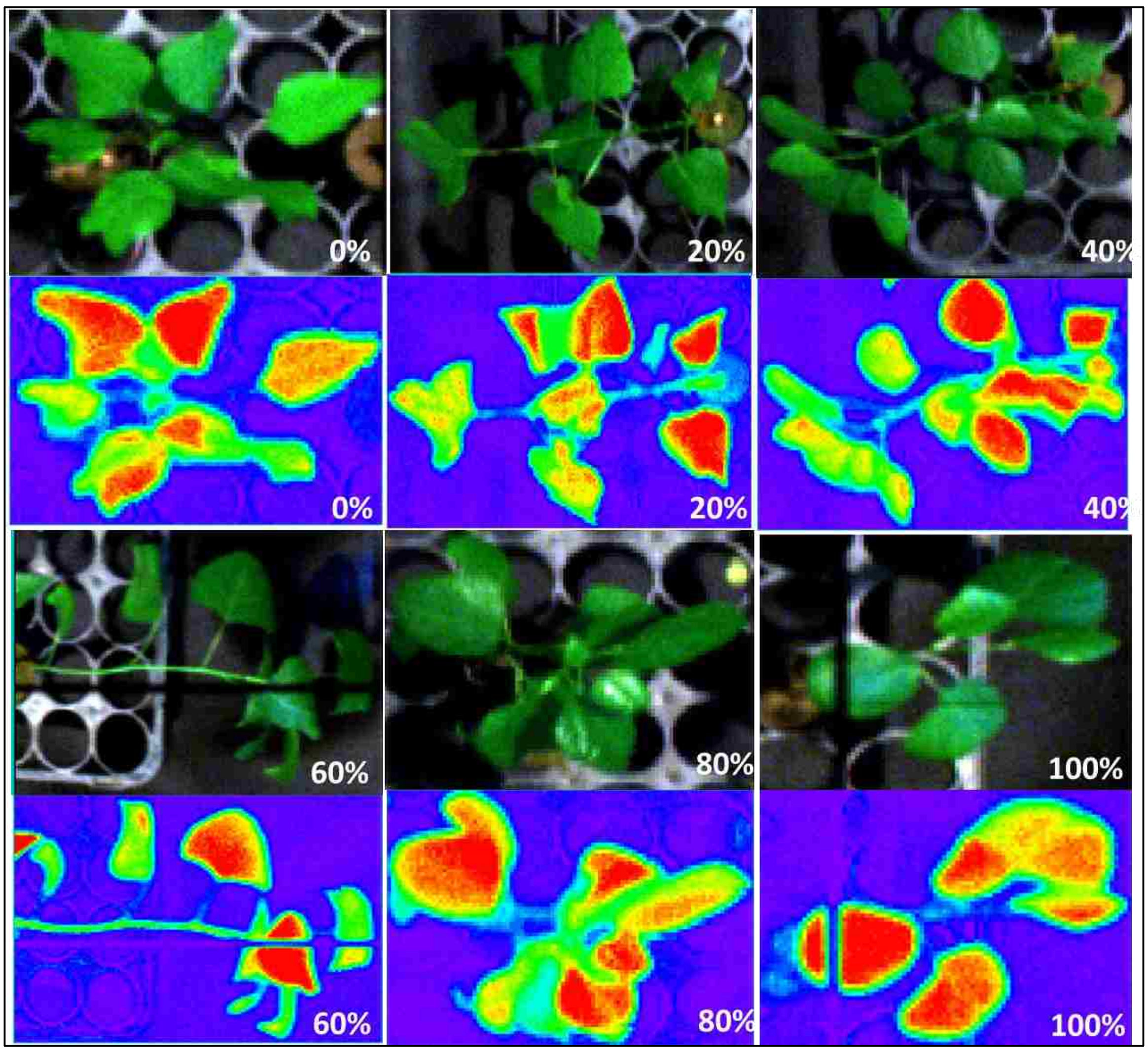


Figure 4.11: Differential Vegetation Index images obtained from hyperspectral images of *Populus* at various leachate doses using ENVI (colorized images show red as high DVI and blue with lowest DVI)

Table 4.5: Pearson's correlation coefficient (*r*) and *p*-value between leaf counts (after 50 days) for 20% to 100% leachate dosages and vegetation indices for *Populus*, *Salix*, *Vetiveria*, and *Festuca* using hyperspectral camera (shaded values show strong correlation ($r > \pm 0.70$) and bold font show statistical significance $p \leq 0.05$)

Dicots					
Vegetation Index	**Information about**	***Populus***		***Salix***	
		r	***p***	***r***	***p***
NDVI750	Chlorophyll	0.775	0.12	0.858	0.06
NDVI	Biomass	**0.874**	**0.05**	0.848	0.07
NDVI2	Biomass	**0.892**	**0.04**	0.857	0.06
WI	Water Content	0.273	0.65	-0.668	0.22
WBI	Water Status	-0.282	0.64	0.668	0.22
MCARI1	Chlorophyll	**0.882**	**0.04**	0.769	0.13
MCARI2	Chlorophyll	**0.887**	**0.04**	0.818	0.09
PSRI	Plant Senescence	**-0.895**	**0.04**	-0.790	0.11
GM2	Chlorophyll	0.810	0.09	**0.871**	**0.05**
R801/R550	Photosynthesis	0.146	0.81	**0.892**	**0.04**
R740/R850	Stress	0.768	0.13	0.724	0.17
LIC1	Stress	**0.891**	**0.04**	0.854	0.06
LIC2	Stress	0.320	0.60	0.680	0.206
GM1	Chlorophyll	0.423	0.48	**0.944**	**0.01**
VOG1	Chlorophyll	0.809	0.09	0.806	0.09
VOG2	Chlorophyll	-0.832	0.08	-0.736	0.15
VOG3	Chlorophyll	-0.809	0.09	-0.766	0.13
Carter1	Stress	-0.793	0.11	-0.690	0.19

In Table 4.5, the correlation between leaf count data obtained after 50 days and vegetation indices calculated after 53 days is shown. As a result, a strong correlation was observed in both the dicots with many vegetation indices listed in Table 4.5.

In poplars, statistical significance was observed only in with vegetation indices such as NDVI, NDVI2, MCARI1, MCARI2, PSRI, and LIC1. These observations show that in poplars, vegetation indices related to biomass production, chlorophyll content, plant senescence and plant stress were in strong significant correlation with measured shoot biomass. In willows, a strong significant correlation was only observed in vegetation indices which are related to chlorophyll content and photosynthesis (GM2, R801/R550, and GM1). These differences in dicots can be considered natural, as poplars and willows have a different genetic makeup and both dicots cope with leachate exposure in a different way.

For example, MCARI1 and leaf count are showing a strong significant correlation in poplars (r= 0.882 and p= 0.04) with similar patterns unlike willows (r= 0.769 and p= 0.13). In willows, GM1 and leaf count are statistically significant (r= 0.944 and p= 0.01) while poplars are not (r= 0.423 and p= 0.48). Figure 4.12 shows plots with VIs (MCARI1 and GM1) and leaf counts for *Populus* and *Salix*.Plants are known to respond to decrease in oxygen in the rhizosphere, which leads to suppressed respiration of root system, eventually impacting plant survivability (Hoeks, 1972; Gilman et al., 1982).

Also, early detection of changes in reflectance in plants exposed to herbicide is noted by researchers (Carter et al., 1994; Carter et al., 1996). Similarly, associations between leaf counts and vegetation indices indicate the potential for early detection of changes in pigment content, leaf anatomy, plant senescence etc., using hyperspectral image analysis.

These relationships between leachate exposure of plants and detection of changes in plant anatomy can be used to monitor plant health on a landfill site to predict leachate outbreaks. However, monitoring the changes in spectral reflectance over the entire period of the experiment could provide a better resolution with respect to early detection of stress in plant health.

