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Using *Tillandsia recurvata* (Ball Moss) as a Biological Indicator to Monitor Air Pollution and Retain Oil Pollution

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Abstract

Air pollution is defined as the presence of a substance in the atmosphere that is harmful to human health, living things, and/or has a negative impact on the environment. A plant such as *Tillandsia recurvata*, ball moss, could be used as an inexpensive biological indicator for urban pollution. The purpose of this research was to determine if ball moss could be used as a biological indicator of urban pollution and retain oil pollution. Multiple sites were identified and grouped by vehicular traffic frequency (counts) using the Louisiana State Department of Transportation and Development (LaDOTD) traffic data to randomly select five low (0.0 - 7000), and five medium/high frequency (7001 to >14,000) traffic counts in locations within Baton Rouge, La. city limits. Differential analysis determined that harvested ball moss tissue levels from areas with low traffic (<0.05 level) contained lower S concentrations than plants tested from high traffic counts. In a second study, dried *Tillandsia recurvata* plant tissue accumulated greater oil weight than absorbent paper towels. *Tillandsia recurvata* absorbed and/or retained oil at a greater ratio of oil than its own mass. Therefore, the results of each experiment indicated that *Tillandsia recurvata* may successfully function as a biological indicator and serve as an oil retentionist on a small-scale test. Further research is needed on a larger-scale area to confirm the efficacy of ball mosses for controlling water pollution *in-situ*.

Keywords

Greenhouse Gases, Pollution, BTEX, Air Quality, Urban Ecology

1. Introduction

Pollution worldwide has become a major problem, especially concerning human health issues. Air, water, and soil contamination is a costly and difficult problem to remediate. Urban cities areas are particularly susceptible to ecological disturbances due to large, concentrated industry and human activities. Major cities in the United States have reported poor air quality due to heavy automobile usage expelling combustion engine fumes and industrial activities. Between 2016 and 2018, pollution increased 5.5% and resulted in an estimated 9700 premature deaths accounting for a conventional valuation of $89 billion in damages [1]. If pollution levels continue increasing, a greater percentage of individuals will be exposed to pollutants causing a further increase in disease occurrence, monetary loss, and increased health care costs.

In 2015, EPA designated the five-parish area near Baton Rouge, La. to be in non-compliance with the United States Clean Air Act law and the 1990 Amendments enacted into the United Stated code [2]. The purpose of the amended Clean Air Act in 1990 was to improve the nation's air quality and improve the stratospheric ozone levels. Mandated corrective actions to achieve compliance required specialized annual car inspections in the metropolitan Baton Rouge area, establishing pollution monitoring stations and a reduction of industrial emissions. Technological advances have developed digital monitoring devices; however, biological indicators can be used as a screening method for pollution. In 2019 Baton Rouge was ranked 35th worst polluted city in the nation due to high ozone levels by the American Lung Association [3] [4]. Pollution from combustion engine emissions consists of benzene, toluene, ethylbenzene, xylene (BTEX) and other contaminants such as sulfur dioxide [5]. Each compound in BTEX is classified as a volatile organic compound (VOC) as well as a potential threat causing health issues [5]. Additionally, VOCs can be formed during the production of industrial products and machines used by homeowners. Volatile organic compounds can easily evaporate, which is otherwise known as high vapor pressure. Along with high vapor pressure, VOC's dissolve easily in water, which has low water solubility, contaminating other resources. Additionally, sulfur dioxide (SO_2) levels were found to be at a hazardous level in Louisiana. Sulfur dioxide emissions can be detected from combusted fuel emissions. Main contributors of sulfur dioxide are chemical plants, ports, and other industries using products that contain sulfur dioxide. A substantial volume of ingested sulfur dioxide can result in problems regarding lung diseases [3]. Studies from the U.S. EPA (United States Environmental Protection Agency) detected sulfur dioxide in the vicinity of chemical plants, specifically in St. Bernard Parish [6]. Air monitors were placed throughout the state to monitor the rate of pollution. The facilities in Louisiana and the United States are required to uphold pollution standards ensuring they do not exceed EPA limits [6]. Measures to reduce sulfur dioxide are the key to managing harmful VOCs in the environment. Louisiana waterways were ranked 3rd most polluted in the nation [7]. In 2012 scientists determined that 12.6 million toxic chemicals were sent into Louisiana's waters, which accounted for 6% of total toxic chemicals released that year worldwide.

