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Rapid-Throughput Analysis of 1,4-Dioxane in Plants by Centrifugal Sampling and Phytoforensic Analysis for Site Delineation and Assessing Enhanced Rhizodegradation

Anthony Eberechukwu Oha
Missouri University of Science and Technology

Department:

RAPID-THROUGHPUT ANALYSIS OF 1,4-DIOXANE IN PLANTS BY CENTRIFUGAL SAMPLING AND PHYTOFORENSIC ANALYSIS FOR SITE DELINEATION AND ASSESSING ENHANCED RHIZODEGRADATION

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ANTHONY EBERECHUKWU OHA

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ABSTRACT

Owing to its broad use and environmental persistence, 1,4-dioxane (dioxane) poses a notable threat to public health and is recalcitrant to traditional remedial systems. Dioxane moves readily in an aqueous environment and is an emerging contaminant in drinking water, surface water, groundwater, and wastewater. The extent of dioxane in the biosphere is also difficult to delineate, particularly in the subsurface. Recent findings indicate that plant-microbial symbiosis may be more advantageous for limiting potential transport and transfer into plant tissues and the atmosphere. Biochar, as a soil amendment is evaluated to attenuate chemical activity and aid microbial survival and degradation of dioxane in the rhizosphere.

This study investigated the ability of plants to work symbiotically with rhizosphere-dwelling microorganisms, Pseudonocardia dioxanivorans (CB1190), to enhance the treatment of 1,4-dioxane in bench-scale experiments. Concurrently, phytoforensic analytical methods were developed to rapidly detect dioxane in plant tissues which was also applied in a field study to delineate an existing plume. The dioxane concentrations found in the inoculated trees were significantly lower ($p < 0.05$) than those in the trees with no microbial inoculation. Furthermore, the impact of biochar on stimulating microbial degradation was not significant in this study, which could be related to the physicochemical properties of the type of biochar used in this study. Overall, this study shows that plant tissue analysis can be effective for the site characterization and distribution of contaminants in the subsurface environment, as well as being a sensor of subsurface degradation.

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NOMENCLATURE

SYMBOL	DESCRIPTION
BTEX	Benzene, Toluene, Ethylbenzene and Xylenes
PDMS	Polydimethylsiloxane
cDCE	cis-1,2-Dichloroethylene
μ	Micro
EPA	Environmental Protection Agency
GC	Gas Chromatography
HS	Headspace
K_H	Henry's constant
K_{OW}	Octanol-water partitioning Coefficient
MCL	Maximum contaminant level
MDL	Method Detection Limit
PCE	Tetrachloroethylene
PDMS	Polydimethylsiloxane
SPME	Solid-Phase MicroExtraction
TCE	Trichloroethylene
TSCF	Transpiration stream concentration factor
USGS	U.S. Geological Survey
VC	Vinyl chloride

1. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. 1,4-DIOXANE

For decades, industries have used 1,4-dioxane (dioxane) to make products, such as shampoos, shower gels, dish soaps, and laundry detergents. Dioxane is primarily used as a stabilizing agent in chlorinated solvent mixtures, and 90% of the production is in excess of 29 million pounds annually; in 1985, it was used as a stabilizing agent for TCA and other chlorinated solvents in the United States (HSDB, 1995). Additionally, dioxane is used as a solvent for paper and textile processing, and in paint and cosmetic production. With the massive production, widespread use, and transportation of dioxane, contamination of soil and groundwater is also widespread, as is common with large production compounds (Teng et al., 2014). Dioxane is fully miscible in water, highly mobile in soil, and has the potential for vegetative uptake (Aitchison et al.,2000). Due to its hydrophilic nature, dioxane is transported efficiently in groundwater and is difficult to extract from water.

1,4-dioxane is an emerging contaminant that poses a risk to human health. Dioxane has been detected in surface water and groundwater resources in the US for decades (Zenker et al., 2003). The United States Environmental Protection Agency (USEPA) has classified 1,4-dioxane as a Class B2 (potential) human carcinogen. This compound was found to induce liver and nasal cancer in rats and mice through drinking water containing 10,000 milligrams per liter (mg/L) of dioxane (Klaassen et al., 1986). The effects of dioxane on human health depend on the magnitude, frequency, and

duration of exposure (ITRC, 2021). Limited available environmental monitoring data suggest that the level of exposure to the public is significantly lower than that used for experimental studies with animals (ITRC 2021). It should be noted that the magnitude of exposure to contaminated soil or groundwater is site specific.

As of 2022, no drinking water standard or Maximum Contamination Level (MCL) for dioxane has been established at the federal level in the U.S. Some states have imposed regulatory standards for dioxane (Chiang et al., 2016). As an emerging contaminant, dioxane is a candidate for the development of an MCL to protect drinking water quality. Health advisories are issued by the USEPA to provide information on drinking water contaminants that pose a risk to human health. In 2018, the USEPA, after revising the health advisory established in 1987 for a 1-day and 10-day exposure, lowered the advisory levels to 4,000 micrograms per liter (µg/L) and 400 µg/L, respectively (ITRC 2021). For the risk of cancer, USEPA in its 2012 Edition of the Drinking Water Standards and Health Advisories, listed a health advisory of 0.35 µg/L for 1,4-dioxane based on a 1×10^{-6} cancer risk.

1.2. PHYSICAL AND CHEMICAL PROPERTIES

1,4-dioxane is cyclic with two ether bonds that make the molecule hydrophilic and completely miscible in water (Zenker et al., 2003). The physical and chemical properties of 1,4-dioxane are listed in Table 1.1 below. Figure 1.1 shows the structure of dioxane.

Table 1.1. Physical and Chemical Properties of 1,4-Dioxane

Chemical Abstracts Service (CAS) number	**123-91-1**
Physical description (physical state at room temperature)	Clear, flammable liquid with a faint, pleasant odor
Molecular weight (g/mol)	88.106
Water solubility	Miscible
Melting point (°C)	11.75
Boiling point (°C) at 760 mm Hg	101.2
Vapor pressure at 25°C (mm Hg)	38.1
Specific gravity at 20°C	1.0337
Octanol-water partition coefficient (log K_{ow})	-0.27
Measured K_{oc} values	17 and 29
Henry's law constant at 25°C (atm-m^3/mol)	4.8 X 10^{-6}

(Adapted from Clu-in, 2022)

1.3. FATE AND TRANSPORT

Following the release of 1,4-dioxane into the environment, its fate and transport are a function of the chemical and physical properties of the compound, as well as the environmental properties. These properties are used to assess and understand the subsurface distribution of dioxane, and the risks involved in its migration. Zenker et al.,

(2003) noted that dioxane is persistent and mobile in aquatic environments. A defining characteristic of 1,4-dioxane is its low organic carbon partitioning coefficient (k_{oc}), making it highly mobile in groundwater, increasing concerns that contaminant plumes will expand further compared to those of other co-contaminants such as chlorinated volatile organic compounds (CVOCs) that have a higher k_{oc} (ITRC, 2021).

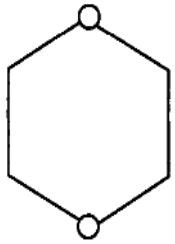

Figure 1.1. Structure of 1,4-Dioxane

Another characteristic considered is the low Henry's constant (K_H) of dioxane, which governs the partitioning of contaminants from an aqueous solution to air. The vapor pressure is lowered owing to the van der Waals force of attraction between the dipoles of 1,4-dioxane and water molecules. Dioxane volatilizes readily as a pure product or from dry soil; however, based on its miscible properties and low sorption to soil, dioxane does not volatilize from water or sorb to soil as a natural attenuation mechanism (Zenker et al., 2003).

Dioxane is recalcitrant to biodegradation under ambient subsurface conditions. However, recent studies have evaluated the dioxane degradation potential of bacteria, with positive results. Adamson et al. (2015) and Gedalanga et al. (2016) documented the natural attenuation of 1, 4-dioxane at field sites. Stevenson and Turnbull (2017) mapped

the 1,4-dioxane degradation pathway in several strains of Pseudonocardia through their work. Some field and laboratory studies have shown that aerobic dioxane degradation is favorable, but the presence of chlorinated solvents and metals inhibits this effect (Adamson et al., 2015; Zhang et al., 2016). Dioxane is photooxidized by hydroxyl radicals when released into air, with an estimated half-life of 33 h (HSDB, 2015). Similar findings by ATSDR (2012) and DHHS (2011) revealed that the half-life of dioxane in the environment was only 1 to 3 days. Dioxane has low bioaccumulation potential and persists moderately in the environment (USEPA, 2017a). The fate and transport of dioxane in the environment is demonstrated in Figure 1.2.

1.4. EXPOSURE ASSESSMENT

According to the U.S. Department of Health and Human Services (USDHHS) (2011), potential exposure pathways to 1,4-dioxane for humans include inhalation, ingestion, and dermal contact. The World Health Organization International Agency for Research on Cancer (IARC) (1999) reported that dioxane is readily absorbed into the body via ingestion and inhalation; however, absorption through the skin is poor. Besides contaminated groundwater, exposure sources for 1,4-dioxane include contact with residues contained in some detergents, shampoos, surfactants, food additives, pharmaceuticals, adhesives, and antifreeze products (USDHHS, 2011). Another possible means of exposure discussed by the IARC is occupational exposure during the manufacturing and management of dioxanes by workers. Overall, the primary source of exposure to dioxane is groundwater contamination due to industrial mismanagement due to its physical and chemical properties. The contaminant typically makes its way to tap

water through contaminated groundwater. Kraybill (1977, as cited in Zenker et al., 2003) mentioned the detection of dioxane (1µg/L) as early as 1975 in the U.S. Other occurrences of dioxane in groundwater have been cited in this paper. Figure 1.3 depicts the different exposure routes for humans.

