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EXPLORATORY ANALYSIS OF NUTRIENT USE EFFICIENCY VERSUS BIOACCUMULATION ON TWO NATIVE SPECIES IN A COLLEGE CAMPUS

Partial requirement for the earning of the Master's Degree in Sciences in Environmental Management in Conservation and Management of Natural Resources

> By: David T. Nachi Vázquez May 19, 2014

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THESIS SUMITTED AS A PARTIAL REQUIREMENT FOR THE TITLE OF

MASTER IN SCIENCE IN ENVIROMENTAL MANAGEMENT IN CONSERVATION AND MANAGEMENT OF NATURAL RESOURCES

UNIVERSIDAD METROPOLITANA SAN JUAN, PUERTO RICO 2014

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DEDICATION

This project is dedicated lovingly to my nuclear and extended family, especially my wife, Jeniffer Rivera, and my sister, Minami Nachi, for giving me the critical support when I needed it the most. Without your understanding, love, and passion, this weight over my shoulders would have been too much to bear and I would have not come this far to celebrate this collective triumph that marked significantly our lives.

ACKNOWLEDGEMENTS

I want to thank all of the faculty members of the School of Environmental Affairs at the AGMUS UMET, Cupey Campus, fellow graduate students, and people responsible for encouraging the accomplishment of this achievement.

To my dear Committee Director, Beatriz Zayas, for your help and support with open arms despite your tight agenda; I will never forget I owe you free Karate classes in exchange for your gift of time and effort. To dear Committee Member, Juan C. Musa-Wasil, for your understanding, inspiration, and mentoring all along my Graduate School life. Thank you for your supportive, honest, and fun way of teaching and mentoring me. I will always cherish your friendship and passion. To dear Committee Member, Professor Carlos Morales, for giving me critical feedback for the written thesis paperwork and for your availability and noble participation at my defense. To my dear mentor from my Bachelor in Science at the University of Puerto Rico, Cayey Campus, Dr. Robert Ross, and the staff members at the RISE Program, for guiding me through my younger academic years. Because of you, I've improved my critical thinking and my English writing skills, helping myself ease the pace to success at Graduate School.

To former and fellow Graduate students, Wilmer Rivera, Carla Mejías, Marixa Maldonado, Luis Colón, because the little details that helped improve my academic work counted and mattered deeply. To Mrs. Yamilis Ocasio, Mrs. Zidnia Nieto, Mrs. Priscilla Casanova, Mario Maceira, and Director Julia O' Hallorans, from the Agricultural Experimental Station at the University of Puerto Rico Botanical Garden. Thank you for the suggestions, your accurate recommendations, expertise and availability to help me work this project out. To fellow laboratory trainees, María Yadira Berríos, Jelissa Reynoso, and Gabriela. To my sister, Minami Nachi, for giving me hints on how to get things better with organization on sampling, besides helping me do the samples collection. To my dear wife, Jeniffer Rivera, for helping me sacrifice some of our Sundays to finish the samples collection, getting stung by mosquitoes with me in the process.

I also want to give my best regards to the staff of the Department of Agriculture Agrological Laboratory, specially to Mrs. Sonia Carrasquillo, for my first referenced analyses on the first samples collection.

I thank to Professor Félix Velázquez and his staff at the Office of Safety, Occupational Hazard and Environmental Protection at the University of Puerto Rico, Cayey Campus: your disposition to allow me access to the Park of the Green Shadows really helped me to feel like at home.

My deepest displays of gratitude to all at the School of Environmental Affairs: to former secretary, Nilda Rivera, for welcoming me every time I stepped into the School, to current secretary, Ms. Ginasarely Quiles, for your mellow attention; to Mrs. Sharon Matos, for your sweetness in your concerns when I needed you; to Academic Coordinator, Mr. Alex Rodríguez, for giving me your fast, accurate, and heart-warming service. To former Graduate Student and Statistics expert, Mr. Iván Montilla, and fellow Graduate student, Mr. Rafael Díaz, for the flexibility and the concern in helping me with the Statistics for my thesis. To the Dean of the School of Environmental Affairs, Professor María C. Ortiz-Rivera, for being supportive and empathic throughout my academic growth from the very beginning.