The Oil Pollution Act of 1990 (OPA 90) amended the Clean Water Act (CWA) to prevent and clean oil and chemical spills in waterways. The federal government empowered the EPA to regulate offshore oil and gas activities and subsequent amendments. Unfortunately, oil spills still occur either accidentally or naturally polluting water bodies. Therefore, monitoring pollution is essential to protect the environment.

Historically, biological indicators have been used for decades to warn people of dangerous atmospheric conditions. Coal miners used canaries (*Serinus canaria forma domestica*) as an early warning sign of the presence of dangerous carbon monoxide and gases in mines to protect workers from asphyxiation [8]. Canaries were used in British mines to alert miners of poisonous gas accumulation as late as 1986. Electronic technology was used subsequently instead of canaries. Zebra fish (*Danio rerio*) have been used as biological indicators for environmental contamination of waterways [9]. Leyrer *et al.* [10] determined that crickets could be used as biological indicators following an oil spill to determine the critical threshold levels of oil pollution in marshland. BTEX thresholds levels were monitored throughout the experiment. Frogs, toads, bacteria, insects, microbes and other organism populations have all been used as biological indicators [11]. Mandelik [12] determined that the integrated use of technology, insects, and plants gave a much more comprehensive representation of environmental conditions which correlate to the ecosystem's health compared to any one indicator. A single bioindicator may not result in the best information in all situations. Additionally, an integrated approach may result in the most economical [12].

A plant's survival and growth, and chemical analysis can inexpensively reflect the severity of pollution. Plants have been used as biological indicators. Sunflowers (*Helianthus annuus*) and Indian mustard (*Brassica juncea*) are effective soil Pb hyperaccumulators [13]. Spanish moss (*Tillandsia usneoides*) has been used as an indicator of poor air quality where high carbon monoxide levels occur [14]. Spanish moss populations have decreased where human activities have increased [15]. Since 1970, an introduced invasive epiphytic plant species, ball moss (*Tillandsia recurvata*) began spreading throughout the Baton Rouge, La. metropolitan area [16]. Both Tillandsia species are in the family Bromeliaceae and a relative of the pineapple. Spanish moss has trichomes (hairs) that absorb moisture and assimilate its nutrients from the atmosphere. The difference morphologically between ball moss and Spanish moss is that ball moss forms a golf ball size mass of leaf tissue instead of the graceful, long filamentous draping Spanish moss. Ball moss forms a 3-dimensional spherical lattice structure of filamentous living plant tissue. Ball moss has an aggressive growth habit and spreads prolifically. Due to its widespread growth in the warm moist climate in Louisiana, researchers wanted to determine the feasibility of using ball moss as a biological indicator.

The objective of this study was to determine the feasibility of using ball moss as a biological indicator and an oil retentionist.