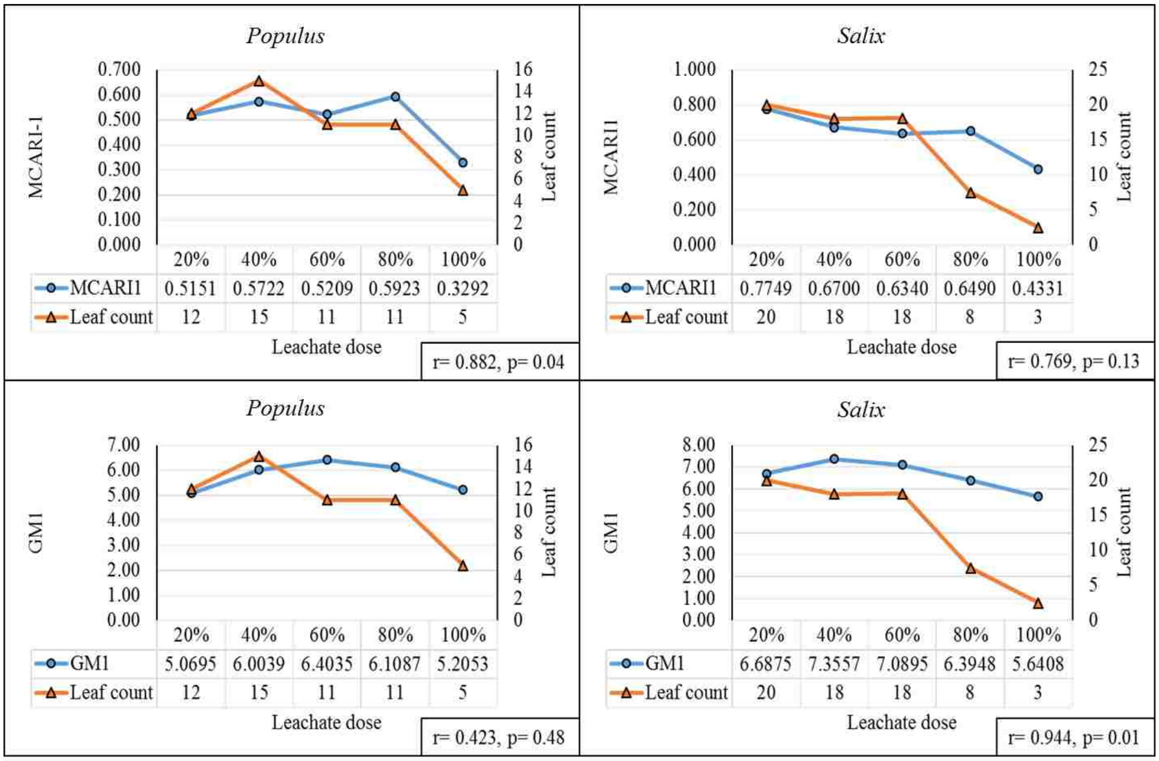


Figure 4.12: VIs and leaf count (50 days) in dicots at 20% to 100% leachate doses (with *r* and *p* values).

4.3.2. FieldSpec-Pro Results. After 74 days, using FieldSpec-Pro (Analytical Spectral Devices (ASD) Inc.) hyperspectral reflectance was recorded, covering a wavelength range from 350 to 2500nm. The reflectance was measured a day before harvesting the plants grown for the bioassay.

Hyperspectral measurements by FieldSpec-Pro suggested that vegetation indices can show a hormetic pattern of stimulating health at lower doses and deteriorating health at higher doses for willow, vetiver, and fescue. Poplar did not exhibit such pattern consistently in terms of vegetation indices. For willow, vetiver, and fescue the leachate demonstrated beneficial effects on plant health at lower doses, but deteriorating effects were consistently observed with an increase in leachate concentration above 60% for this study. The vegetation indices for all four plant species indicated highest index values for plant health at 20% and 40% leachate solutions. Similar patterns in the plant health indices with respect to biomass production are consistent with the previous finding that low leachate concentrations can provide nutrient benefits and low toxicity. To show the relationship of remote sensing to plant health, correlation between measured plant biomass production and vegetation indices (from 20% to 100%) is shown in Table 4.6.

Table 4.6: Pearson's correlation coefficient (r) and p-value between measured aboveground biomass for 20% to 100% leachate dosages and vegetation indices for *Populus*, *Salix*, *Vetiveria*, and *Festuca*, using FieldSpec-Pro (shaded values show strong correlation ($r>\pm0.70$) and bold fonts show statistical significance i.e. $p\leq0.05$)

Vegetation Index	**Dicots**				**Monocots**			
	Populus		***Salix***		***Vetiveria***		***Festuca***	
	r	***p***	***r***	***p***	***r***	***p***	***r***	***p***
NDVI750	0.593	0.29	**0.945**	**0.02**	**0.932**	**0.02**	0.697	0.19
NDVI	0.597	0.29	**0.918**	**0.03**	0.697	0.19	0.492	0.40
NDVI2	0.592	0.29	**0.918**	**0.03**	0.69	0.20	0.463	0.43
WI	0.739	0.15	**0.945**	**0.01**	0.707	0.18	0.777	0.12
WBI	-0.738	0.15	**-0.947**	**0.01**	-0.704	0.19	-0.772	0.13
MCARI1	0.718	0.17	**0.888**	**0.04**	0.574	0.31	0.728	0.16
MCARI2	0.669	0.22	**0.91**	**0.04**	0.83	0.08	0.719	0.17
PSRI	-0.667	0.22	**-0.907**	**0.03**	-0.06	0.92	**0.877**	**0.05**
GM2	0.647	0.24	**0.919**	**0.04**	**0.897**	**0.04**	0.654	0.23
R801/R550	0.334	0.58	**0.926**	**0.02**	**0.884**	**0.05**	**0.885**	**0.05**
R740/R850	0.804	0.10	**0.903**	**0.03**	**-0.871**	**0.05**	**-0.897**	**0.04**
LIC1	0.604	0.28	**0.921**	**0.03**	0.696	0.19	0.473	0.42
LIC2	**0.904**	**0.04**	0.769	0.14	0.032	0.96	**-0.972**	**0.01**
GM1	-0.517	0.49	**0.944**	**0.02**	**0.881**	**0.05**	0.877	0.05
VOG1	0.413	0.37	**0.954**	**0.02**	**0.962**	**0.01**	0.788	0.11
VOG2	0.523	0.65	**-0.976**	**0.01**	**-0.956**	**0.01**	-0.865	0.06
VOG3	-0.275	0.62	**-0.977**	**0.01**	**-0.955**	**0.01**	-0.864	0.06
Carter-1	-0.662	0.22	**-0.962**	**0.01**	-0.031	0.96	**0.942**	**0.02**

For example, LIC2 and shoot biomass is showing a significant correlation in poplars (r= 0.904 and p= 0.04) with similar patterns unlike willows (r= 0.769 and p= 0.14). In willows, GM1 and shoot biomass are statistically significant with strong positive correlation (r= 0.944 and p= 0.02) while poplars are not (r= -0.517 and p= 0.49). Figure 4.13 shows plots with VIs (LIC2 and GM1) and shoot biomass for *Populus* and *Salix*.