Special thanks to the UMET Chancellor and former Dean of the School of Environmental Affairs, Carlos Padín, for your unconditional interest in the growth of my academic career, helping me with the last minute details regarding my thesis, and the Secretary of the Chancellor, for your quick response in terms of the disbursements for the funding of my work.

Last, but not least, I want to thank my nuclear family, Belén Aponte and Carmen Vázquez, because the circumstances between us helped me build a strong and die-hard spirit. To my extended family, Norma Álvarez, Wilfredo Rivera, Carmen E. Rivera, Stephanie Rivera, and Erica Rivera, for your good vibes and your support. To my friends, or the family I chose, Elvin Santana, María Meléndez, Christian López, Zenaida Suárez, Carla Pérez, Maribel Rosario, José Daniel Núñez, and every friend from my Karate circles that helped contributed in my training, contributing to the discipline I have made for every aspect of my life through the years. I apologize if there is any key person I may have missed, making the most of my effort to share this triumph with all that is concerned. To everyone, my most profound of gratitude's, because each grain of sand built the dune that I am today.

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LIST OF ABBREVIATIONS

- NUE = nutrient use efficiency
- UPR-CUC = University of Puerto Rico, Cayey University Campus
- OSSOPA = Office of Safety, Occupational Hazard and Environmental Protection
- Tg = *Thespesia grandiflora* (Maga tree)
- Gg = *Guarea guidonia* (Guaraguao tree, American muskwood tree)
- N = nitrogen
- P = phosphorus
- K = potassium
- NPK = nitrogen, phosphorus, and potassium
- RUE = resource use efficiency
- %RT = retranslocation percentage/nutrient reabsorption efficiency/retranslocation efficiency
- SLA = specific leaf area
- MRT = mean residence time
- [NPK] = concentration of nitrogen, phosphorus, and potassium
- DNER = Department of Natural and Environmental Resources
- LMA = leaf mass per leaf area
- JCA = Junta de Calidad Ambiental (Environmental Quality Board)
- JP = Junta de Planificación (Board of Planning)
- NEPA = National Environmental Policy Act
- EIA = Environmental Impact Assessment

- DEI = Declaration of Environmental Impact
- USEPA = United States Environmental Protection Agency
- CEQ = Council of Environmental Quality
- CWA = Clean Water Act
- SDWA = Safe Drinking Water Act
- ESA = Endangered Species Act
- USFWS = United States Fish & Wildlife Service
- USGS = United States Geological Survey
- UTM = Universal Transfer Mercator
- CAL = Central Analytical Laboratory
- AES = Agricultural Experimental Station
- TKN = Total Kjeldahl Nitrogen
- TKP = Total Kjeldahl Phosphorus
- Sn = senescent
- Mt = mature
- %BCF = Bioaccumulation percentage of nutrients
- [NPKleaves] = concentration of NPK in leaves
- [NPKsoil] = concentration of NPK in soil
- NRCS = Natural Resources Conservation Service
- GPP = gross primary productivity
- NPP = net primary productivity