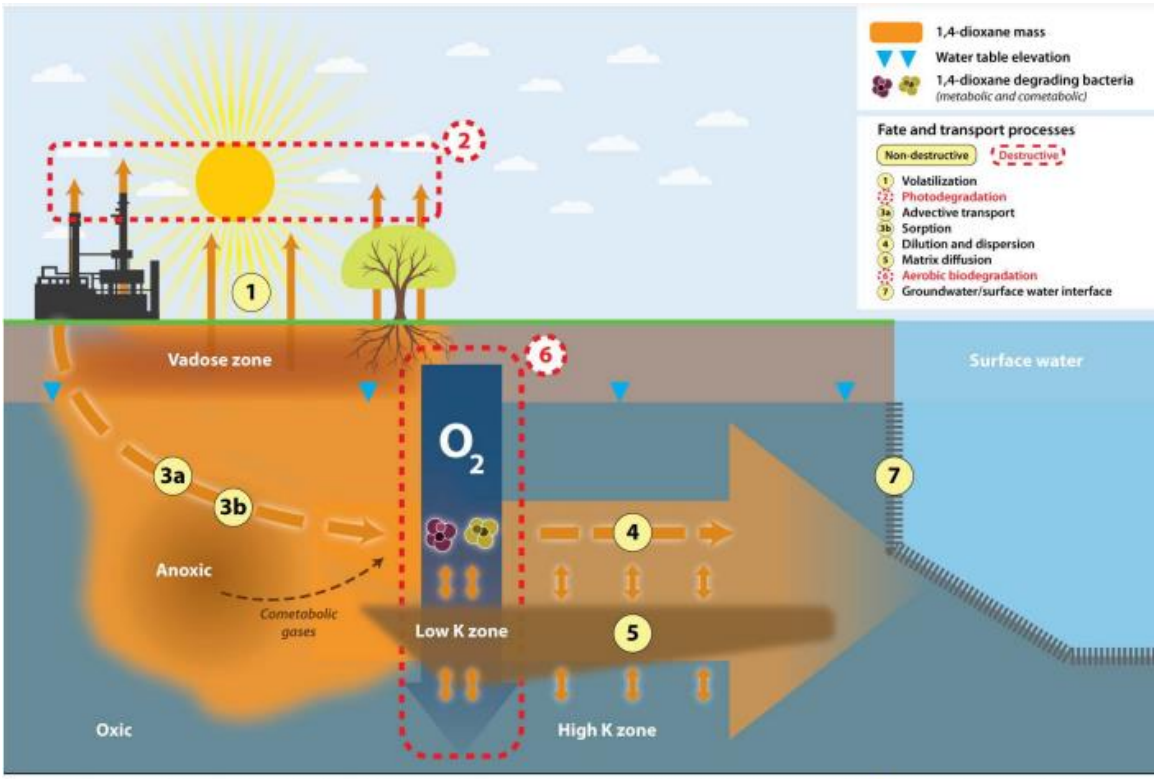

Figure 1.2. Fate and transport of 1,4-Dioxane in the environment (ITRC, 2021)

1.5. SITE CHARACTERIZATION AND ANALYTICAL METHODS

Cleaning up dioxane-contaminated sites is challenging because of the biochemical recalcitrance of dioxane, its low tendency to volatilize, and its high mobility in groundwater. As a result, the plumes tend to be large. More than 34 sites on the EPA

National Priorities List (NPL) have been identified with dioxane as of 2016, and many untested sites have the potential to be contaminated with the pollutant (EPA 2016b).

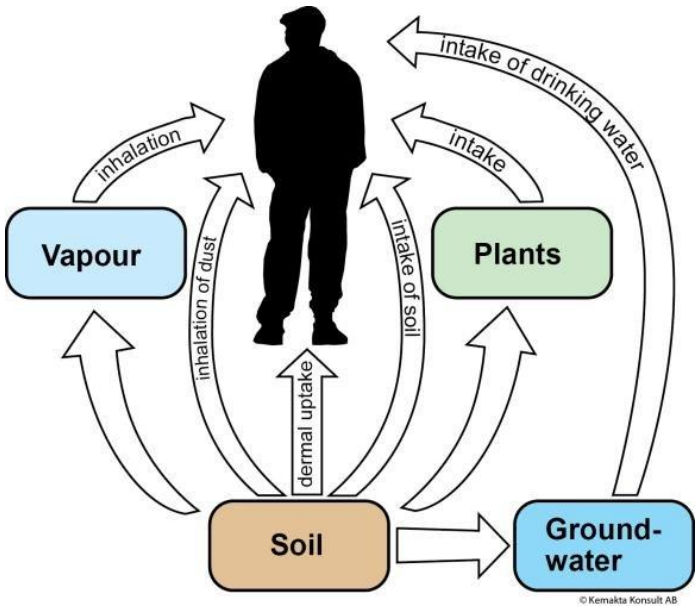

Figure 1.3. Human exposure pathways (Suer et al., 2012)

Given the widespread use of dioxane, environmental sampling should be performed with attention to contamination. Dioxane can be present in detergents used to decontaminate sampling equipment if not rinsed sufficiently. Additional field or equipment blanks are advised before and during sampling for dioxane to ensure that no residual dioxane remains in the sampling equipment (Baker et al., 2015).

The analysis of dioxane in environmental samples presents a challenge as it is a small hydrophilic molecule. Various extraction techniques have been developed to separate dioxanes from water. Shirley and Linton (2006) developed methods for dioxane extraction from water using 80-µm carboxen–polydimethylsiloxane solid-phase microextraction (SPME) fibers, followed by gas chromatography (GC)–Flame ionization

detection (FID) or GC–Mass Spectrometry (MS). An extensive list of the available analytical techniques is presented in Table 1.2.

1.6. REMEDIATION AND TREATMENT TECHNOLOGIES

Remediation of contaminated sites is conducted either *in situ* (degradation of the pollutant in the subsurface) or *ex situ* (extraction from the subsurface followed by treatment). Due to the high mobility of dioxane in groundwater, which results in large plumes, traditional remediation strategies such as pump and treat have proven to be costly and ineffective. The pump and treat method with air stripping or adsorption with granular activated carbon as a separation technique were ineffective in removing dioxane from water (Suthersan et al., 2016). Considering the large, dilute plume of dioxanes, using energy-intensive *ex situ* techniques, such as advanced oxidation, is economically impractical (Simon, 2015c The use of UV radiation, distillation, and chlorination are all efficient treatment techniques for the oxidized, hydrophilic molecule. Chiang et al., (2016) in their investigative study found that dioxane is susceptible to chemical oxidation. Their work showed that sodium persulfate, ozone, peroxide, and a modified Fenton's reagent can be used to treat dioxane in groundwater; however, sodium permanganate is not effective in treating dioxane. Air stripping/sparging or soil vapor extraction (SVE) is ineffective for dioxane treatment because of its relatively low Henry's constant (ITRC, 2021).

In situ bioremediation is another treatment strategy that has been documented to induce the metabolic biodegradation of dioxane. Chiang et al., (2016), through their practical review of treatment technologies for dioxane, reported that the addition of

Table 1.2. 1,4-Dioxane Analytical Methods.

MATRIX	METHOD	INSTRUMENTATION	DETECTION LIMIT
Soil, Water	EPA SW846 Method 8015	GC/FID	15 µg/L (MDL)
Soil, Water	EPA SW846 Method 8240	GC/MS Purge and trap or direct injection	
Soil, Water	EPA SW846 Method 8260	GC/MS	*
Soil, Water	EPA SW846 Method 8260 SIM	GC/MS-SIM	0.5-10.0µg/L (MDL)
Soil, Water, Tissue	EPA SW846 Method 8261	VD/GC/MS	1.1 µg/L (MDL)
Soil, Water	EPA SW 846 Method 8270	GC/MS	0.23-1.0µg/L (MDL)
Soil, Water	EPA SW 846 Method 8270 SIM	GC/MS-SIM	
Air	EPA Method TO-15	GC/MS	
Water	EPA Method 1624 (Note compound listed as a method analyte)	ID GC/MS	
Air	NIOSH 1602	GC/FID	
Water	EPA Method 522	SPE, GC/MS-SIM	0.020-0.036µg/L (DL)
Soil, Water	EPA Method 625 (Note: compound not listed as a method analyte)	GC/MS	

* When direct injection is not conducted, a purge and trap extraction procedure (SW 846 5030 or 5035) is typically used to analyze the presence of other potential contaminants. With a high detection limit, this extraction technique was highly inefficient for 1,4-dioxane. A technique for extracting volatile, impure, and water-soluble chemicals, such as Azeotropic Distillation. Table Source: U.S. Environmental Protection Agency, 2013, as cited by Mohr (2001).

oxygen and an appropriate substrate induces cometabolic biodegradation of dioxane. Bioaugmentation being important to the remediation of dioxane, Zhang et al. (2017) tabulated dioxane-degrading microbes and their degradation rates. Additionally, Zhang et al. (2016) showed that biodegradation of dioxane is also inhibited by CVOCs, which are common co-contaminants. This implies that before using the bioremediation approach for dioxane plumes, CVOCs should also be treated, which can render biodegradation impractical.

One possible technique to speed up the treatment of dioxane by phytoremediation is to pump low concentrations of contaminated water onto plantations of trees (sub-surface irrigation) and to bioaugment the rhizosphere with dioxane-degrading bacteria. Figure 1.4 shows the various remedial technologies that has been applied for dioxane clean up.

1.7. PHYTOREMEDIATION OF 1,4-DIOXANE

Phytoremediation involves the use of plants to clean contaminated sites and groundwater. This remediation strategy offers economic, aesthetic, and environmental benefits. Henry et al., (2013) stated that phytotechnologies have the potential to reduce the amount or toxicity of chemicals and agents, thereby reducing human exposure to these substances. Researchers have found that plants are tolerant to translocating a variety of contaminants including chlorinated solvents, petroleum hydrocarbons, pesticides, radionuclides, explosives, and metals. Potential plants include herbs, shrubs, and trees. Edwards et al., (2011) mentioned the effectiveness of phytoremediation in removing organic chemicals, reducing chlorinated benzenes, and absorbing excess nutrients. Figure

1.5 represents typical phytoremediation processes. Pulford and Watson (2003) as cited by Edwards et al., (2011), noted that tree species like poplar (*populus spp.*) and willow (*salix spp.*) have been effective for phytoremediation due to their extensive root systems and high evapotranspiration (ET) rates. Briggs et al. (1982) demonstrated a relationship to predict the efficiency of plant uptake as a function of the hydrophobicity of organic

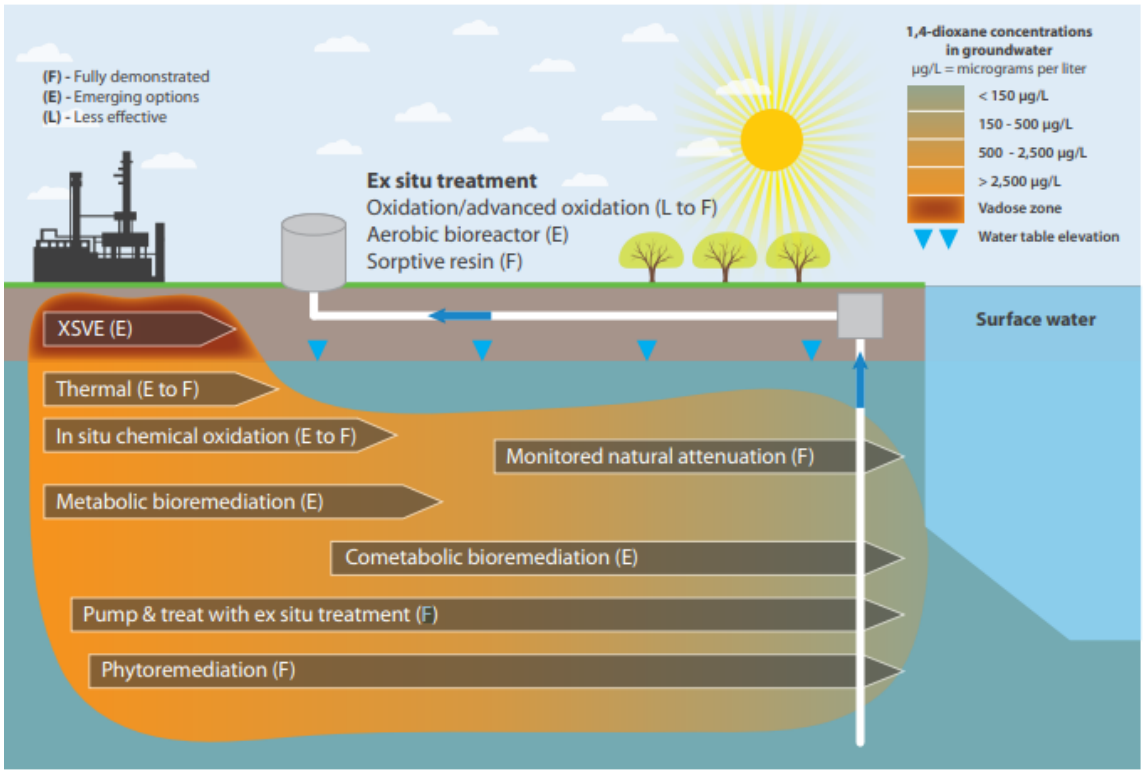

Figure 1.4. Remedial technologies for 1,4-dioxane plume (ITRC, 2021).

contaminants. The transpiration stream concentration factor (TSCF) was used to quantify the uptake efficiency. The TSCF was defined as the pollutant concentration in the transpiration stream (i.e., within the plant) divided by the aqueous solution concentration. Values were assigned to indicate the movement of chemicals in the plants. A TSCF value

of 1 indicates that the chemical moves freely with water into the plants, while a TSCF value of 0 shows a lower tendency for the chemical to move freely with water into the plants.