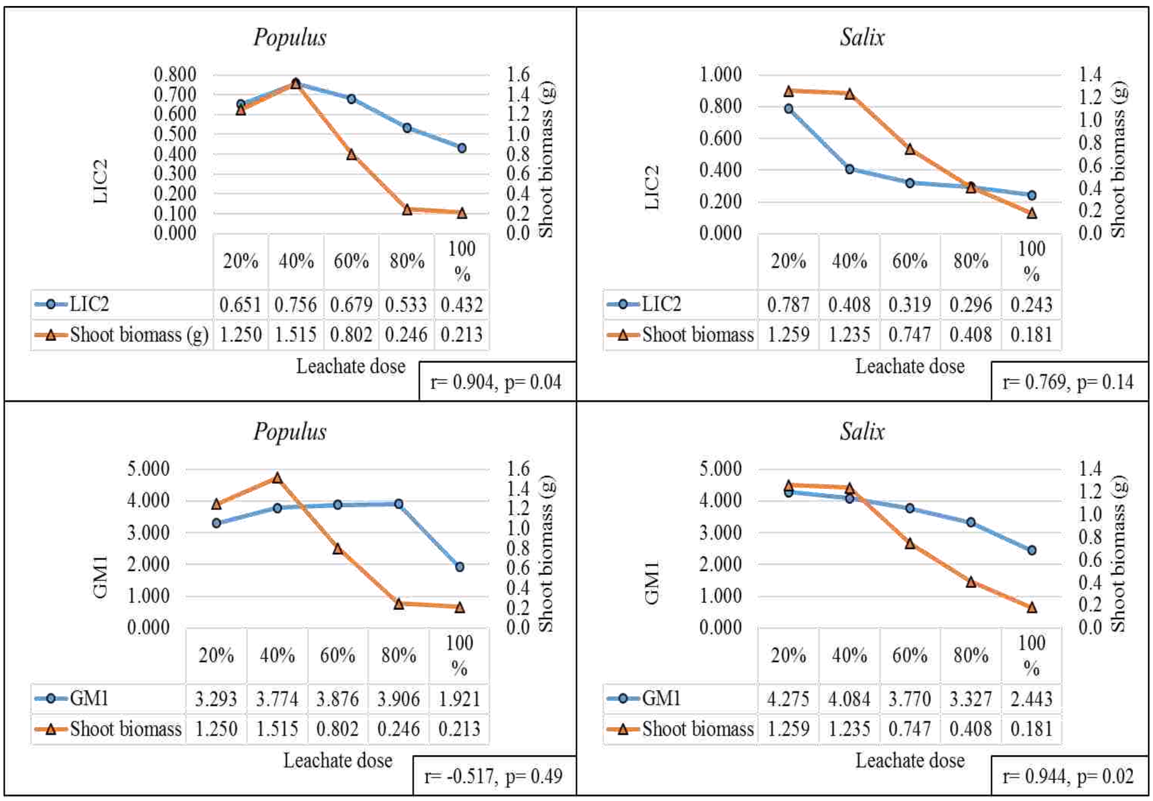


Figure 4.13: VIs and shoot biomass in dicots at 20% to 100% leachate doses (with *r* and *p* values).

For example, NDVI750 and shoot biomass is showing a significant correlation in vetiver (r= 0.932 and p= 0.02) with similar patterns unlike fescue (r= 0.697 and p= 0.19). In fescue, LIC2 and shoot biomass are statistically significant with strong positive correlation (r= -0.972 and p= 0.01) while vetivers are not (r= 0.032 and p= 0.96).

Figure 4.14 shows plots with VIs (NDVI750 and LIC2) and shoot biomass for *Vetiveria* and *Festuca*.

All plants have varying degrees of differences, which make each plant unique. Plants grown in similar environmental conditions may react differently. Such differences in plants are observed not just physically but also in the reflectance spectra. A statistically significant correlation describes the associations of a measured parameter such as above ground biomass and vegetation health indices. Ranking the plants in descending order of number of vegetation indices showing a strong correlation with measured above ground biomass, it is evident that in willow, a strong correlation with statistical significance was observed in multiple vegetation indices. Willow was followed by vetiver and fescue which showed a strong correlation in 7 and 4 vegetation indices, respectively. In poplar, the vegetation indices were not showing a significant correlation except LIC-2. Similar trends were observed in the correlation between leaf counts (75 days) in dicots and vegetation indices calculated using FieldSpec-Pro, see Table 4.7.

At the end of the bioassay experiment, leaf counts in willows exhibited a strong correlation with all the vegetation indices, while poplar only showed a strong correlation in vegetation indices related to water content, chlorophyll content and plant stress. These differences among plant species are due to several differences inherited by the plants. Correlations could also aid in segregating stress caused by specific compounds, which in future can establish better detection and monitoring tools for hazard mitigation in a landfill site and beyond. For example, LIC2 and leaf count is showing a significant correlation in poplars ($r= 0.921$ and $p= 0.02$) with similar patterns unlike willows ($r= 0.766$ and $p= 0.13$). In fescue, GM1 and leaf count are statistically significant with strong positive correlation (r= 0.485 and p= 0.40) while poplars are not (r= 0.950 and p= 0.01). Figure 4.15 shows the plots with VIs (LIC2 and GM1) and shoot biomass for *Populus* and *Salix*.

Table 4.7: Pearson's correlation coefficient (r) and p-value between leaf count (75 days) for 20% to 100% leachate dosages and vegetation indices for dicots, using FieldSpec-Pro (shaded values show strong correlation (r>±0.70) and bold fonts show statistical significance i.e. p≤0.05)

Vegetation Index	**Information**	**Dicots**			
		Populus		***Salix***	
		r	***p***	***r***	***p***
NDVI750	Chlorophyll	0.654	0.23	**0.927**	**0.02**
NDVI	Biomass	0.661	0.22	**0.939**	**0.01**
NDVI2	Biomass	0.657	0.22	**0.942**	**0.01**
WI	Water Content	0.759	0.13	**0.916**	**0.02**
WBI	Water Status	-0.760	0.13	**-0.917**	**0.02**
MCARI1	Chlorophyll	0.762	0.13	0.744	0.12
MCARI2	Chlorophyll	0.724	0.16	0.818	0.09
PSRI	Plant Senescence	-0.724	0.16	**-0.921**	**0.02**
GM2	Chlorophyll	0.706	0.16	**0.901**	**0.04**
R801/R550	Photosynthesis	0.411	0.49	**0.949**	**0.01**
R740/R850	Stress	0.833	0.08	0.837	0.08
LIC1	Stress	0.668	0.21	**0.939**	**0.01**
LIC2	Stress	**0.921**	**0.02**	0.766	0.13
GM1	Chlorophyll	0.485	0.40	**0.950**	**0.01**
VOG1	Chlorophyll	0.585	0.30	**0.927**	**0.02**
VOG2	Chlorophyll	-0.340	0.57	**-0.943**	**0.02**
VOG3	Chlorophyll	-0.365	0.54	**-0.943**	**0.02**
Carter-1	Stress	-0.678	0.20	**-0.949**	**0.01**

Stress caused due to contaminants is considered to be dose- dependent (Lichtenthaler, 1988), showing a hormetic pattern with stimulated metabolism and plant health at low concentrations and adverse effects on plant health at higher doses. Also, vegetation indices and biomass production were strongly correlated with a variety of health indices (Calabrese and Blain, 2009).

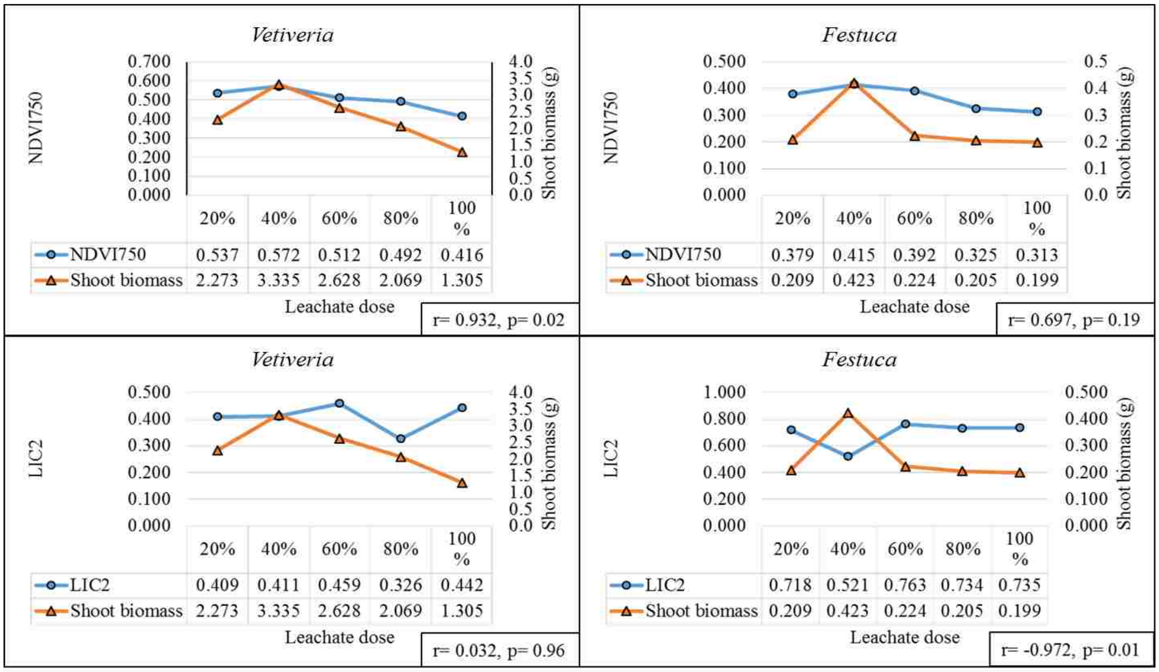


Figure 4.14: VIs and shoot biomass in monocots at 20% to 100% leachate doses (with *r* and *p* values).