ABSTRACT

Nutrient use efficiency (NUE) and bioaccumulation are two important forestal parameters that allow us to understand the ecophysiology of plants in terms of nutrient conservation and nutrient cycling mechanisms between the plant species and the ecosystems, being all these related to productivity and biomass generation, among other ecological energy fluxes (Rivera, 2011; Lugo, 1998). A good approach to quantify NUE is by measuring retranslocation efficiency (%RT), which represents mobilization of nutrients from the senescing leaf to the phloem of the tree, which leads to biomass generation and productivity increase. Bioaccumulation is the parameter that studies the behavior of nutrient mobilization from soil to the plants leaves via radicular nutrient uptake. Cayey's urban development has received peculiar impacts for more than a century, turning the vast majority of the forest coverage to secondary forests. Our area of study has been altered by the human hand for more than a century at the University of Puerto Rico, Cayey Campus. The Park of the Green Shadows is located in a former riparian ecosystem, but the re-channeling due to the construction of the gym, the Athletic racetrack, the Miguel Meléndez Muñoz High School and the Ramon E. Betances Middle School caused dynamic changes that scarred the activity of the ecosystem. The Park of the Green Shadows (PGS) is now an urban forest with a focus on native species revitalization. Though there is not enough information regarding past impacts or current impacts, this investigation pursued in an exploratory manner the observation of the present status of PGS in terms of productivity, biomass, and nutrient flux. The physiological mechanisms used by these trees are diverse, but our focus was mainly to make an exploratory analysis of nutrient retranslocation versus bioaccumulation in both *Thespesia grandiflora* and *Guarea guidonia* tree species in the same restoration conditions. We found that G. guidonia had higher retranslocation rates than T. grandiflora. There was no difference in %RT between, but K had the highest %RT in the 1st collection, which could indicate this is the less available nutrient in soil when precipitation occurs. There were significant changes in BCF between the first and second samplings, being P the macronutrient that had higher mobility in the first and N and K had better mobilization in the second, which could indicate that both species can be sensitive to changes in weather. This research protocol (Protocol No. B01-019-14) was submitted to and approved by the Institutional Biosafety Committee (IBC) at AGMUS.

CHAPTER I INTRODUCTION

Problem Background

In Puerto Rico, it has been well-documented the loss of up to 90% of the forests to agriculture until the 1940's. The natural recovery of them by formation of secondary forests through abandonment of land due to shift from agriculture to manufacture (Pascarella, Mitchell-Aide, Serrano, Zimmerman, 2000), has created an environment approachable in terms of agroforestry and ecophysiology studies. Since we are working on forests with a background of anthropogenic perturbations for more than a hundred years, measures are to be strict about conservation and management. Studies of how the productivity, mass and energy fluxes between ecosystems, biogeochemical cycling, biomass production and nutrient use efficiency (NUE) and nutrient use proficiency (Killingbeck, 1996) are to be taken into consideration since all these variables have been significantly affected by human sources.

Cayey, municipality of the Central-South-East area of Puerto Rico, had only less than 20% of forest coverage by 1937, which was then recovered naturally by secondary forest growth up to 62% by 1995 (Pascarella et al., 2000). In the 1990's, Cayey began to experience a fast-growing urban development since its induction to the Metropolitan San Juan Area, which also encompasses the municipalities of San Juan, Guaynabo and Caguas. According to Google Maps® and Carta Natal Online, the coordinates for the University of Puerto Rico, Cayey Cam-

pus are 18°06′53″N (latitude) 66°09′08″W (longitude) in geodesic coordinates (Hernández & Arévalo-Hernández, 2012), and 18.1183, -66.1621 (Dices: Directorio Cartográfico, 2012) in Universal Tranverse Mercator (UTM) coordinates (Hernández & Arévalo-Hernández, 2012), and a specific above-sea elevation of 395m (Dices: Directorio Cartográfico, 2012). With a maximum above-sea elevation of 1300m and a mean annual precipitation of 2,540mm, the Cayey zone consists mostly of subtropical rain forests. By 1990, there was a record of 530mm of rainfall in twenty-four hours. The record minimum temperature to date is 7°C, and 34°C as a maximum. Due to the urban development and the cool temperatures, many multinational companies established some manufacturing units, which emissions, combined to the characteristic fogs and recurrent precipitations, tend to prolong greenhouse gases in the lower atmospheric layers. Also, the private and public transport systems contribute to induce stress on trees, which are the largest living, non-human population at a macro-level in Cayey.