Other hydrophilic substances with log K_{ow} of 1.0, such as methyl-tert-butyl ether (MTBE), which has a log K_{ow} of 0.94 and a high TSCF of 5, enter the transpiration stream via hydrogen bonding with water molecules as opposed to sorption with the cellular membrane (Burken, 2003; Hansch et al.,1996; Howard, 1990; Rubin and Ramaswami, 2001). These compounds have characteristics similar to those of water, making them candidates for phytoremediation (phytovolatilization). Preliminary research has focused on substances with a log $K_{ow} < 1.0$, such as 1,4-dioxane and MTBE (Aitchison et al. 2000; Burken 2003; Rubin and Ramaswami 2001).

Phytoremediation of 1,4-dioxane is not a new strategy. Aitchison et al., (2000) cited phytoremediation as an early technology that was studied as in-situ treatment remediation for dioxane and have been successfully implemented on sites. DiGuiseppi et al. (2016) cited studies that demonstrated the use of phytoremediation for dioxane. Investigative research by Aitchison et al., in 2000 showed the feasibility of plant uptake of 1,4-dioxane. Hybrid poplar cuttings were used to rapidly remove 23 mg/L of dioxane in a hydroponic study. They reported the removal of 54.0 ± 19% of dioxane in nine days. Poplars effectively remediated the dioxane-spiked soil (10 mg/kg). After 15 days, 18.8 ± 7.9% of the initial dioxane remained in planted soil compared with 72.0 ± 7.7%, which remained in unplanted soil. Aitchison et al., (2000) concluded that phytoremediation is an effective alternative to remove dioxane from contaminated sites and can be considered for other hydrophilic contaminants.

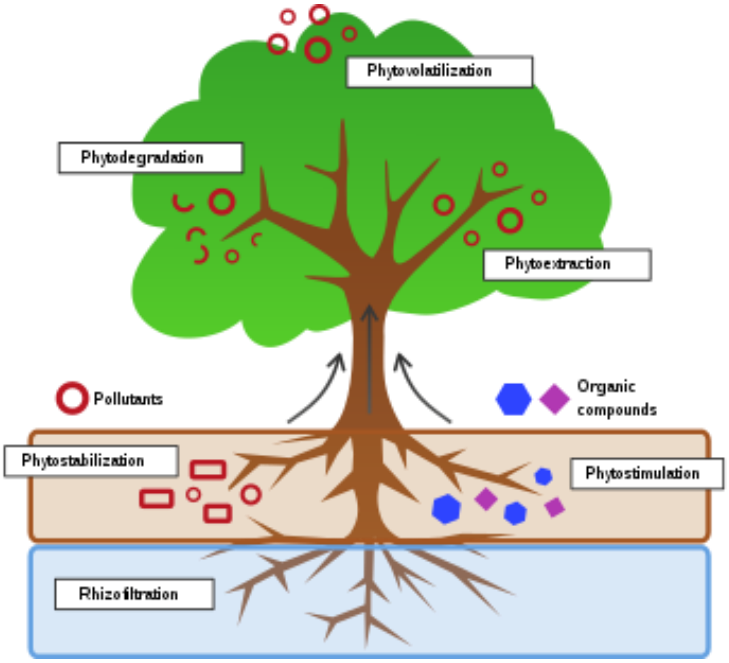

Figure 1.5. Phyto processes (Wikipedia)

Field tests employing conifers have indicated that MTBE can be successfully removed from contaminated groundwater (Hong et al., 2001). Ma et al. (2004) studied the uptake and volatilization of methyl tert-butyl ether (MTBE) in lab-scale hydroponic systems, as well as the fate and transport of MTBE in phytoremediation. According to this study, MTBE was taken up by hybrid poplar cuttings before being volatilized into the atmosphere. Both stems and leaves allowed MTBE to volatilize.

Phytoremediation does not solely focus on plant growth; it is a remediation system that consists of selecting the type of system and its location. Trees act as pumping wells in this case, taking up contaminants and water from the surrounding plume. Gestler (2016) revealed that phytoremediation systems with multiple units create a cone of depression; therefore, to estimate the treatment potential of phytoremediation systems,

groundwater in the area should be compared with the volume of groundwater transpired by each tree and the number of trees.

In 1990, a phytoremediation system was first applied in New Jersey. This comparatively simple system consists of a 36-inch corrugated steel pipe buried vertically in the ground up to the surface of the aquifer. Hybrid poplar saplings (diameter: approximately 2.5 in) were inserted into the pipe, which was subsequently filled with soil with amended nutrients. Although the intended depth was 5 m, deeper aquifers may be accessed by employing larger trees. Over the ensuing decades, this technique has been modified and improved and is now referred to as the Treewell® technology (Gatliff et al., 2016). The depth at which the trees are planted should be considered. Applications are more efficient with high water tables and when the dioxane plume is close to the top of the water table. This implies that trees should be planted such that they can engage contaminated groundwater and not transpire shallow soil moisture in the vadose zone.

1.8. BIOAUGMENTATION

Another mechanism that favors the removal of 1,4-dioxane is the stimulation of microbial activity and biochemical transformation in the rhizosphere through the release of root exudates and plant enzymes (Kelley et al., 2001). Bioaugmentation is a process of using microbes to their advantage to break down or degrade contaminants. Plants can enhance this process (rhizodegradation in the rhizosphere). Specific microbes are used to target specific pollutants and use contaminants as energy sources (metabolism). To achieve successful bioaugmentation, the environment must be suitable for microbial activity. Initially, 1,4-dioxane was thought to be an inert industrially manufactured

chemical. However, recently published research on the biodegradation of 1,4-dioxane has indicated the potential for bioremediation in this area. Kelley et al., (2001), investigated the use of dioxane-degrading microbes CB1190, to enhance dioxane biodegradation in both planted and unplanted soil. They reported that poplar root extracts stimulated dioxane degradation in bioaugmented soil (100 mg/L dioxane was removed within 45 d). In another experiment, hybrid poplars were planted in the soil and bioaugmented. A higher concentration of dioxane was removed within 26 days than in the unplanted reactors, regardless of the addition of CB1190. These results suggest that bioaugmented phytoremediation is an effective way to remediate dioxane from shallow-contaminated sites.

It is important to consider the impact of chlorinated compounds on the biodegradation of dioxane given that dioxane is mostly associated with chlorinated solvent solutions. Zhang et al., (2016), in their study showed that individual chlorinated solvents inhibited biodegradation of 1,4-dioxane in the following order: 1,1-dichloroethene (1,1-DCE), cis-1,2-diochloroethene (cDCE), trichloroethene (TCE), and 1,1,1-trichloroethane (TCA). These findings have an impact on the type of bioremediation strategy for dioxane that can be used at sites with chlorinated solvents as co-contaminants. Figure 1.6 shows the concurrent application of phytoremediation and bioaugumentation in the clean-up of 1,4 dioxane.

Other sample collection methods, such as passive samplers, can be used instead of actively pumping and collecting groundwater. Passive samplers rely on diffusive chemical movement through a polymer and partition it into a sampling phase (Limmer et al., 2014). To understand groundwater contamination by sampling plants growing above

plumes, phytoforensics combines the natural ability of plants to sorb organic contaminants and their capacity to pump groundwater using solar energy to drive transpiration.

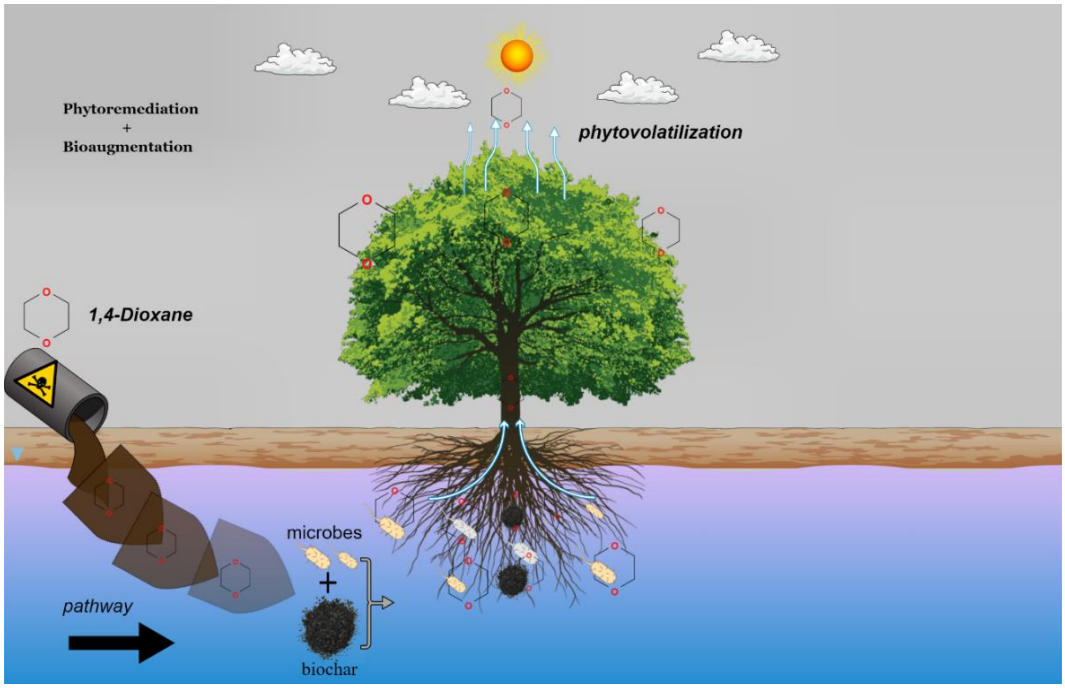

Figure 1.6. Phytoremediation + Bioaugmenation symbiotic process

This method is similar to passive sampling. Burken et al., (2011) in their work revealed that dissolved components are simultaneously transported while water is transpired by plants, consequently, the chemistry of groundwater is partially reflected in the water and wood of trees. They further defined phytoforensics as the examination of pollutant levels in plants to understand environmental chemistry. When used together, phytoscreening and phytomonitoring offer techniques that can be applied to environmental forensic investigations. There have been reports of pollutant uptake at

field sites (Burken et al., 2011; Karnjanapiboonwong et al., 2012; J. Wilson et al., 2013; J. L. Wilson et al., 2017; Vroblesky et al., 2004). In a 36.5-cm diameter Loblolly pine (Pinus taeda), Vroblesky et al., (2004), observed sectored absorption that related to a steep groundwater contamination gradient, where TCE concentrations ranged from 10 mg/L (north) to less than 0.1 mg/L (south) across 10m. The potential transmembrane migration into plant roots transport through vascular trees presents the potential for sampling hydrophilic contaminants such as dioxane and MTBE. The use of plants as a monitoring tool for dioxane and characterization of the contaminated site before applying remedies depends on a thorough understanding of dioxane biodegradation pathways and particular enzymatic reactions.