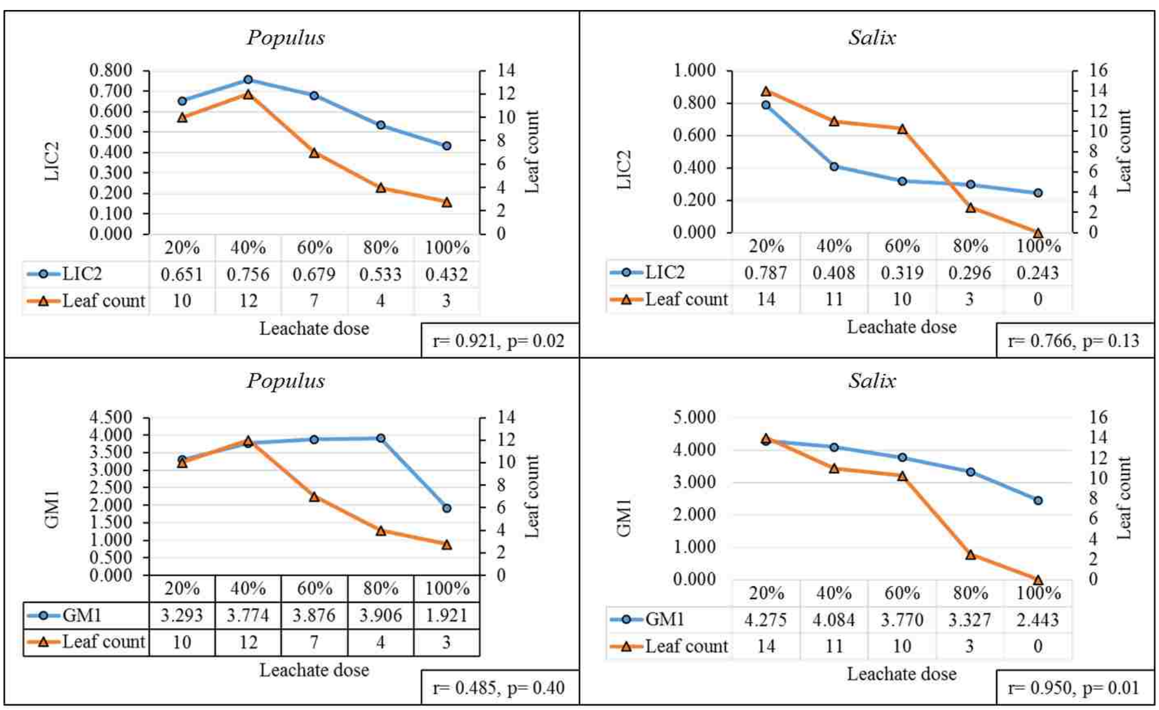


Figure 4.15: VIs and leaf counts (75 days) in dicots at 20% to 100% leachate doses (with r and p values)

The presence of contaminants in the media can cause changes in the immediate surroundings of plant roots which consecutively affects the root system adversely, causing stress. The defense system of the plant in return gets activated to cope with such stressed conditions. In this case, differences in vegetation indices among all four plants species justify that stress tolerance differs from plant to plant. Natural and anthropogenic stressors can induce changes in metabolic and genetic expressions, which in addition causes changes in pigment and biomass production (Lichtenthaler, 1998). Periodic collection of spectral data can aid in monitoring changes occurring in plants due to constant and prolonged leachate exposure. These changes can cause the plants to perform differently (better or worse) and can be detected using various vegetation indices.

4.3.3. Impacts of Hyperspectral Assessments of Plant Health. Stressed conditions induce several changes in morphology and metabolism of plants. These changes occur at an anatomical level such as changes in pigmentation, rate of photosynthesis, and structural changes in leaves (Davids and Tyler, 2003). These changes can be detected by using remote sensing technologies. Correlation between VIs and plant growth parameters can indicate plant health and stress from environmental conditions. Plants exposed to different dilutions of same leachate solution impacted biomass production, leaf count, root traits and vegetation indices. Impacts were positive at low concentrations and negative at high concentrations, consistent with hormetic patterns previous demonstrated for leachate exposure (Del Moro et al., 2014, Calabrese, 2007). Vegetation indices, which were strongly correlated with leaf count and biomass, can be considered for detection of plant attributes in the field test as well. However, the four tested plant species has demonstrated correlations between plant health parameters and different VIs, indicating variable stress response among the species. While hyperspectral imaging data did indicate leachate stress anomalies, multiple indices should be evaluated for any specific species. The remote sensing of plant stress from environmental pollutants can indicate toxic impacts early, potentially preventing continued exposure or spreading to prevent impacts to human health and environment. In vegetation based leachate treatment system, early detection of plant stress caused by leachate exposure can preserve plant survival by controlling leachate concentrations and application rates, which may also minimize plant mortality and complete failure of the phytotechnologies (Jones et al., 2006).

5. CONCLUSIONS

The study successfully assessed plant stress and health for use in phytotechnologies of leachate treatment for landfills. The relationships between plant health parameters such as biomass production and leaf counts, root phenotyping and vegetation indices were successfully evaluated. Specific conclusions are as follows:

The greenhouse bioassay experiment provided evidence of plant hormesis in all four plant species with stimulated growth at lower leachate concentrations and decrease in growth with an increase in leachate concentrations. In dicots, shoot biomass production was observed to be higher around 20%-40% leachate concentrations and higher root biomass allocation towards the root system in plants growing in nutrient deficient water (0%) and at 40% leachate concentrations. Whereas, in monocots, the shoot biomass production was following a similar pattern as in the roots, with higher overall (shoot and root) biomass production around 40% leachate concentration. Therefore, from the bioassay of plants, we can conclude that plant systems can efficiently balance between leachate treatment and survival, provided the leachate concentrations are controlled and provide nutrients and water necessary for enhanced growth and irrigation of the plants. In general, the methods for evaluating plant health and stress were consistent in showing a hormetic pattern, including toxic impacts at higher leachate concentrations.

Hyperspectral measurements revealed similar patterns and were statistically correlated to leaf counts (in dicots) and biomass assessments. The correlation indicates that remote sensing can assess plant stress and health effectively, with low cost of resource investment. In dicots, vegetation indices exhibited relationships with growth parameters such as leaf counts and shoot biomass and vegetation indices obtained using a hyperspectral camera and a radio-spectrometer. Such relationships between plant growth parameters and vegetation indices effectively display a potential for early detection of plant stress and effects on pigments and photosynthesis on a landfill site. Also, these relationships vary in different plant species and are relative to certain vegetation indices which show stronger relationships with ground truth measurements. Further research is required to detect changes in plants with respect to leachate exposure in a field setting.