Very little has been studied about Cayey's environmental issues. There has been a series of projects that changed the face of urbanization and economic growth in Cayey, provoking also the perturbation to environment. The issue is, if the authorities are making environmental decisions, these are not as rapidly implemented as the urbanization and industrial development decisions. In the case of our area of study, both the impacted zone and the nursery are being taken care of at the University of Puerto Rico, Cayey University Campus (CUC), established in 1967. The CUC was previously a Spanish military camp upon the Hispanic American War until it was occupied by US Military Forces in 1898 for three years, and then turned into the Henry Barracks military unit (its name honoring the third military governor since the occupation), then into the National Guard base until it was designated the new University of Puerto Rico (UPR) Cam-

pus in 1967. All of the activities created a peculiar impact of the current College area to be studied in terms of nutrients fluxes and nutrient conservation.

For purposes of this research study, we are only focusing on how the internal nutrient fluxes within two native species of trees are able to overcome the dramatic changes, both in retranslocation efficiency and bioaccumulation efficiency towards the maturation of the forest within the Park of the Green Shadows. We might consider that the harvest of native tree species at the Park of the Green Shadows, which is under jurisdiction of the CUC was, in fact, a mitigation strategy to revitalize and provide for the necessities of the native species to be strengthened and favored and how the nutrient use efficiency (NUE) distinguishes these species from those in the human-impacted zones. Determination of NUE gives us hints on which species we can harvest in impacted and degraded areas for soil recovery (Rivera, 2011). We might also consider more specifically in future investigations variables such as productivity, biomass measurements, nutrient donation and recycling, among others.

In the past, the Park of the Green Shadows (PGS) belonged in a watershed where rivers flowed through the present UPR-CUC athletic track, the Miguel Meléndez Muñoz High School, and the Ramón Emeterio Betances Middle School, but became dried and great pieces of cortex were cleaned up to advance the Cayecan -Arahuacan/Tainian name given honoring the village of the tribal chief Cayeco- urbanization development. Since then, it became an uncultivated land with a slow process of recovery, with the former nursery at the main campus being transplanted at the Park as a place to harvest the species.

Problem of study

Human perturbations on soil and vegetation are most likely the main causes of acidification, nutrients leaching, run-offs, sedimentation, and eutrophication of watersheds, which all affect directly in the autonomy of the ecosystems to renew their biogeochemical cycles and to provide food for the rest of the trophic net (Balasubramanian, G., Udayasoorian, C., Prabu, P.C., 2007; Zhang, J.P., Wang, J.J., Zhao, Z.M., Dou, W. & Chen, Y., 2004). There are natural and anthropogenic causes of scarcity of nutrients in ecosystems, which among them might be acidification due to excess of airborne sulphur and nitrogen (Göransson, Falkengren-Grerup, & Andersson, 2011). However, most (if not all) trees manifest a smart alternative in cases there has been nutrient deficiency of soil and there is not much to be reabsorbed (Covelo et al., 2008). Trees tend to react by incurring to nutrient conservation or reabsorption, although certain species have advanced radicular adaptations that allow them to reach deeper in the soil (Aerts, 1999).

Our area of study has been altered by the human hand for more than a century with the Spanish military occupation in the late 1890's until the 1960's at the lands that are now the UPR-CUC. The UPR-CUC college houses more than 5,000 trees, which means lots of work of silvicul-ture, forest engineering, and forestry to be made. The physiological mechanisms used by these trees are diverse, but our focus is to make a quantitative analysis of nutrient retranslocation and bioaccumulation fluxes within two native species in a four-week period (i.e., nutrient efficiency vs. nutrient proficiency vs. bioaccumulation). This way we can trace and understand, if not predict, certain parameters such as leaf lifespan (i.e., life cycle from maturity to abscission), retranslocation percentage by the time of last collection, among other patterns and mechanisms.

The establishment of nurseries are a way of rehabilitation and enhancement on the environmental conditions for the trees to grow. The purpose of these is to restore the ecosystems' integrity, regulate the development of invasive species, enhance the quality of the sites, decrease of the distribution and flux of pollutants, among other environmentally profitable activities (Arana, 2008; Rivera, 2011). Mitigation methods such as nurseries revitalize the accumulation, fixing and flux of nutrients, while leading to forest recovery and increase of biodiversity (Francheschini, 2008; Rivera, 2011). Through these projects we can get basic information such as forest profiles, description of impacts and uses, biomass generation and nutrient uptake through litterfall (Arana, 2008; Rivera, 2011).