Analytical chemistry techniques for dioxane concentration in solutions have been developed for various applications (Kawata et al., 2001; Yano et al., 2005; Shirley & Linton, 2006). However, the analysis of plant tissue samples is limited. Aqueous methods often rely on relatively large samples and present challenges in the analysis of plant tissues. The rapidity, ease of use, detection limit, and solvent elimination of solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) have made it a well-liked method for analyzing volatile compounds in plant tissues (Silva et al., 2017; Zhoa et al., 2007; Krzyzanowski et al., 2008). Figure 1.7 shows the summary of the method development for the application of phytoforensics using the SPME.

1.9. RESEARCH OBJECTIVE

The primary aim of this study was to develop and assess methods for the rapid-throughput analysis of 1,4-dioxane for phytoforensic analysis for site delineation and

assessing enhanced rhizodegradation. The target application targets hybrid poplar DN91 as the representative plant species for the simple and sensitive method used for plume delineation and monitoring of groundwater concentrations.

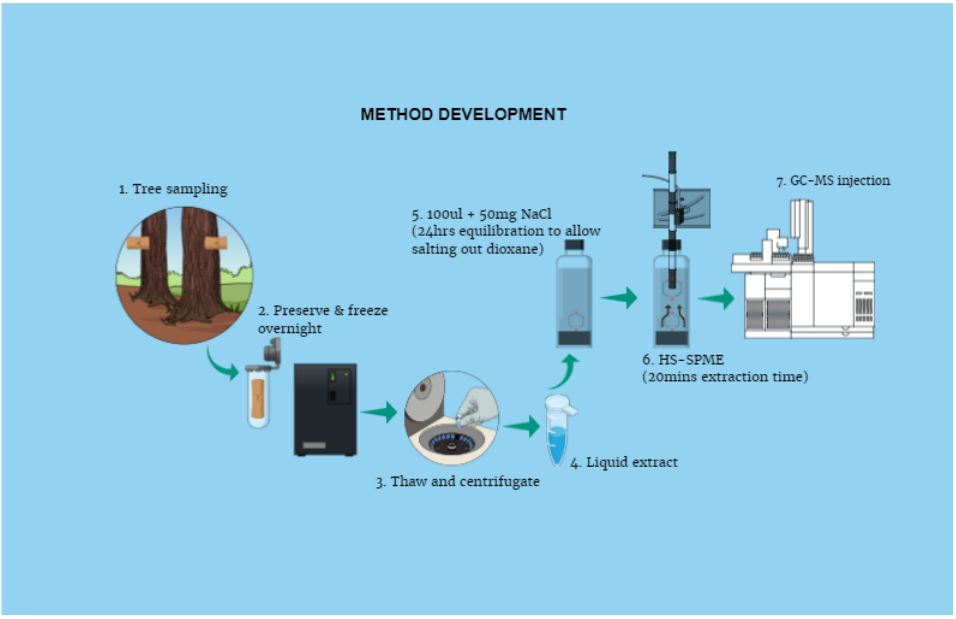

Figure 1.7. Method Development

We studied the impact of using perlite as the medium, bioaugmenting the rhizosphere with dioxane-degrading bacteria, Pseudonocardia dioxanivorans (CB1190), and the effect of biochar as an amendment/stimulant for microbial degradation. The measured aqueous concentration of dioxane in the plants will reflect the impact of phytovolatilization, degradation, and the possible sorption of 1,4-dioxane to biochar.

The plant samples were prepared for analysis by freeze-thaw procedure and centrifugation, followed by headspace-solid-phase microextraction (HS-SPME) and GC-MS detection to determine the aqueous concentration of 1,4-dioxane. This method can be

effective in using plants as subsurface sensors for site characterization and distribution of contaminants in the environment.

A set of specific objectives and corresponding hypotheses were developed to achieve the overall goal.

- Objective 1: To Develop a SPME sampling and paired analytical method for measuring 1,4-dioxane aqueous concentrations in the stems and leaves of plants.

Hypothesis: Dioxane translocation in plants moves subsurface solutions above the ground. The method will extract dioxane from the plant tissues, partition dioxane from the liquid phase to the gas phase and measure the aqueous concentration in the sample.

- Objective 2: To assess/analyze the impact of bioaugmentation on the degradation of dioxane by analyzing the bulk concentrations and concentrations in the stem and leaves.

Hypothesis: Dioxane will be degraded by the bacteria in the rhizosphere, and this can be observed through the analysis of the plant tissues using the developed method

- Objective 3: To evaluate the impact of biochar as an amendment on dioxane degradation and uptake in plants using the developed method.

Hypothesis: Biochar amendment will reduce the chemical activity of dioxane and may be a source of nutrients and attachment sites for enhanced microbial growth and degradation.

- Objective 4: To assess the dioxane fate and evaluate its transpiration with water into the atmosphere and the possible accumulation in leaves.

Hypothesis: Transpiration occurs in the leaves of plants and dioxane is phytovolatilized to the atmosphere, with Low K_H, which may accumulate.

PAPER

I. RAPID-THROUGHPUT ANALYSIS OF 1,4-DIOXANE IN PLANTS BY CENTRIFUGAL SAMPLING AND PHYTOFORENSIC ANALYSIS FOR SITE DELINEATION AND ASSESSING ENHANCED RHIZODEGRADATION

ABSTRACT

Owing to its broad use and environmental persistence, 1,4-dioxane (dioxane) poses a notable threat to public health and is recalcitrant to traditional remedial systems. Dioxane moves readily in an aqueous environment and is an emerging contaminant in drinking water, surface water, groundwater, and wastewater. The extent of dioxane in the biosphere is also difficult to delineate, particularly in the subsurface. Phytoremediation has demonstrated the potential for dioxane groundwater remediation. Recent findings indicate that plant-microbial symbiosis may be more advantageous for limiting potential transport and transfer into plant tissues and the atmosphere. Bioaugmentation of the rhizosphere paired with soil amendments has been proposed as a sustainable approach to improve phytoremediation applications. Biochar, as a soil amendment is evaluated to attenuate chemical activity and aid microbial survival and degradation of dioxane in the rhizosphere.

This study investigated the ability of hybrid poplar trees (*DN91*) to work symbiotically with rhizosphere-dwelling microorganisms, *Pseudonocardia dioxanivorans* (CB1190), to enhance the treatment of 1,4-dioxane in bench-scale experiments. Concurrently, phytoforensic analytical methods were developed to rapidly detect dioxane in plant tissues which was also applied in a field study to delineate an existing plume.

The impact of phytovolatilization, microbial degradation, and the possible sorption of 1,4-dioxane onto biochar was quantified by HS-SPME analysis of dioxane in the stems and leaves of our cuttings using GC-MS. The dioxane concentrations found in the inoculated trees were significantly lower ($p < 0.05$) than those in the trees with no microbial inoculation. This finding suggests that an efficient degradation pathway for the cleanup of dioxane exists in the symbiotic interactions between plants and microbes. Furthermore, the impact of biochar on stimulating microbial degradation was not significant in this study, which could be related to the physicochemical properties of the type of biochar used in this study. Overall, this study shows that plant tissue analysis can be effective for the site characterization and distribution of contaminants in the subsurface environment, as well as being a sensor of subsurface degradation.

1. INTRODUCTION

For decades, industries have used cyclic ether1,4-dioxane (dioxane) to stabilize chlorinated solvents such as 1,1,1-trichloroethane (TCA) and trichloroethylene (TCE) (Anderson et al., 2012; Mohr et al., 2010). Dioxane-contaminated sites are challenging to remediate because dioxane is resistant to both microbial and abiotic degradation, has a low potential to evaporate from water, and is highly mobile in groundwater because it has a low tendency to bind to soil (Aitchison et al., 2000; Howard, 1990). 1,4-dioxane is an emerging contaminant that poses a risk to human health and has been found in US groundwater for decades (Zenker et al., 2003). The USEPA has classified 1,4-dioxane as a Class B2 (potential) human carcinogen.

As of 2022, no drinking water standard or Maximum Contamination Level (MCL) has been set for dioxane at the federal level in the U.S. Some states have imposed regulatory standards for dioxane (Chiang et al., 2016). As an emerging contaminant, dioxane is a candidate for the development of an MCL to protect drinking water quality. According to EPA risk assessments, the amount of 1,4-dioxane in drinking water that poses a 1×10^{-6} cancer risk is 0.35 µ/L (EPA IRIS 2013).

Phytoremediation of dioxane is not a new strategy. Aitchison et al., (2000) proposed phytoremediation as a technology for remediation for dioxane showed the feasibility of plant uptake, and phytoremediation has been successfully implemented on sites. DiGuiseppi et al. (2016) cited several studies that demonstrated the use of phytoremediation for dioxane. To quantify uptake efficiency, the transpiration stream concentration factor (TSCF) was used, and Briggs et al. (1982) demonstrated a relationship to predict the efficiency of plant uptake as a function of organic contaminant hydrophobicity. The TSCF was defined as the pollutant concentration in the transpiration stream (i.e., within the plant) divided by the aqueous solution concentration. Values were assigned to indicate the movement of chemicals in the plants. A TSCF value of 1 indicates that the chemical moves freely with water into plants, while a TSCF value of 0 shows a lower tendency for the chemical to move freely with water into plants. Other hydrophilic substances with log K_{ow} values less than 1.0, such as methyl-tert-butyl ether (MTBE), which has a log K_{ow} of 0.94, enter the transpiration stream via hydrogen bonding with water molecules, as opposed to sorption with the cellular membrane (Burken, 2003; Hansch et al.,1996; Howard, 1990; Rubin and Ramaswami, 2001). These

compounds have characteristics similar to those of water, which makes them candidates for phytoremediation via uptake and potential phytovolatilization or phytodegradation.

Despite these encouraging findings, it remains unclear whether phytoremediation alone can safely reduce dioxane levels in groundwater to those required by health advisories, and if uptake alone may lead to concentrations in the plant tissue or transfer to the atmosphere. Bioaugmentation is a possible *in situ* method for treating dioxane plumes. Numerous dioxane-degrading bacteria have been discovered, some of which can only use dioxane as a carbon and energy source (Chen et al., 2016; Goodfellow et al., 2004; Huang et al., 2014; Kampfer and Kroppenstedt, 2004; Kim et al., 2009; Matsui et al., 2016; Nakamiya et al., 2005; Parales et al., 1994; Sei et al., 2013a). Kelley et al., (2001), investigated the use of dioxane-degrading microbes CB1190, to enhance dioxane biodegradation in both planted and unplanted soil. They reported that poplar root extracts stimulated dioxane degradation in bioaugmented soil (100 mg/L dioxane was removed within 45 days). A recent study by Simmer et al. (2020) demonstrated the effectiveness of combining phytoremediation and bioaugmentation to treat dioxane-contaminated groundwater to low-risk levels (~1µ/L).