Methods of assessing root architecture and growth also indicated that leachate exposure can not only affect biomass production in roots but can also affect root development and traits. Relationships between root biomass and root traits were successfully established using root image analysis. Information about root phenotyping can also help in screening plants, which have tolerant root traits, and for plant breeding to produce high performing plants for phytotechnologies where plants are actively interacting with pollutants and root structure plays a key role.

Root establishment is a strong indicator of plants creating a healthy foundation for growth, which also contributes to enhanced treatment of leachate for prolonged periods while preventing soil erosion, providing soil stabilization and promoting soil ecology. Results obtained from image analysis of roots were correlated to root biomass production. These results conclude that image analysis can be used as a tool for screening plants with relatively higher treatment efficacy. Image analysis can also be used as a screening tool for selection of plants with dense and robust root structure for landfill applications.

Relationships of hyperspectral data with biomass production and leaf counts also indicates that remote sensing relates to the comprehensive health of plants and can be used for early detection of toxic impacts before mortality sets in. The use of hyperspectral imaging also offers potential to identify potential fugitive leachate plumes that may impact human health and environment, if left undetected for long periods. Also, hyperspectral imaging can be utilized for monitoring plants which are involved in leachate treatment systems and for other phytoremediation applications. Use of such nondestructive and low-cost plant health assessments will be beneficial for detection of pollutants interacting with plants.

6. RECOMMENDATIONS FOR FUTURE RESEARCH

1. Exposure of leachate to a wide number of plant species to categorize variations in stress symptoms.
2. Effect of change in leachate composition on plant health should be assessed.
3. Field scale tests of plants exposed to leachate are required to study *in situ* plant response.
4. Monitoring changes in vegetation indices over the entire period of leachate exposure can help document the spectral changes occurred over the exposure period.
5. Evaluate contaminant degradation potential of individual plant-soil systems to create better phytotechnologies for landfill stabilization and leachate treatment systems.
6. Measurement of biological activity in soil of each plant-soil systems can provide information about plant-microbes association best suitable for remediation of contaminants.

APPENDIX

Shoot biomass measurements for individual plants

	Dry Biomass (g)							
	Populus (g)				*Salix (g)*			
Leachate dose	1	2	3	4	1	2	3	4
0%	0.83	1.098	0.813	0	0.619	0.624	0.503	0.6
20%	1.155	1.125	1.36	0	0.787	1.355	1.448	0
40%	1.712	1.924	1.211	0	1.255	1.451	1.103	1.129
60%	0.725	0.87	0.805	0	0.751	0.557	0.752	0.929
80%	0.25	0.346	0.129	0.185	0.389	0.324	0.524	0.394
100%	0.286	0.252	0.194	0	0	0.275	0.087	0
Hoagland's	3.276	3.67	3.023	0	1.85	1.244	1.756	2.647
	Vetiveria (g)				*Festuca (g)*			
Leachate dose	1	2	3	4	1	2	3	4
0%	1.162	1.938	2.328	0.905	0.054	0.045	0.05	0.047
20%	1.759	2.524	2.457	2.352	0.198	0.301	0.155	0.183
40%	3.162	3.624	3.549	3.005	0.398	0.462	0.288	0.542
60%	2.424	2.44	2.259	3.387	0.193	0.151	0.251	0.299
80%	2.98	0.95	1.898	2.448	0.179	0.209	0.213	0.219
100%	0.919	1.308	1.917	1.077	0.208	0.222	0.179	0.188
Hoagland's	4.147	4.21	3.847	3.951	0.801	1.817	1.072	1.747

Root Biomass measurements for individual plants

	Populus (g)				*Salix (g)*			
Leachate dose	1	2	3	4	1	2	3	4
0%	4.06	3.825	3.918	4.176	3.271	2.161	2.528	2.617
20%	3.108	1.942	2.154	2.197	1.639	2.535	1.887	2.456
40%	2.604	3.256	3.294	3.147	2.436	3.064	2.585	2.534
60%	1.573	2.77	2.742	1.826	2.177	1.556	2.316	2.162
80%	1.818	2.045	1.816	1.976	1.61	1.344	1.478	1.729
100%	1.953	1.966	2.27	1.357	1.629	1.114	1.355	1.436
Hoagland's	1.415	2.317	2.377	2.041	2.678	2.729	3.191	2.753
	Vetiveria (g)				*Festuca (g)*			
Leachate dose	1	2	3	4	1	2	3	4
0%	0.934	1.156	1.154	0.615	0.174	0.097	0.081	0.079
20%	0.86	1.753	1.647	1.49	0.254	0.296	0.164	0.352
40%	2.124	1.818	2.135	2.03	0.171	0.219	0.203	0.165
60%	1.703	1.507	1.542	2.233	0.171	0.122	0.095	0.135
80%	1.977	0.669	1.459	1.974	0.099	0.096	0.128	0.125
100%	0.566	0.838	2	0.681	0.121	0.154	0.161	0.168
Hoagland's	1.958	1.519	0.859	1.423	0.345	1.593	1.071	1.154

D.I.R.T. parameters with description:

Sr. No.	Trait Name	Trait Description
1	Stem Diameter	Stem Diameter derived from the medial axis
2	Simple Stem Diameter	(beta) Simple Stem Diameter as calculated in Shovelomics 2.0 from the ETH
3	Projected Root Area	number of pixels belonging to the root (as in GIA roots)
4	Average Root Density	ratio of foreground to background pixels with in the root shape
5	Median Tip Diameter	Median Tip Diameter estimated from the medial circle over all detected tips
6	Mean Tip Diameter	Mean Tip Diameter estimated from the medial circle over all detected tips
7	Median width of root system	Median width of root system measured horizontally from the first to the last foreground pixel
8	Maximum width of root system	Maximum with of root system measured horizontally from the first to the last foreground pixel

Sr. No.	Trait Name	Trait Description
9	Accumulated width over 10 percent depth	Percentage of width accumulation at 10% depth
10	Accumulated width over 20 percent depth	Percentage of width accumulation at 20% depth
11	Accumulated width over 30 percent depth	Percentage of width accumulation at 30% depth
12	Accumulated width over 40 percent depth	Percentage of width accumulation at 40% depth
13	Accumulated width over 50 percent depth	Percentage of width accumulation at 50% depth
14	Accumulated width over 60 percent depth	Percentage of width accumulation at 60% depth
15	Accumulated width over 70 percent depth	Percentage of width accumulation at 70% depth
16	Accumulated width over 80 percent depth	Percentage of width accumulation at 80% depth
17	Accumulated width over 90 percent depth	Percentage of width accumulation at 90% depth
18	Slope of the graph of central path length vs accumulated width at 10 percent of accumulated width	Slope at the D10 value that represents the rate of accumulation
19	Slope of the graph of central path length vs accumulated width at 20 percent of accumulated width	Slope at the D20 value that represents the rate of accumulation
20	Slope of the graph of central path length vs accumulated width at 30 percent of accumulated width	Slope at the D30 value that represents the rate of accumulation
21	Slope of the graph of central path length vs accumulated width at 40 percent of accumulated width	Slope at the D40 value that represents the rate of accumulation
22	Slope of the graph of central path length vs accumulated width at 50 percent of accumulated width	Slope at the D50 value that represents the rate of accumulation
23	Slope of the graph of central path length vs accumulated width at 60 percent of accumulated width	Slope at the D60 value that represents the rate of accumulation
24	Slope of the graph of central path length vs accumulated	Slope at the D70 value that represents the rate of accumulation