At the UPR-CUC, there is still much to be studied about the past impacts and the management methods applied on the study sites due to lack of information generated, but the initiatives carried on by the Office of Safety, Occupational Hazard and Environmental Protection (OSSOPA) within the campus are a good start to a successful restoration project, now that the nurtured species became transplanted in the lands that belong to the Park of the Green Shadows new habitat. Though the Park of the Green Shadows was established for the recovery and maturation of the species, there is still much impact in the surroundings to be mitigated or diminished, or much to study about the resiliency of the species in the park.

Cayey (from the Arahuacan Word that means 'land of waters') urban development has received peculiar impacts for more than a century, turning the vast majority of the forest coverage to secondary forests. Our area of study has been altered by the human hand for more than a century (for military purposes) at the University of Puerto Rico, Cayey Campus. The Park of the Green Shadows is located in a watershed that belonged in the past to a riparian ecosystem, but the re-channeling due to the construction of the gym, the athletic racetrack, the Miguel Melendez Munoz High School, and the Ramon E. Betances Middle School caused dynamic changes that scarred the activity of the ecosystem. The Park of the Green Shadows is now an urban forest with a focus on ecosystem revitalization using native and introduced species. Though there is not enough information regarding past impacts or current impacts, this investigation wants to explore the actual status in terms of productivity, biomass, and nutrient flux. The physiological mechanisms used by these trees are diverse, but our focus is to make an exploratory analysis of nutrient retranslocation versus bioaccumulation in both *Thespesia grandiflora* and *Guarea guidonia* tree species in the same nutrient exchange conditions.

Though the mechanisms involved are not fully understood (Chapin, F. S., Matson, III, P. A. & Mooney, H. A., 2002) due to the complex net of causes that influence these packed traits within the plants incurring in such activities as CO2-related dynamics (Norby, Long, Hartz-Rubin & Neill, 2000) or increase in latitude of origin (Oleksyn, J., Reich, P.B., Zytkowiak, R., Karolewski, P. & Tjoelker, M. G., 2003), nutrient use efficiency (NUE) is a forestal measurement that helps manage how tree species are able to transfer all the necessary nutrients from the senescent leaf (i.e., abcissed, or cut down to be added into the litterfall) to younger leaves for photosynthetic rates renewal through the phloem (Chapin et al., 2002; Del Arco, Escudero & Garrido, 1991; Imbert & Blanco, 2004). This does not necessarily mean that NUE is exclusively from leaves to branches, and studies of other retranslocation routes have been documented, such as retranslocation from the heartwood to the sapwood (Attiwill & Leeper, 1987; Imbert & Blanco, 2004). This

does not also mean that all of the senescent leaves nutrients move back to the internal tree fluxes: part enriches the soil as the leaves fall into the litter bank (Allison & Vitousek, 2004).

The relevance of these parameters is that allows us to identify and suggest tree species we might harvest to improve their conditions for growth and development, restoring the ecosystems' default properties where they once were limited and degraded (Cuevas & Lugo, 1998; Montagnini & Jordan, 2002; Rivera, 2011). Among the reasons for the scarcity of nutrients in certain environments, loss of the predominant nutrients, such as phosphorus (P) to agriculture by runoffs, leaching and other processes contribute to the stimulation of the biochemical traits of the plant species to retranslocate their resident nutrients in leaves (McDowell, Biggs, Sharpley & Nguyen, 2004). To raise an interesting topic, deciduous trees are not necessarily different in their ecology than their evergreen counterpart, though this bout disseminated lots of questions (Del Arco et al., 1991).