A phytoforensic technique, also known as phytoscreening, identifies areas of contaminated groundwater by analyzing chemical concentrations in plant tissues (Burken et al., 2011). This research revealed that dissolved components are simultaneously transported while water is transpired by plants, and the groundwater chemistry is partially reflected in the water and wood of trees, confirming the uptake of pollutants at field sites and delineation of contaminant plumes (Burken et al., 2011; Karnjanapiboonwong et al., 2012; Wilson et al., 2013; Wilson et al., 2017; Vroblesky et al., 2004).

Multiple techniques have been developed for measuring dioxane concentrations in solutions (Kawata et al., 2001; Yano et al., 2005; Shirley & Linton, 2006). However current aqueous methods are challenging to apply for plant tissue analysis with limited aqueous volumes available in the plant transpiration stream. Given the multimedia characteristics of plant tissues, advanced sampling and analytical techniques are required. Sampling requires the separation of multimedia to analyze the concentration in the tissues, specifically in the aqueous transpiration stream. To detect hydrophilic compounds such as dioxane in plant tissue, partitioning of dioxane from the aqueous phase to the gas phase for SPME analysis is necessary. Due to their strong interactions with water dipoles, salts that form structures, such as NaCl and other salts with small ions, improve the structuring of aqueous phases and, subsequently, the cohesive energy in water (Endo et al., 2012). The addition of salt to the aqueous solution results in dioxane being driven to the gaseous phase. Studies have demonstrated the salting-out effect on aqueous samples combined with SPME analysis (Niu et al., 2017; Jonker et al., 2010; Wang et al., 2014).

The rapidity, ease of use, detection limit, and solvent elimination of solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) have led to the development of methods for the analysis of volatile compounds in plant tissues (Silva et al., 2017; Zhoa et al., 2007; Krzyzanowski et al., 2008). A recent study by He et al. (2021) developed a method for analyzing 1,4-dioxane and TCP in the stems of annual monocots tomatoes, corn, and wheat using SPME-HS analysis. The results of their study also provide a substantial contribution to the understanding of how EFCs are transported in plants and the ability to evaluate the possible risks that EFCs pose to human health and food safety. Similarly, studies have developed methods to measure the concentrations of

pesticide residues and volatile compounds in mangoes, grapes, and vegetables by SPME−SPME-GC-MS and SPME-GC-ECD (Chen, 2017; Menezes et al., 2010; Sanchez et al., 2005). Combining novel methods to isolate the aqueous phase and analyzing these minute volumes of solution was developed in this study. The developed method was applied both in a controlled greenhouse study and in the analysis of tissues from a known dioxane-contaminated site. In this greenhouse study, we developed an analytical technique to detect 1,4-dioxane in plant tissues, utilizing hybrid poplar DN91 as a representative plant species for a quick, simple, and sensitive method. To investigate the potential of applying this method to use plants as biomonitors for subsurface remediation and assessing enhanced rhizodegradation, the rhizosphere was bioaugmented with the dioxane-degrading bacteria *Pseudonocardia dioxanivorans* (CB1190), and biochar was used as an amendment. The measured aqueous concentration of dioxane in tree tissues reflects the impact of phytovolatilization, biodegradation, the impact of biochar on microbial growth, and the possible sorption of 1,4-dioxane to biochar.

2. METHODOLOGY

2.1. CHEMICAL AND MATERIALS

The 1,4-Dioxane standard was purchased from Sigma Aldrich (St. Louis MO, USA). Sodium chloride (≥99%) and methanol (LC-MS grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Biochar was supplied by the Coolplanet Energy System (California, USA). The properties of the biochar are listed in (Table 1). Unrooted hybrid poplar cuttings (DN91) were obtained from EcoloTree, Iowa City, Iowa.

Hoagland solution was purchased from Phyto Technology Laboratories (Lenexa, KS, USA).

Table 1. Biochar Properties

Property	Cool Terra (CF11)
Surface Area (m^2/g)	230.5
Pore Volume (cm^3/g)	0.09
Electrical Conductivity (ds/m)	2.11
pH	7.2
Bulk Density (Ib/ft^3)	35
Crush Strength (Ibs/mm)	50
Particle Size (%>0.5mm)	80+
Ash (wt %)	4.1
Total usable porosity (%vol)	66
AEC/CEC(meq/l)	37/43
Saturates Hydraulic Conductivity (in/hr)	5.3

(Adapted from Coolplanet Energy System)

2.2. EXPERIMENTAL SETUP AND ANALYSIS

2.2.1. Reactor Setup and Plant Growth. A greenhouse study was conducted to test the attenuation and degradation of dioxane through plant tissue analysis. One liter glass reactors were filled with 100 g perlite. Perlite was used in place of soil because of its high porosity and low water-holding capacity, preventing waterlogging. The reactors were wrapped in aluminum foil to prevent algal growth. All cuttings were of similar size and length (20 cm) and were pregrown for two weeks in the reactors while being fed with 10% Hoagland solution before dosing. Six treatment combinations were set up for the experiments, which were performed in triplicate. Controls without dioxane dosing were also grown. The resulting treatment combinations were as follows: (1) perlite, (2) perlite + 10% biochar (low), (3) perlite + 20% biochar (high), (4) Perlite + CB1190, (5) Perlite +

CB1190 + 10% biochar (low), and (6) Perlite + CB1190 + 20% biochar (high). The biochar-amended treatment sets had two application rates: low – 10% (10 g) and high – 20% (20 g) of biochar amendment mixed properly with perlite. The application rates were based on the percentage of total mass of perlite (100 g) in the reactors. When cuttings were actively growing, the reactors were periodically dosed with dioxane solution. Two dosing concentrations, 50 μg/L and 1000 μg/L, were used for an exposure duration of 35 days. To maintain the soil moisture at approximately 70% of the field capacity, the reactors were weighed initially and every third day, and then watered to replace the transpiration volume. The transpired water volumes were recorded, and the transpiration of each reactor was recorded. On average, about 2500 mL of dioxane solution was dosed for the duration of this experiment. Figure 1 shows the experimental setup.

Figure 1. Reactor set up.

2.2.2. Strain Cultivation and Bioaugmentation. *Pseudonocardia dioxanivorans* (CB1190) liquid cultures were grown as described by Simmer et al. (2020). The culture was grown by adding 500 mg/l dioxane to liquid ammonium mineral salt (AMS) media and incubated at 30 °C on an orbital shaker (150 rpm). To guarantee adequate oxygen, the liquid culture volume was limited to 20% of the flask volume. After 5-7 days of a 10% transfer from the plates, rapid growth was observed, and the strain was ready for transfer to the reactors. The liquid cultures were sonicated for 30 min before inoculation into the planted reactors, and the optical density of the culture was obtained at OD_{600} = 0.9. Each reactor was inoculated with 20 ml of the liquid culture using a 100 mL glass syringe and a Teflon tube to inject the culture into the bottom of the reactor.

Samples from the bottom of the reactors were taken to evaluate the sustainability and presence of the CB1190 by the end of the experiment. Approximately 2 g of perlite samples were collected around the root zone of the reactors and placed in 20 mL vials for culturing. DI water (1 mL) was then added to each vial and sonicated for 30 min. The liquid from each vial was serially diluted ten folds before being cultured on agar plates. Cell growth was observed after 3 days, and to identify the CB1190 strains, 16S PCR was used to confirm the CB1190 presence of the strains in the reactor.

2.2.3. Sample Preparation and Analysis. Poplar cuttings with their leaves were harvested by cutting the stems above the ground. A 2 cm segment of each plant was cut and stored in a freezer (-20°C) overnight to freeze the samples. Tissue sampling and analytical methods were adapted from the initial work by He et al. (2021). The leaves from each cutting were harvested and frozen. A centrifugation technique was developed to quickly separate the transpiration liquid from the plant tissue for aqueous dioxane

analysis. Upon thawing, the tree xylem sections were cut to fit into a 1.5 mL centrifugal tubes and centrifuged for 10 min at 15,000 rpm to extract the liquid from the tissues. A 100 µL aliquot of the liquid extract was taken using a 100 µL gas syringe and injected into a 20 mL vial containing 50 mg of sodium chloride (NaCl) salt. The injected samples were left to equilibrate for 24 h to allow the salting out of dioxane from the 100 µL aliquot to the headspace. The salting-out step increased the dioxane concentration in the gas phase for headspace SPME analysis. In cases of limited water content in the tissues, two or more 1.5 mL tubes were used to fit the cut sections of each sample, and the liquid extracts were combined before taking the desired volume (100 µL). The leaf tissues were prepared in the same steps by fitting them into tubes and centrifuging them at the same time and speed, as mentioned above.

Prior to the plant tissue analysis, the aqueous concentrations in the reactors were also sampled. After 25 days of periodic dosing, 1 mL of the aqueous solution around the root zone in each reactor was extracted for analysis. Before extraction, the aqueous solution in the reactors was left to equilibrate for 4 h after dosing for the day. For SPME analysis, 100 µL was injected into a 20 mL vial with 50 mg of NaCl salt to obtain the aqueous concentration in the reactors.

2.2.4. Field Site Tree Sampling. Poplar trees were used as biosensors to characterize a contaminated site and to assess the distribution of 1,4-dioxane in the area. The endophyte-assisted poplar trees phytoremediation system has been actively growing for four years before using their tissues to delineate the contaminated site. The dioxane plume was not specified at this site but was inferred to be widespread. Before the analysis, 3–4 cm tree trunk cores were bored from the trees using a tree coring device at a

height of approximately 18 inches above the ground and placed in headspace vials that were labeled, sealed, and shipped on ice. The tree core samples underwent the freeze-thaw-centrifugal sample preparation process before running the SPME to determine the levels of 1,4-dioxane present. Fifty-one tree core samples, grouped into five areas across the site, were analyzed.

2.2.5. HS-SPME-GC-MS Method. An SPME method using gas chromatography-mass spectrometry (GC-MS) was developed to detect 1,4-dioxane in the liquid extract. The GC-MS parameters and standard preparations were adapted from He et al. (2021). The SPME was performed using an autosampler. The headspace aqueous concentration of volatile compounds in the capped 20 mL vials was extracted using 85 μm Carboxen/PDMS fibers purchased from Sigma-Aldrich (Bellefonte, PA, USA). The extraction time was set to 20 min, after which the fiber was desorbed for 4 min at 250 °C using a splitless injector. An Agilent Mass Spectrometer 5973 and an Agilent GC Model 6890 with an electronic pressure control system and HP-5 column (30 m 0.25 mm I.D, 0.25 m film thickness) were used. The inlet temperature was maintained at 250 °C. With the inlet "pulse" pressure set to 40 psi for 0.2 minutes, the inlet pressure was 5.0 psi. A 75.0 mL/min septum purge to split the vent was set for 0.5 minutes. Pulsed splitless injection of 1 μL was used to minimize the residence time and improve the resolution. The carrier gas flow (H_2) in the column was constant at 0.8 mL/min. The oven temperature was first maintained at 40 °C for 2.0 minutes, followed by a 20 °C/min ramp to 280 °C, which was maintained isothermally for 3.0 minutes. The entire run lasted for 10.0 minutes. The solvent delay was set to 1.0 minutes, and the EM offset was set to 200. For quantification and confirmation, the monitored dioxane ions were 58 m/z and 88 m/z,

respectively. Using these parameters, instrument calibration was linear from 0.5 to 1000 µg/L. The MSD acquisition was performed using selected ion monitoring (SIM) with a dwell time of 100 µs.