Sr. No.	Trait Name	Trait Description
	width at 70 percent of accumulated width	
25	Slope of the graph of central path length vs accumulated width at 80 percent of accumulated width	Slope at the D80 value that represents the rate of accumulation
26	Slope of the graph of central path length vs accumulated width at 90 percent of accumulated width	Slope at the D90 value that represents the rate of accumulation
27	Spatial Root Distribution X	spatial distribution of the root shape in X. This is the x component of the vector pointing from the center of the bounding box of the root shape to the center of mass of the root shape
28	Spatial Root Distribution Y	spatial distribution of the root shape in Y. This is the y component of the vector pointing from the center of the bounding box of the root shape to the center of mass of the root shape
29	Rooting depth skeleton	(beta) Rooting depth calculated from the RTP skeleton
30	Skeleton width	(beta) Width calculated from the medial axis
31	Number of Root Tip Paths	Corresponds to the overall number of tips detected in the image
32	Root Top Angle	Root Top Angle measured at depth of the D10 value
33	Root Bottom Angle	Root Bottom Angle measured at depth of the D80 value
34	Soil Tissue Angle Range	range of STA angles present in the root
35	First Dominant Soil Tissue Angle	Average of the 1st significant peak in the histogram of calculated soil tissue angles binned in 1 degree steps
36	Second Dominant Soil Tissue Angle	Average of the 2nd significant peak in the histogram of calculated soil tissue angles binned in 1 degree steps
37	STA 25% 1	1st dominant angle at 25% of the RTP length
38	STA 25% 2	2nd dominant angle at 25% of the RTP length
39	STA 50% 1	1st dominant angle at 50% of the RTP length
40	STA 50% 2	2nd dominant angle at 50% of the RTP length
41	STA 75% 1	1st dominant angle at 75% of the RTP length

Sr. No.	Trait Name	Trait Description
42	STA 75% 2	2nd dominant angle at 75% of the RTP length
43	STA 90% 1	1st dominant angle at 90% of the RTP length
44	STA 90% 2	2nd dominant angle at 90% of the RTP length
45	RTA dominant angle 1	Average of the 1st significant peak in the histogram of calculated root tissue angles binned in 1 degree steps
46	RTA dominant angle 2	Average of the 2nd significant peak in the histogram of calculated root tissue angles binned in 1 degree steps
47	Minimum Soil Tissue Angle	Minimum Soil Tissue Angle measured over all RTPs
48	Maximum Soil Tissue Angle	Maximum Soil Tissue Angle measured over all RTPs
49	Median Soil Tissue Angle	Median Soil Tissue Angle measured over all RTPs
50	Root Tissue Angle Range	range of RTA angles present in the root
51	Minimum Root Tissue Angle	Minimum Root Tissue Angle measured over all RTPs
52	Maximum Root Tissue Angle	Maximum Root Tissue Angle measured over all RTPs
53	Median Root Tissue Angle	Median Root Tissue Angle measured over all RTPs
54	Roots Seg 1	(beta) number of RTPs emerging from the Hypocotyl (Root seg 1)
55	Roots Seg 2	(beta) number of RTPs emerging from the taproot (Root seg 2)
56	Number of adventitious roots	(beta) Number of adventitious roots estimated from root seg 1
57	Number of basal roots	(beta) Number of basal roots estimated from root seg 2
58	Adventitious root angels	(beta) Adventitious root angel estimated from the paths detected in the number of adventitious roots
59	Basal root angles	(beta) Basal root angles estimated from the paths detected in the number of basal roots
60	Hypocotyl Diameter	(beta) Hypocotyl Diameter estimated over the detected hypocotyl region as the average of diameters of medial circles
61	Tap root diameter	(beta) Tap root diameter estimated over the detected taproot region as the average of diameters of medial circles

Sr. No.	Trait Name	Trait Description
62	Maximum diameter at 90-100 percent depth	Maximum diameter found in the interval of 90-100 percent rooting depth
63	50 percent drop	(beta) depth value were 50% of the RTPs emerged from the central path (hypocotyl+taproot)
64	Tap root diameter at 25 percent of depth	Tap root diameter at 25 percent of depth
65	Tap root diameter at 50 percent of depth	Tap root diameter at 50 percent of depth
66	Tap root diameter at 75 percent of depth	Tap root diameter at 75 percent of depth
67	Tap root diameter at 90 percent of depth	Tap root diameter at 90 percent of depth
68	Average lateral root length	Average length of lateral roots emerging along the central path of the excised root
69	Nodal root path length	Length of the central path of the excised root
70	Lateral branching frequency	Lateral branching frequency
71	Mean nodal root diameter	Mean nodal root diameter measured along the medial axis of the excised root sample
72	Lateral mean angle	Mean angle of all lateral roots emerging from the excised root sample
73	Lateral angular range	Range of angles of the lateral root sample
74	Lateral minimum angle	minimal lateral angle present in all measurements of the excised root sample
75	Lateral maximum angle	maximal lateral angle present in all measurements of the excised root sample
76	Distance to first lateral	Distance to first lateral along the medial axis of the excised root
77	Median diameter of lateral roots	Median diameter of lateral roots estimated from the medial axis
78	Mean diameter of lateral roots	Mean diameter of lateral roots estimated from the medial axis

D.I.R.T. data for Poplars

Leachate dose	Projected root area	Maximum width	Skeleton depth	Skeleton width	Number of root tips	Adventitious roots	Basal roots
0%	541.91	31.22	74.69	39.05	146	12	21
20%	335.79	25.93	42.56	28.20	92	12	11
40%	438.33	26.80	53.24	29.80	126	14	13
60%	98.99	7.53	30.73	8.54	20	4	2
80%	123.61	5.59	41.34	6.49	23	4	5
100%	55.16	4.46	17.74	6.15	14	4	3
Hoagland's	553.63	31.95	98.28	41.53	136	26	15

D.I.R.T. data for Willows

Leachate dose	Projected root area	Maximum width	Skeleton depth	Skeleton width	Number of root tips	Adventitious roots	Basal roots
0%	958.60	26.17	163.18	43.19	220	23	34
20%	182.15	7.86	35.43	8.84	57	7	7
40%	278.93	14.17	59.47	21.09	50	7	7
60%	185.06	11.47	49.11	13.99	43	8	8
80%	136.99	4.81	44.76	8.08	15	3	4
100%	15.34	2.59	8.36	2.51	8	2	2
Hoagland's	1017.29	43.71	124.77	52.42	178	19	24.5

D.I.R.T. data for Vetiver

Leachate dose	Projected root area	Average root density	Skeleton depth	Skeleton width	Number of basal roots
0%	1591.20	2.57	153.67	38.47	41
20%	1145.86	2.72	123.29	33.44	27
40%	1323.87	2.90	111.64	35.85	31
60%	1215.37	2.85	114.11	35.84	31
80%	1125.44	2.56	101.47	34.44	26
100%	794.18	2.52	100.24	31.58	34
Hoagland's	1891.91	3.86	139.07	40.06	27

D.I.R.T. data for Fescue

Leachate dose	Projected root area	Average root density	Skeleton depth	Skeleton width	Number of basal roots
0%	320.47	3.20	89.30	17.82	34
20%	637.12	3.32	89.78	21.26	22
40%	843.92	7.35	95.05	21.60	27
60%	756.99	8.01	86.01	23.01	20
80%	441.02	6.23	74.72	17.84	20
100%	544.65	5.58	85.06	19.43	21
Hoagland's	951.12	9.29	82.47	28.57	22

Vegetation indices calculated for Poplars (FieldSpec-Pro)

Index	0%	20%	40%	60%	80%	100%	Hoagland's
NDVI750	0.339	0.389	0.455	0.442	0.415	0.169	0.350
NDVI	0.756	0.809	0.789	0.738	0.784	0.326	0.733
NDVI2	0.744	0.795	0.780	0.726	0.780	0.380	0.717
WI	1.018	1.021	1.013	1.026	0.993	0.961	1.028
WBI	0.982	0.979	0.987	0.975	1.007	1.041	0.973
MCARI1	0.996	1.180	1.057	1.026	0.918	0.295	0.572
MCARI2	0.949	1.080	0.997	0.919	0.927	0.275	0.659
PSRI	-0.004	0.003	0.004	0.023	0.023	0.163	-0.009
GM2	2.590	3.058	3.611	3.380	3.153	1.487	2.670
R801/R550	2.780	3.325	3.834	3.953	4.106	2.135	2.755
R740/R850	0.960	0.961	0.941	0.938	0.880	0.781	0.972
LIC1	0.745	0.795	0.778	0.726	0.768	0.311	0.720
LIC2	0.667	0.651	0.756	0.679	0.533	0.432	0.791
GM1	2.745	3.293	3.774	3.876	3.906	1.921	2.741
VOG1	1.254	1.294	1.390	1.384	1.352	1.150	1.272
VOG2	-0.036	-0.042	-0.061	-0.060	-0.058	-0.037	-0.038
VOG3	-0.038	-0.045	-0.066	-0.065	-0.062	-0.038	-0.040
Carter-1	2.208	2.462	1.754	1.856	2.466	2.708	1.598