We want to calculate NUE of nitrogen, phosphorus, and potassium (NPK) (Lugo, 1998; Rivera, 2011; Vitousek, 1982; 1984) by also measuring retranslocation percentage and bioaccumulation of the nutrients previously mentioned (Aerts, 1996; Allison & Vitousek, 2004; Lugo, 1998) on the native tree species, being their taxonomical names *Thespesia grandiflora* (Spanish common name: maga), and *Guarea guidonia* (Spanish common name: guaraguao), in order to analyze ecophysiological features and differences between these trees at the Park of the Green Shadows. We also want to track how nutrient use proficiency, which is the percentage of nutrients left on the leaf at the litterfall (Killingbeck, 1996), can help us determine the better species in terms of nutrient contribution to the litterfall and nutrient resorption and the bioaccumulation to the soil. The greater the retranslocation percentage (%RT) of NPK, the greater the nutrient recycling and reuse (Aerts, 1996; Allison & Vitousek, 2004; Lugo, 1998; Rivera, 2011). Justification of study

Nutrient use efficiency, or NUE has been well-documented. Genetic traits allow trees to adapt to nutrient-poor environments, making them tend to rely less on soil nutrients uptake and are more inclined in nutrient conservation mechanisms (Lugo, 1998; Rivera, 2011). According to Funk & Vitousek (2007), high NUE maximizes resource-use efficiency (RUE). Thus low-nutrient environments select for plants with the following traits - slower growth rates, longer leaf lifespan, higher concentrations of chemical defenses, and thicker leaves with lower nutrient contents. NUE is a component of the eco-physiology of trees and helps mobilize nutrients and sustains trees in the ecosystem (Allison & Vitousek, 2004). It also favors biomass generation, primary productivity, and other benefits (Lugo, 1998). The most appropriate mathematical definition of NUE is the primary net productivity (taking aerial and underground variables into consideration) per unit of reabsorbed nutrient per year (Martín, A., Santa Regina, I. & Gallardo, J. F., 1996). These measures are possible if taking into account under controlled conditions, but are hardly put on practice on Nature (Birk & Vitousek, 1986).

In the young years of the plant species in general, concentrations of NPK are far superior to the concentrations of foliage development and shoot growth (Escudero, A., Del Arco, J. M., Sanz, I. C. & Ayala, J., 1992; Martín et al., 1996; Gallego, H. A., Santa-Regina, I., Rico, M. & Rapp, M., 1994) than in the stable phase. Mobilization of these nutrients decrease until the maximum foliage cover comes with a stable concentration of the nutrients (Martín et al., 1996). Some species exhibit maximum N concentration two or three months after the leaves begin to grow (Martín et al., 1996; Escudero et al., 1992). Nutrients stabilization phase extends to the end of the leaves' life (Gallego et al., 1994), approximately four or five months for the majority of the deciduous species that are more vulnerable to herbivory and genetic diseases.

Hemminga, Marbà & Stapel (1999) defined accurately what Killingbeck (1996) stated the difference in the insights of reabsorption efficiency and reabsorption proficiency. Reabsorption efficiency is considered as the degree to which nutrient investment stored in leaves stands for conservation in plants, while reabsorption proficiency is the value related to the traits of the plants that distinguish minimization of nutrient loss that is invested to the litterfall (Hemminga et al., 1999). These traits depend directly of the latitude, type of ecosystem, nutrient-availability in soil, type of soils, watersheds perturbations, among other reasons. Examples like the one presented by Mejías-Rivera, Musa-Wasil & Otero (2013), showed the ability for even the mangrove tree forests to bio-accumulate heavy metals by retranslocation to their tissues.

Retranslocation, or nutrient reabsorption, especially the one from senescing leaves, is so far the more contributing of the internal nutrient remobilization processes occurring in perennials to nutrient cycling in plants and soils (Milla, Castro-Díez, Maestro-Martínez & Montserrat-Martí, 2005), leading us to conclude that the leaves are the main warehouses of life chemistry in plants. From this process on, there is equal contribution to the litter bank and to the bank within the species, promoting in the meantime self-sustainability and nutrient conservation mechanisms (Milla et al., 2005). Another way in which we find usefulness in the NUE parameter is by observing the retranslocation percentage, or %RT, which is commonly used as a species-level measurement of internal nutrient transfer (Kobe, Lepczyck & Iyer, 2005; Allison & Vitousek, 2004; Pérez, C. A., Armesto, J. J., Torre Alba, C. & Carmona, M. R., 2003; Wright & Westoby, 2003; Aerts, 1996; Killingbeck, 1996). %RT, also known as nutrient resorption efficiency (Killingbeck, 1996) or retranslocation efficiency (Aerts, 1996), is also the amount of nutrients in the foliar tissue per specific leaf area (SLA) unit (Allison & Vitousek, 2004; Lugo, 1998). Its calculation is made as the ratio between total nutrient contents in senescent leaves and total nutrient contents in mature leaves (Allison & Vitousek, 2004). The correlation between NUE and %RT is positive: both indexes point to the same direction (Lugo, 1998; Pérez et al., 2003).