2.2.6. Standard Calibration. A primary stock solution of 1,4-dioxane was prepared in methanol at a concentration of 10,000 mg/L. A secondary standard stock solution was prepared by diluting the primary stock solution in DI water to a concentration of 1000 mg/L. The calibration standard solutions were prepared by further diluting this secondary standard solution with DI water at a series of concentrations (0.5, 1, 2, 5, 10, 20, 50, 100, 500, and 1000 µg/L). Each concentration was prepared in a 4 ml vial. The standards were prepared by injecting 100 µL of the series of concentrations into a 20-mL vial containing 50 mg NaCl for SPME HS analysis. Prior to analysis, the calibration standards were equilibrated for 24 h. A linear calibration plot was obtained from the standard sets. Figure 2 shows the summary of the method development for the application of phytoforensics using the SPME.

2.2.7. Statistical Analysis. The influence of treatments on dioxane concentrations in plant stems and leaves was tested using a one-way analysis of variance (ANOVA). All data were checked for normality and homoscedasticity of residuals. A log transformation was applied to normalize the data when violations of these assumptions were observed. When significant differences occurred between treatments, multiple comparisons of mean values were made using post-hoc Tukey's HSD tests. Differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using the Minitab software.

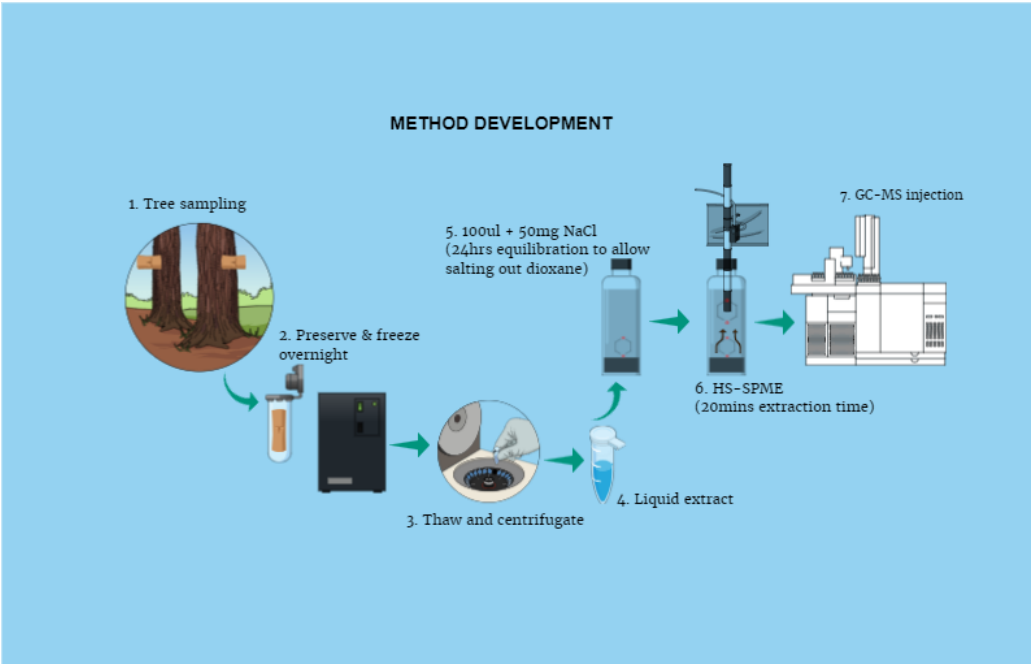

Figure 2. Method Development

3. RESULTS AND DISCUSSION

3.1. ANALYTICAL METHOD

3.1.1. Method Performance. The freeze-thaw process was successful in extracting aqueous samples from tree tissues by centrifugation. The performance of the method was validated by evaluating the limits of detection (LODs), limits of quantitation (LOQs), linearity of calibration, spike recovery, repeatability of retention time (RT), and sensitivity of dioxane. The repeatability of RT and concentration was determined by analyzing six different sections of the same sample. The percent relative deviation (%RSD) of the six replicates was 0.1 %, indicating good repeatability of RT. The concentrations in the stems also showed good repeatability, with an RSD% of 7.02 %.

Ten series of 100 µl standard concentrations were injected into capped vials with 50 mg of NaCl salt to generate a linear regression calibration curve (R^2 = 0.995) to obtain the LOD and LOQ. The linearity of the calibration curve started from 0.502 µg/L, hence the LOQ of this analytical method was determined to be 0.502 µg/L and the LOD was 0.201 µg/L (Table 2). Spike recovery confirmed the analytical accuracy. 10 µg/L and µg/L (low and high) dioxane were used to spike six different liquid extracts (100 µL) of the same sample for both the stem and leaf extracts. The spike recovery in the leaf tissues were relatively low in this study which could be related to the possible high organic matter content in leaf tissues. Tables 2 and 3 summarize the performance methods and spike recovery in the stems.

Table 2. Performance of the methods.

RT (min) ± RSD (%)	**RSD (%) of peak area**	**LOD (µg/L)**	**LOQ (µg/L)**	**Linearity (R^2)**
3.12 ± 0.1	7.02	0.201	0.502	0.995

Table 3. Spike recoveries of dioxane in stems.

Sample type	**Spike (µg L^{-1})**	**n**	**Mean recovery (%) (range)**
Stem	10	6	85.88 (76.21 - 90.21)
	20	6	92.09 (85.42 - 100.82)

3.1.2. Analyzing Dioxane in the Bulk Concentration. The analysis of the bulk solution in the reactors showed higher aqueous concentrations in the reactors of the treatments without CB1190 compared to the inoculated sets (Figure 3). Given the TSCF, that is, an uptake efficiency of less than 1.0 (TSCF = 0.72) as reported by Aitchison et al.

(2000), dioxane can be expected to build up outside the root membrane at higher concentrations than the feed solution for the reactors. The reactors inoculated with CB1190 showed either no accumulation or elevated aqueous concentrations (Figure 3).

The aqueous concentrations found in the CB1190 inoculated sets were significantly ($P < 0.05$) lower than treatments without inoculation. This result is evidential to support active biodegradation of dioxane in the rhizosphere before plant uptake. Another supporting evidence of biodegradation in the rhizosphere is the culturing of the aqueous samples from the reactors and identification of the presence of our inoculated specie, as mentioned above. Figure 4 shows the colonies formed on agar plates after 5 days. Grostern et al., (2012) gave a good depiction of how dioxane is metabolized as a sole carbon and energy source for strain CB1190.

3.1.3. Analyzing Dioxane in Tree Tissues. All treatment combinations were grown in triplicate, and the aboveground tissues were harvested for analysis. No signs of toxicity were observed during the study period. Two sections from each plant were cut and analyzed for dioxane concentrations, as described above. Duplicate samples of same tree stems were analyzed, and results were averaged. Figure 5 shows the average aqueous concentration of 1.4-dioxane in the stems of the hybrid poplar DN91 species, which were dosed with two concentrations of 50 and 1000 µg/L.

For the six treatment combinations, after dosing for 35 days, dioxane concentrations were significantly ($p < 0.05$) reduced in the stems of trees inoculated with the CB1190 strains and no biochar amendment compared to the trees with no bioaugmentation for the two dosing concentrations (Figure 5). The concentration of dioxane found in trees grown with biochar + CB1190 showed that biochar amendment

did not have a positive impact on enhancing the biodegradation of dioxane. This could be related to the physicochemical properties of the biochar limiting microbial activity and decreasing the degradation rate of dioxane in the reactors. In this case, the poor performance of the microbes could be related to the type of biochar used in this study. The effects of biochar as a soil amendment and on microbial growth have been well reported (Gul et al., 2015; Quilliam et al., 2012; Whitman et al., 2015; Al-Lami et al., 2019; Al-Lami et al., 2022). Furthermore, the presence of biochar despite not being effective in enhancing microbial degradation, had resulted in attenuating the chemical activity of dioxane in the trees compared to the control set. The lower concentration observed in the stems could be related to the possible sorption of dioxane to biochar. The adsorption of 1,4-dioxane has been previously reported (Ouyang et al., 2017; Myers et., 2018). Information regarding the different treatment concentrations in tree stems is shown in Figure 5.

This study also investigated potential dioxane accumulation in the leaves of trees. The concentrations found in the leaves showed similar trend like the concentrations found in the stems. Though there were low concentrations and no dioxane accumulations observed in the leaves, which supports Aitchison et al. (2000) work, where they reported that 76 to 83% of dioxane uptake by poplars transpired from leaves to the atmosphere where it can easily be dispersed and photodegraded, however, the result was inconclusive with respect to the low spike recovery observed during the analytical method development. Owing to this effect, the low concentrations observed in the leaves could be associated with their higher rate of metabolism and high organic matter content. Leaves are responsible for photosynthesis, which is a process that requires a significant amount

of energy, leading to a high metabolic activity that can help detoxify or metabolize dioxane through oxidation and sequestration.

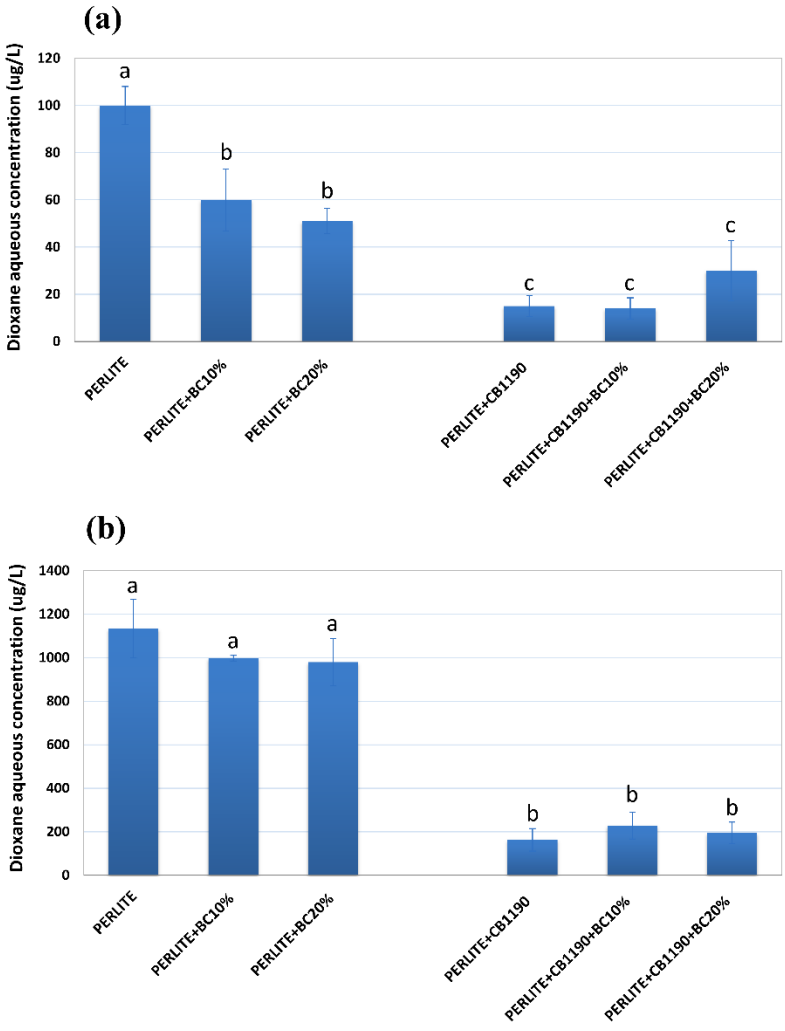

Figure 3. Average concentrations of 1,4 dioxane in the reactors. Two dosing levels: (a) 50 µg/L and (b) 1000 µg/L. Values are expressed as the mean ± SE per treatment (n = 3). Different letters above the bars indicate significant differences between the treatments (one-way ANOVA, $p < 0.05$).