2. Materials and Methods

On October 13, 2020, vehicular frequency data from LaDOTD was chosen by location and daily average vehicular count records [17]. The records established several areas that were randomly selected where ball moss could be collected. Multiple sites were selected and classified as daily low (0.0 - 7100 average vehicle counts) to medium/high (7100 to >14,000 average vehicle counts) vehicle counts. The plant tissue was analyzed for S content by the LSU AgCenter Soil Testing Lab. Nutrient analysis was initiated by harvesting plants' tops at the termination of the project, and dried at 60˚C. One gram of ground plant material was transferred into a 20 ml scintillation vial and placed in an oven at 50˚C for 1 h to remove residual moisture. Vials were transferred to desiccators for 1 h to further remove moisture and cool the sample to room temperature. The caps of each sample were tightened upon removal from the desiccators to prevent moisture from re-entering. Elements were analyzed by placing 0.5 g of tissue into a 50 ml tube (SCP Scientific digiTUBE). Funnels were placed in each tube, and samples were placed into an automatic digester (Thomas Cain, DEENA) for digestion using nitric acid. During the digestion, the samples are heated for 6 s at 60˚C and 2.2 ml of distilled water is added. After 2 m, 5 ml nitric acid (SCP Science, 67% to 70% HNO_3, reagent grade) was dispensed into each tube, and the temperature was increased 10˚C every 10 m from 60˚C to 110˚C. The temperature was increased to 125˚C and held for 45 m, and then held for 50m at 128˚C, and cooled for 2 m. One ml of hydrogen peroxide (Macron Fine chemicals, 30% solution) was dispensed into each tube, cooled for 5 m and reheated for 5 m to 128˚C. One ml of hydrogen peroxide was dispensed, and another 1ml of hydrogen peroxide was dispensed into each tube. Samples were cooled for 5 minutes and heated for 30 minutes at 122˚C, cooled for 6 seconds to 20˚C and cooled for 1 more minute. The volume of each sample was brought to 20 ml using distilled water. Samples were removed from the digester and vacuum filtered using a 1.0 micron Teflon membrane filter (SCP Science) into another 20 ml tube. ICP was performed for S using a Spectro Arcos according to the LSU Soil Testing and Plant Analysis Lab's AgMetals procedure. The instrument was calibrated using one blank and 6 standard samples. Samples were run in sets of 60 (2 blanks included) with two National Institute of Standards and Technology (NIST) peach samples and an internal standard for every 20 samples. The data was verified to ensure it was within the tolerant ranges of the NIST and internal standards. Nutrient levels were reported as % (dry weight).

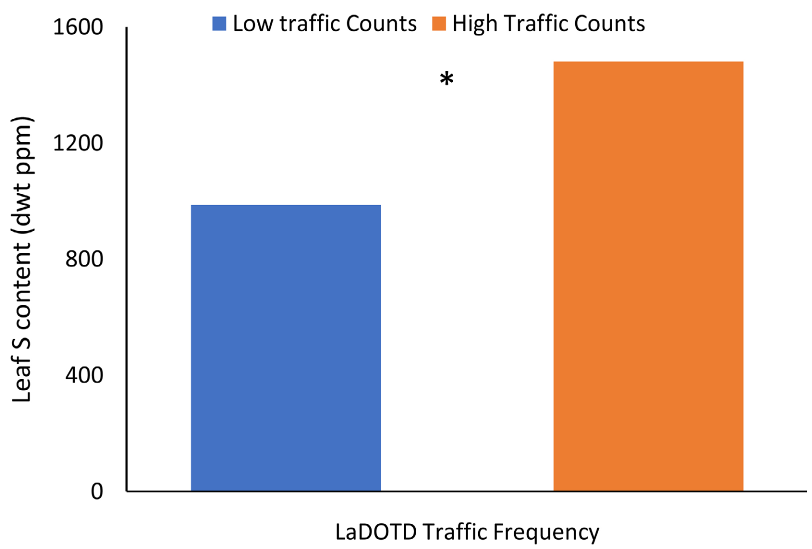
Ten ball moss sub-samples were collected from each respective site. The plant material was dried at 60˚C for 48 h and ground using a Whiley Mill passing through a #20 sieve. Additionally, absorbent paper towels were formed into a similar sphere as the ball moss (average 4.7 cm diameter). Data was statistically analyzed using an ANOVA at the 0.05 level of significance.

During the second experiment, *Tillandsia recurvata* tested for oil retention. A 5.7 L plastic tub was filled with water, and then 50 mL of motor oil serving as the experimental contaminant. Six wooden skewers pierced 18 *Tillandsia recurvata* balls and 18 paper balls were used to clean oil from the water surface. Skewered ball moss and paper towels (oil cleaning booms) were passed over the water surface 6 times for the opportunity to retain as much oil as possible. After the ball moss and paper towels absorbed oil, the treatment balls were re-weighed (g) and a ratio was established by placing the prior plant weight in the denominator and the weight after imposing the treatments establishing a ratio (weight in grams after treatment/weight prior to treatment in grams). A control treatment (no oil) consisting of plants passed over a clean water surface 6 times in a similar manner as the oil contaminated water.