Vegetation indices calculated for Willows (FieldSpec-Pro)

Index	0%	20%	40%	60%	80%	100%	Hoagland's
NDVI750	0.364	0.474	0.390	0.301	0.260	0.084	0.313
NDVI	0.749	0.817	0.637	0.577	0.477	0.208	0.563
NDVI2	0.739	0.808	0.631	0.580	0.481	0.243	0.544
WI	1.015	1.020	1.006	0.992	0.988	0.971	1.033
WBI	0.985	0.980	0.994	1.008	1.012	1.030	0.968
MCARI1	1.255	0.899	0.891	0.310	0.535	0.082	0.454
MCARI2	1.025	0.958	0.756	0.360	0.479	0.108	0.472
PSRI	0.000	-0.001	0.112	0.164	0.221	0.454	0.044
GM2	2.748	3.864	2.770	2.156	1.897	1.204	2.275
R801/R550	3.084	4.354	4.253	4.064	3.562	2.755	2.519
R740/R850	0.956	0.938	0.902	0.850	0.866	0.796	0.985
LIC1	0.738	0.808	0.624	0.557	0.457	0.189	0.549
LIC2	0.696	0.787	0.408	0.319	0.296	0.243	0.731
GM1	3.050	4.275	4.084	3.770	3.327	2.443	2.524
VOG1	1.288	1.415	1.355	1.265	1.230	1.078	1.260
VOG2	-0.041	-0.064	-0.060	-0.049	-0.042	-0.026	-0.036
VOG3	-0.044	-0.070	-0.065	-0.051	-0.044	-0.027	-0.039
Carter-1	2.012	1.862	2.804	3.539	4.243	4.756	1.624

Vegetation indices calculated for Vetiver (FieldSpec-Pro)

Index	0%	20%	40%	60%	80%	100%	Hoagland's
NDVI750	0.407	0.537	0.572	0.512	0.492	0.416	0.190
NDVI	0.900	0.931	0.914	0.878	0.899	0.812	0.367
NDVI2	0.904	0.934	0.916	0.874	0.900	0.806	0.362
WI	1.055	1.075	1.090	1.048	1.064	1.046	1.026
WBI	0.948	0.931	0.918	0.954	0.940	0.956	0.975
MCARI1	0.930	0.648	0.944	1.220	0.553	0.682	0.336
MCARI2	1.138	0.978	1.154	1.200	0.810	0.819	0.314
PSRI	-0.018	-0.013	-0.010	-0.006	-0.002	-0.010	0.079
GM2	3.190	4.716	5.051	4.192	4.041	3.086	1.593
R801/R550	3.048	4.402	4.826	3.888	4.005	3.120	1.732
R740/R850	0.935	0.887	0.858	0.897	0.900	0.907	0.959
LIC1	0.904	0.933	0.915	0.873	0.898	0.801	0.361
LIC2	0.340	0.409	0.411	0.459	0.326	0.442	0.642
GM1	2.978	4.212	4.564	3.750	3.857	3.029	1.709
VOG1	1.342	1.560	1.689	1.534	1.480	1.398	1.180
VOG2	-0.053	-0.099	-0.137	-0.097	-0.081	-0.067	-0.031
VOG3	-0.057	-0.110	-0.156	-0.107	-0.089	-0.073	-0.033
Carter-1	13.577	51.067	4.820	4.331	7.402	3.651	1.847

Vegetation indices calculated for Fescue (FieldSpec-Pro)

Index	0%	20%	40%	60%	80%	100%	Hoagland's
NDVI750	0.220	0.379	0.415	0.392	0.325	0.313	0.501
NDVI	0.635	0.755	0.767	0.768	0.708	0.656	0.783
NDVI2	0.629	0.751	0.761	0.767	0.708	0.650	0.775
WI	1.036	1.033	1.042	1.015	1.022	1.021	1.057
WBI	0.966	0.968	0.960	0.985	0.979	0.979	0.946
MCARI1	0.327	0.651	0.814	0.374	0.605	0.480	0.307
MCARI2	0.387	0.739	0.856	0.492	0.669	0.536	0.427
PSRI	0.023	-0.008	0.024	-0.004	-0.023	-0.001	0.017
GM2	1.840	2.788	3.043	2.957	2.430	2.315	3.854
R801/R550	2.182	3.072	3.793	3.104	2.587	2.501	4.438
R740/R850	0.945	0.917	0.890	0.915	0.918	0.935	0.854
LIC1	0.629	0.750	0.759	0.762	0.701	0.650	0.773
LIC2	0.598	0.718	0.521	0.763	0.734	0.735	0.799
GM1	2.125	2.974	3.629	3.020	2.520	2.443	4.195
VOG1	1.175	1.353	1.414	1.358	1.277	1.274	1.594
VOG2	-0.028	-0.064	-0.081	-0.062	-0.048	-0.047	-0.134
VOG3	-0.029	-0.069	-0.088	-0.067	-0.050	-0.050	-0.149
Carter-1	3.605	2.343	3.099	2.124	1.938	1.865	1.553

Vegetation indices calculated for Poplar (HSI)

Index	0%	20%	40%	60%	80%	100%	Hoagland's
NDVI750	0.336	0.414	0.463	0.489	0.478	0.320	0.487
NDVI	0.844	0.882	0.871	0.889	0.876	0.596	0.871
NDVI2	0.823	0.867	0.857	0.856	0.856	0.569	0.851
WI	1.021	1.009	1.047	1.056	1.101	1.016	1.037
WBI	0.979	0.991	0.956	0.947	0.908	0.984	0.964
MCARI1	0.630	0.515	0.572	0.521	0.592	0.329	0.603
MCARI2	0.810	0.745	0.790	0.762	0.817	0.386	0.820
PSRI	0.166	0.171	0.224	0.248	0.232	1.126	0.188
GM2	2.564	3.306	3.686	3.836	3.871	2.236	4.061
R801/R550	4.450	4.995	5.984	6.817	6.357	5.563	6.010
R740/R850	0.965	0.954	0.928	0.926	0.921	0.887	0.942
LIC1	0.827	0.871	0.860	0.861	0.857	0.564	0.854
LIC2	0.109	0.130	0.141	0.163	0.177	0.122	0.293
GM1	4.279	5.069	6.004	6.404	6.109	5.205	5.964
VOG1	1.270	1.383	1.418	1.442	1.414	1.298	1.465
VOG2	-0.348	-0.469	-0.517	-0.525	-0.518	-0.379	-0.575
VOG3	-0.384	-0.540	-0.609	-0.632	-0.611	-0.418	-0.681
Carter-1	14.280	8.449	7.793	6.763	7.908	11.267	5.661

Vegetation indices calculated for Willows (HSI)