Figure 4. Culture plate.

Organic substances that cross membranes and move to stem and leaf tissues can be transformed (for example, by cytochrome P450s oxidation), conjugated by glutathione or amino acids, and compartmentalized in plant tissues as bound residues (Dietz and Schnoor, 2001). This could be a metabolic pathway of dioxane in plant tissue; however, Aitchison et al. (2000) reported that dioxane did not typically accumulate or undergo metabolic transformation in poplar tree tissue. The metabolism of contaminants in plants has been extensively investigated (Doty et al., 2003; Burken et al 2000; Burken 2003; Doucette 2003).

Additionally, the organic matter in the leaves could contain compounds that are similar in structure to dioxane which could interfere with the sensitivity by binding to the detection reagents or masking the presence of dioxane. An optimization of the developed method would be needed for leaf analysis. Figure 6 shows a summary of average leaf concentrations.

The analysis of the aqueous concentration of dioxane in the reactors provided insight into the distribution of dioxane within the trees and how effectively the trees were taking up and translocating dioxane. Figure 7 shows the ratio of dioxane from aqueous to stem. It can be observed from the data that a larger percentage of dioxane uptake was found in the stem of the trees (Figure 7).

During the development of this analytical method, it is important to note the potential loss mechanisms of dioxane. As a pure substance, dioxane is volatile, and dioxane is semi-volatile from aqueous concentration with Henry's constant of 4.80×10^{-6} atm-m^3/mol. It may tend to volatilize from the soil surface or during sample preparation or analysis. During the active growth of trees in the reactors and the periodic dosing of dioxane, some percentage of dioxane may volatilize.

3.1.4. Field Application of Developed Method. Dioxane was found in 47 out of the 51 tree cores and the rest had concentrations below the detection limit. Concentrations ranged from as low as 0.5 ppb to 5 ppm. Figure 8 shows a map of the site and the five areas where tree samples were collected from the remediation site. This method allowed for the spatial mapping of the distribution of dioxane within the site, providing valuable information for cleanup and remediation efforts. It also allowed for

continuous monitoring of contaminant levels over time, providing important data for understanding the long-term impacts of dioxane contamination.

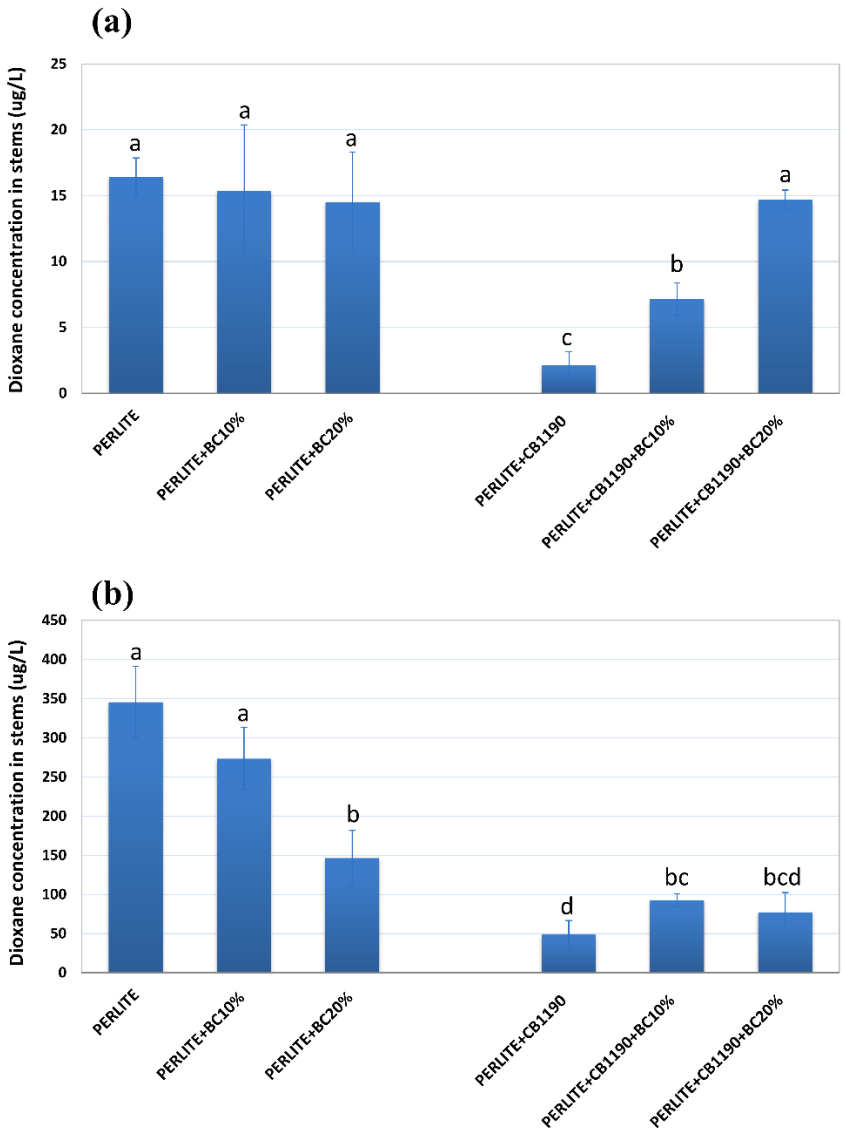

Figure 5. Average concentrations of 1,4-dioxane in hybrid poplar stems. Two dosing levels: (a) 50 µg/L and (b) 1000 µg/L. Values are expressed as the mean ± SE per treatment (n = 3). Different letters above the bars indicate significant differences between the treatments (one-way ANOVA, $p < 0.05$).

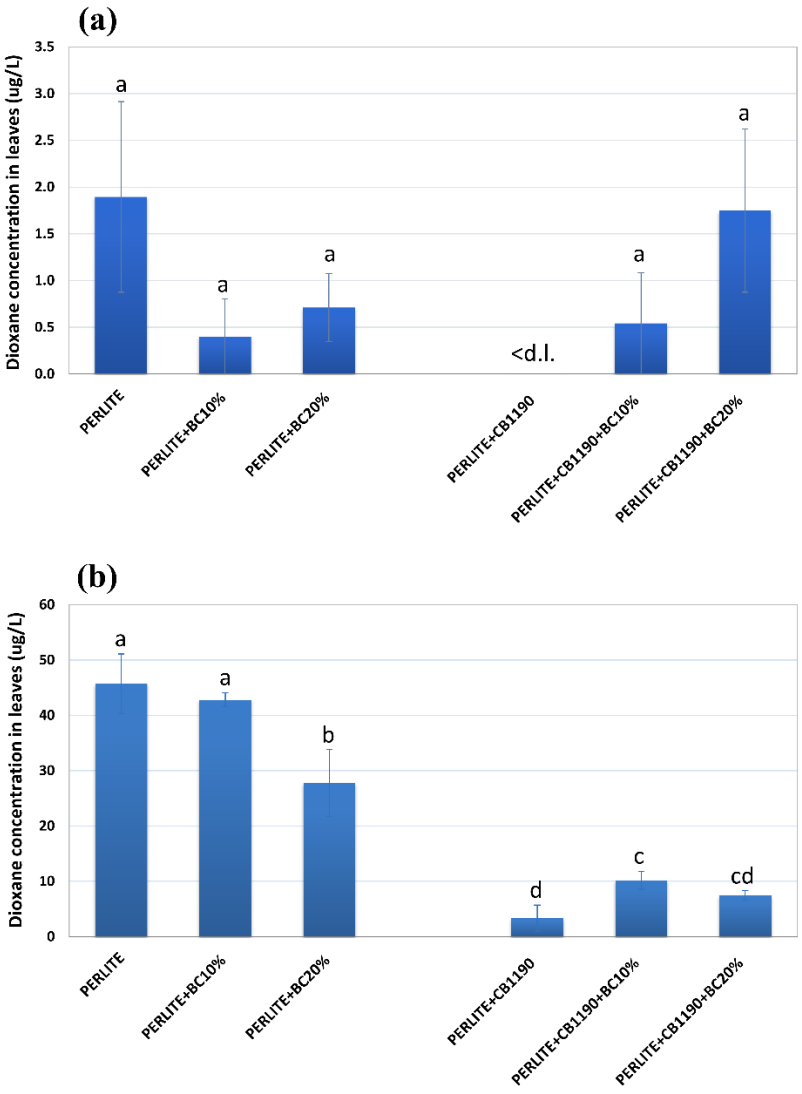

Figure 6. Average concentration of 1.4-dioxane in hybrid poplar leaves. Two dosing levels: (a) 50 µg/L and (b) 1000 µg/L. Values are mean ± SE per treatment (n = 3), where < d. l. = below the detection limit. Different letters above the bars indicate significant differences between the treatments (one-way ANOVA, $p < 0.05$).

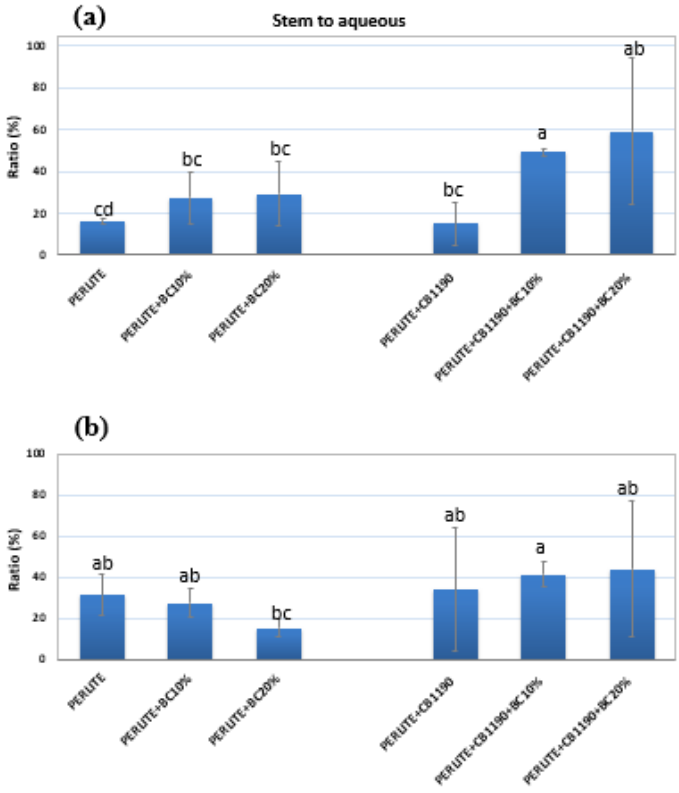

Figure 7. Dioxane uptake ratio in tree tissues. Two dosing levels: (a) 50 µg/L and (b) 1000 µg/L. Values are expressed as the mean ± SE per treatment (n = 3). Different letters above the bars indicate significant differences between the treatments (one-way ANOVA, $p < 0.05$).