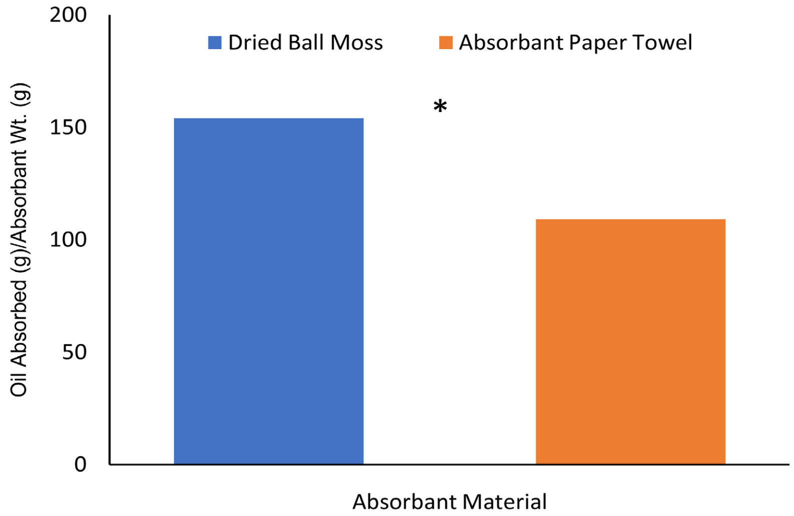
3. Results and Discussion

Results indicated that there was a relationship between S tissue content and the LaDOTD traffic frequency data (**Graph 1**). This research establishes that plant tissue can be used as an inexpensive biological indicator in urban environments. As shown by **Graph 1** there is significantly less S in the low traffic frequency compared to the medium/high frequency sites. Previous research [14] had mentioned that Spanish moss absorbed the atmospheric levels of pollution. Ball moss seemed to also reflect the pollution levels in a similar manner as Spanish moss. This is a logical inference since both plants are in the same family and leaves are both filamentous. Using ball moss, other biological indicators, and digital sensors could give a comprehensive measurement of a polluted environmental site.

Additionally, the results indicated a definite mass difference in absorption of oil between the samples of ball moss passed through water contaminated with oil and absorbent paper tissue (**Graph 2**). Previous research with mosses determined



Graph 1. Comparison of ball moss leaf tissue sulfur levels in low and high traffic areas. *Significant at the 0.05 level.



Graph 2. Comparison of oil absorption from a contaminated water source using different materials. *Significant at the 0.05 level.

that the fibers could absorb up to 30 to 40 times its dry weight in liquids [17]. Research results established *Tillandsia recurvata* can absorb oil from oil:water mixtures. As depicted in **Graph 2**, there was a greater mass difference between the two treatments. The differential mass establishes the potential for *Tillandsia recurvata* to be used as a remediation material. Although data was not shown, a floating boom packed with ball moss maintained a barrier between oil contaminated water and clean water. The 3-D filamentous lattice structure of ball moss seemed to restrict the flow of the oil and absorb it. Past research has suggested that Spanish moss was effective on absorbing many different types of contaminates [18]. This ball moss study reinforces the possibility of using Tillandsia for oil spill remediation. Thomson [19] discussed the development of an oil absorbent towel that has a lattice nano-mesh structure. Future research is needed to establish whether the use of a surface boom filled with ball moss may be used to mitigate surface water contamination.

4. Significance to Society

As time goes by the pollutant rates in Louisiana continue to rise with increasing manufacturing demand. Not only in Louisiana is air pollution a big environmental issue, but also all over the world. *Tillandsia recurvata* was proven to be a successful biological indicator for environmental impact studies or as a useful biological indicator. As the oil industry in Louisiana continues to grow, oil pollutes our waterways as well as coastal areas around the world. The equipment needed to filter the bodies of water is costly. *Tillandsia recurvata* could assist efforts to clean oil spills. Further research should evaluate the usage of ball moss to reduce air and water pollution.

5. Conclusion

Tillandsia recurvata proves to not only be a reliable biological indicator, but also an oil retentionist. Major findings included the absorption of both pollutants in the atmosphere and oil spills in water by ball moss. The first study determined that ball moss could be helpful in monitoring S concentrations in high traffic frequency areas around Baton Rouge. The second study concluded that ball moss can absorb oil and serve as an oil retentionist. Therefore, *Tillandsia recurvata* could be a useful epiphytic plant used to benefit the environment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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