Index	0%	20%	40%	60%	80%	100%	Hoagland's
NDVI750	0.382	0.467	0.516	0.514	0.469	0.319	0.447
NDVI	0.878	0.894	0.892	0.842	0.846	0.696	0.805
NDVI2	0.845	0.879	0.871	0.833	0.829	0.684	0.790
WI	1.028	1.045	1.032	1.071	1.059	1.077	1.039
WBI	0.973	0.957	0.969	0.934	0.944	0.929	0.963
MCARI1	0.665	0.775	0.670	0.634	0.649	0.433	0.754
MCARI2	0.888	0.996	0.912	0.809	0.827	0.520	0.851
PSRI	0.261	0.251	0.289	0.359	0.324	0.857	0.385
GM2	3.002	3.742	4.295	4.115	3.722	2.260	3.353
R801/R550	5.255	6.679	7.454	7.192	6.244	6.204	6.061
R740/R850	0.935	0.944	0.919	0.926	0.929	0.858	0.938
LIC1	0.850	0.880	0.874	0.834	0.830	0.668	0.793
LIC2	0.097	0.138	0.157	0.159	0.158	0.097	0.152
GM1	5.358	6.688	7.356	7.090	6.395	5.641	5.740
VOG1	1.301	1.410	1.487	1.505	1.440	1.275	1.419
VOG2	-0.410	-0.464	-0.570	-0.578	-0.522	-0.373	-0.499
VOG3	-0.469	-0.552	-0.682	-0.695	-0.616	-0.412	-0.582
Carter-1	11.798	12.931	8.870	8.123	8.815	21.352	9.593

Vegetation indices calculated for Vetiver (HSI)

Index	0%	20%	40%	60%	80%	100%	Hoagland's
NDVI750	0.413	0.437	0.447	0.434	0.461	0.427	0.348
NDVI	0.715	0.724	0.753	0.726	0.753	0.734	0.753
NDVI2	0.657	0.673	0.706	0.677	0.715	0.674	0.711
WI	1.078	1.073	1.139	1.143	1.094	1.107	1.128
WBI	0.928	0.932	0.878	0.875	0.914	0.904	0.886
MCARI1	0.338	0.321	0.325	0.316	0.309	0.252	0.462
MCARI2	0.433	0.419	0.435	0.414	0.418	0.344	0.575
PSRI	0.382	0.446	0.361	0.376	0.373	0.368	0.273
GM2	2.913	3.129	3.156	3.138	3.351	3.061	2.524
R801/R550	4.025	4.501	4.512	4.240	4.620	4.184	3.505
R740/R850	1.041	1.013	1.004	1.016	0.989	1.051	1.045
LIC1	0.702	0.710	0.737	0.711	0.740	0.722	0.737
LIC2	0.128	0.133	0.152	0.131	0.163	0.139	0.132
GM1	4.141	4.607	4.345	4.337	4.558	4.514	3.664
VOG1	1.448	1.489	1.484	1.501	1.509	1.418	1.335
VOG2	-0.500	-0.570	-0.572	-0.550	-0.598	-0.505	-0.409
VOG3	-0.594	-0.675	-0.685	-0.667	-0.718	-0.586	-0.466
Carter-1	8.756	13.438	9.508	8.323	6.487	9.428	12.438

Vegetation indices calculated for Fescue (HSI)

Index	0%	20%	40%	60%	80%	100%	Hoagland's
NDVI750	0.205	0.353	0.349	0.310	0.324	0.303	0.384
NDVI	0.595	0.690	0.722	0.676	0.656	0.674	0.814
NDVI2	0.495	0.609	0.658	0.596	0.599	0.594	0.760
WI	1.046	1.122	1.095	1.093	1.082	1.109	1.094
WBI	0.956	0.891	0.913	0.915	0.924	0.902	0.914
MCARI1	0.093	0.161	0.212	0.214	0.204	0.232	0.408
MCARI2	0.128	0.224	0.292	0.283	0.268	0.304	0.560
PSRI	0.539	0.495	0.426	0.417	0.367	0.466	0.279
GM2	1.750	2.456	2.541	2.309	2.333	2.185	2.865
R801/R550	2.768	3.859	4.008	3.620	3.470	3.757	4.621
R740/R850	1.069	1.095	1.106	1.087	1.116	1.075	1.023
LIC1	0.551	0.658	0.705	0.647	0.660	0.648	0.791
LIC2	0.089	0.133	0.084	0.173	0.156	0.132	0.132
GM1	3.001	4.230	4.345	3.665	3.711	3.817	4.670
VOG1	1.111	1.296	1.288	1.276	1.288	1.278	1.364
VOG2	-0.254	-0.374	-0.369	-0.353	-0.356	-0.341	-0.441
VOG3	-0.254	-0.417	-0.408	-0.396	-0.400	-0.387	-0.511

Leachate analysis report for leachate obtained from Prairie Valley Landfill

PDC Laboratories, Inc.
3278 North Highway 67
Florissant, MO 63033
(800) 333-3278

ANALYTICAL RESULTS

Sample: 6060593-01
Name: Leachate Comp.
Matrix: Waste Water
Sampled: 06/01/16 18:00
Received: 06/02/16 15:15

Parameter	Result	Unit	Qualifier	Analyzed	Analyst	Method
Anions - STL						
Fluoride	34	mg/L		06/14/16 04:02	KLA	EPA 300.0*
General Chemistry - STL						
BOD	1200	mg/L		06/03/16 13:44	HIKR	SM 5210B
Solids - total suspended solids (TSS)	39	mg/L		06/06/16 08:12	NS	SM 2540D
Total Metals - STL						
Mercury	< 0.0002	mg/L		06/07/16 14:41	WPS	EPA 245.1 / SW 7470
Arsenic	< 0.015	mg/L		06/06/16 10:37	WPS	EPA 200.7
Beryllium	< 0.0010	mg/L		06/06/16 10:37	WPS	EPA 200.7
Boron	3.2	mg/L		06/06/16 10:37	WPS	EPA 200.7*
Cadmium	< 0.0020	mg/L		06/06/16 10:37	WPS	EPA 200.7
Chromium	0.014	mg/L		06/06/16 10:37	WPS	EPA 200.7
Copper	0.0042	mg/L		06/06/16 10:37	WPS	EPA 200.7
Lead	< 0.010	mg/L		06/06/16 10:37	WPS	EPA 200.7
Molybdenum	< 0.012	mg/L		06/06/16 10:37	WPS	EPA 200.7
Nickel	0.045	mg/L		06/06/16 10:37	WPS	EPA 200.7
Selenium	0.035	mg/L		06/06/16 10:37	WPS	EPA 200.7
Silver	0.0037	mg/L		06/06/16 10:37	WPS	EPA 200.7
Zinc	0.019	mg/L		06/06/16 10:37	WPS	EPA 200.7

Sample: 6060593-02
Name: Leachate grab
Matrix: Waste Water - Grab
Sampled: 06/01/16 18:00
Received: 06/02/16 15:15

Parameter	Result	Unit	Qualifier	Analyzed	Analyst	Method
Field - STL						
pH	7.20	pH Units		06/01/16 18:00	FIELD	SM*
General Chemistry - STL						
Cyanide	< 0.0025	mg/L		06/13/16 14:37	KMM	SM 4500-CN C E*
Hexavalent chromium	< 0.005	mg/L		06/02/16 16:51	KMM	SM 3500-Cr B*
Oil & Grease - total	< 5.3	mg/L		06/09/16 08:03	JS	EPA 1664
Phenol	0.18	mg/L		06/10/16 10:43	KMM	EPA 420.1
Volatile Organics - STL						
Benzene	5.4	ug/L		06/07/16 00:52	KMN	EPA 624
Surrogate: 1,2-Dichloroethane-d4	*83 %*	*55.3-123*		*06/07/16 00:52*	*KMN*	*EPA 624**
Surrogate: Toluene-d8	*85 %*	*67.9-117*		*06/07/16 00:52*	*KMN*	*EPA 624**
Surrogate: Bromofluorobenzene	*112 %*	*69.4-134*		*06/07/16 00:52*	*KMN*	*EPA 624**

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VITA

Rahul Sukharia was born in Rajasthan, India. He received a Bachelor of Science degree in Microbiology in 2010 from Sardar Patel University (India). In 2013, he received a Master of Science degree in Environmental Science and Technology from Sardar Patel University (India). During his master's thesis in India, he worked on performance evaluation of a wastewater treatment plant for future recommendations. In May 2017, Rahul Sukharia graduated with his Master of Science degree in Environmental Engineering from Missouri University of Science and Technology, Rolla, USA. During his master's, he has been fortunate to attend several national and international conferences, to learn about new advancements and present his research work.