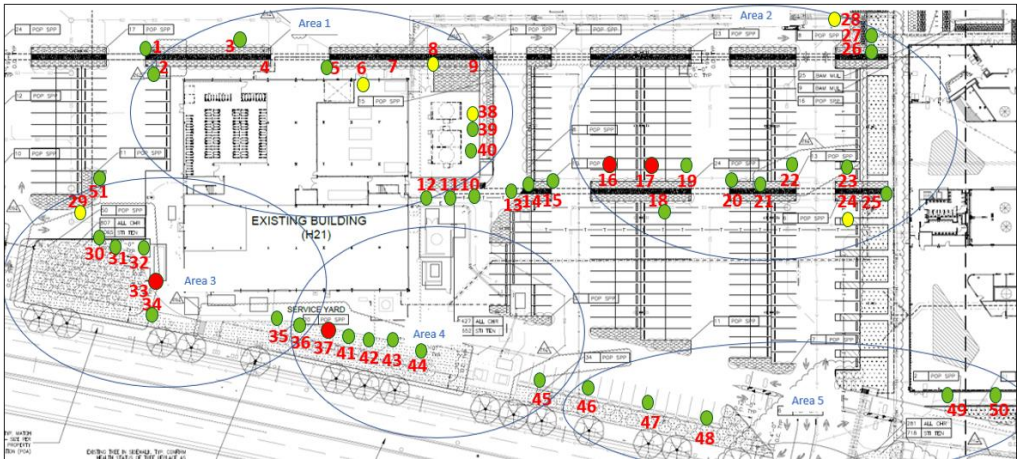

Figure 8. Field Map Distribution of Dioxane

(0.5ppb - 99ppb 100ppb - 600ppb 700ppb - 5554ppb)

4. CONCLUSION

The use of plants as biosensors can provide delineation of organic contamination distribution in the subsurface by utilizing the plants as groundwater and contaminant extraction from the subsurface paired with innovative sampling and analytics of plant tissues. The development of methods for perennial trees is the first published method for 1,4-dioxane detection in tree tissues, and the first application was published for field dioxane detection in plant samples and for phytoforensic analysis. The freeze-thaw-centrifugation process followed by HS-SPME extraction and GC-MS analysis method developed in this study was rapid, inexpensive, and reliable for measuring the 1,4-dioxane concentration in the stems of the hybrid poplar trees. Field identification demonstrated that trees growing over a dioxane plume can be used at a full-scale plume and treatment site and provide for concurrent treatment and delineation using fast-growing poplars in phytoremediation. Through phytoforesincs, the results revealed that sampling can also be used to affirm contaminant degradation in the subsurface, as demonstrated in this study for 1,4-dioxane biodegradation by CB1190 bacteria. This method is cost-effective and provides rapid and cost-effective methods to monitor remediation approaches at field sites, whereas traditional methods for monitoring degradation can be costly and add greatly to the expense of long-term monitoring for site remediation. The successful application of phytoforensics to 1,4-dioxane, a hydrophilic compound, indicates that these methods can be applied to other hydrophilic compounds, particularly for low-volatility compounds, using freeze-thaw methods combined with HS-SPME sampling.

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SECTION

2. CONCLUSIONS AND RECOMMENDATIONS

2.1. CONCLUSIONS

The use of plants as biosensors can provide delineation of organic contamination distribution in the subsurface by utilizing the plants as groundwater and contaminant extraction from the subsurface paired with innovative sampling and analytics of plant tissues. The development of methods for perennial trees is the first published method for 1,4-dioxane detection in tree tissues, and the first application was published for field dioxane detection in plant samples and for phytoforensic analysis. The freeze-thaw-centrifugation process followed by HS-SPME extraction and GC-MS analysis method developed in this study was rapid, inexpensive, and reliable for measuring the 1,4-dioxane concentration in the stems of the hybrid poplar trees. Field identification demonstrated that trees growing over a dioxane plume can be used at a full-scale plume and treatment site and provide for concurrent treatment and delineation using fast-growing poplars in phytoremediation. Through phytoforesincs, the results revealed that sampling can also be used to affirm contaminant degradation in the subsurface, as demonstrated in this study for 1,4-dioxane biodegradation by CB1190 bacteria. This method is cost-effective and provides rapid and cost-effective methods to monitor remediation approaches at field sites, whereas traditional methods for monitoring degradation can be costly and add greatly to the expense of long-term monitoring for site remediation. The successful application of phytoforensics to 1,4-dioxane, a hydrophilic

compound, indicates that these methods can be applied to other hydrophilic compounds, particularly for low-volatility compounds, using freeze-thaw methods combined with HS-SPME sampling.

2.2. RECOMMENDATION FOR FUTURE WORK

This study demonstrates the effectiveness of plant tissue analysis for detecting and monitoring 1,4-dioxane contamination. However, there are areas for further research. First, the equilibration time for the salting-out process should be optimized for quick sampling by running a time-series test to determine the optimal time, considering the minute volume involved. Optimization of the leave tissue sampling as well to grasp the actual cause of the low sensitivity observed during the spike recovery.

Additionally, an understanding of the rate and rate-limiting steps for the microbes to degrade dioxane in the aqueous solution with insight into the time it takes to reflect in the plant tissues would help in understanding the variability in the samples. Moreover, investigating the presence of CB1190 bacteria as endophytes in plant tissues is crucial for determining the extent of their involvement in phytoremediation.

To achieve low-risk concentrations of dioxane, enhanced microbial activity may be required. Furthermore, a study on the metabolism of dioxane in plant tissues could help elucidate the degradation pathways and potential toxicity of the byproducts. Finally, for field applications, collecting at least two or more tree cores from each tree and averaging their concentrations would help minimize sampling variability.

APPENDIX

EXPERIMENTAL PHOTOS

Figure 1. Stem liquid extract

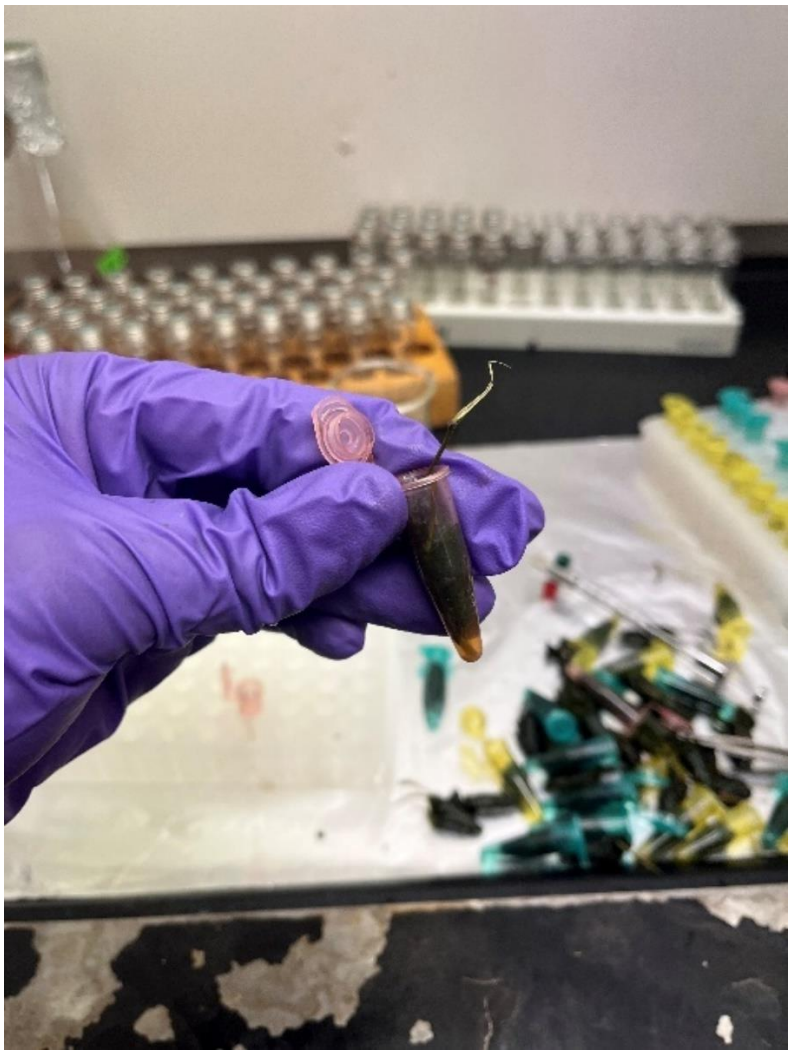

Figure 2. Leaf liquid extract

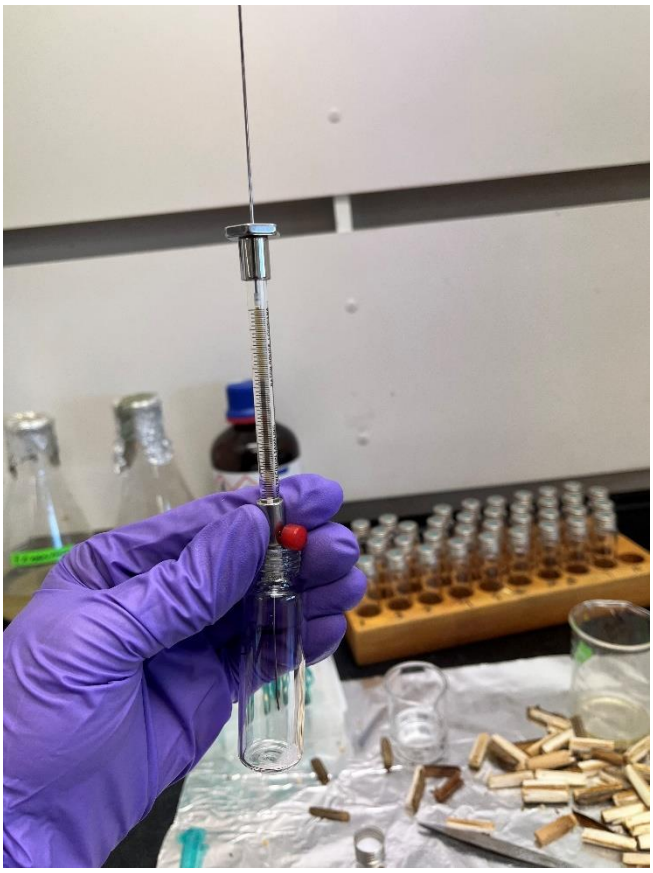

Figure 3. Extract injection into 50 mg of NaCl salt

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VITA

Anthony Eberechukwu Oha was born and raised in Nigeria. He graduated high school from St Anthony's college Aba, Abia state Nigeria. He graduated from Federal University of Technology Owerri in September 2018 with a Bachelor of Engineering in Petroleum Engineering. During his undergrad, he worked internships at the Department of Petroleum Resources, Nigeria. He received a Master of Science degree in Environmental Engineering in May 2023 from the Missouri University of Science and Technology.