The relevance of this research aspect is that ecophysiological traits help plants, including trees, rely on this mechanism as its best, under conditions of hardship in terms of nutrient availability and environmental stress, independently if they are exposed to drought or flood conditions (Allison & Vitousek, 2004; Mediavilla, S., Escudero, A., Heilmeier, H., 2001). These traits of a more complex and extreme nutrient conservation mechanism – nutrient reabsorption – perpetuates for an indefinite lapse of time what studies called mean residence time (MRT), which is the time nutrients stay within the plants in order to satisfy what nutrients uptake cannot do (Eckstein, Karlsson & Weih, 1998; Garnier & Aronson, 1998). Analysis of how much [NPK] is left, predicting leaf lifespan and the time of abscission in case there are no clear observations of leaf abscission by the end of the one-month period of study.

Research Questions

- 1. What are the [NPK]'s for the mature leaves and for the senescent leaves in each species, and for the soil samples for each collection?
- 2. How do any of both species %RT differ one from the other in terms of retranslocation rates and bioaccumulation?
- 3. What species resembles higher retranslocation? What species contributes more to bioaccumulation?

Goal

To analyze in an exploratory manner if due to determination of NPK NUE at the Park of Green Shadows tree species, these are more likely to rely on nutrients retranslocation of their own biomass by unit of reused nutrient (Lugo et al., 1990; Rivera, 2011; Vitousek & Stanford, 1986; Vitousek, 1982; Vitousek, 1984), than uptake of nutrients through their roots available in soil. To also analyze if due to the litterfall contribution, bioaccumulation comparison betweenboth species is closely related or they have unique, distinguishable differences in the soil surrounding the species.

Objectives

- To quantify in an exploratory manner which process, retranslocation efficiency (%RT) and bioaccumulation factor (%BCF) is being benefited, and thereby measure nutrient exchange in the ecosystem.
- 2. To quantify the difference in %RT between *Thespesia grandiflora* (Tg) & *Guarea guidonia* (Gg), and their %BCF in terms of nutrient concentrations in the soil.
- 3. To quantify which nutrient is benefited the most and in which process.

CHAPTER II

LITERATURE REVIEW

Historical Background

Cayey, name of the Central-South-East municipality located between the Central Mountain Chain and the Cayey Mountain Chain, owes its name to the Taino-Arahuacan language that gives the entitlement which means "land of waters". When the Cayey village –known officially as Cayey de Muesas, after the Spanish governor at the time– was separated from the Coamo Valley and then established in 1773, there was first a group of seven houses in the peripherals of the Catholic Church, La Asunción Parish. Since then, Cayey's urban development and eventual growth of agriculture, cattle breeding and farming, and manufacture has drastically changed or shaped the Cayey town landscape. All of those anthropogenic sources of ecological impact, made with no scientific records and with little historical records, among other factors, put pressure on activities such as that rivers' re-channeling and drying by terrestrial cortex removal in order to complete the extension of what later would be the town.

According to the Department of Natural and Environmental Resources (DNER), Cayey's mean annual rainfall of about 530 mm and a mean annual temperature of 72°F, its flora sharing the characteristics of the Sierra de Cayey (Cayey Mountain Chain), encompassing a mixture of volcanic, sedimentary and granitic substrates, and has approximately 9% of subtropical

wet forest, 98.6% of subtropical very wet forest, and 0.5% of subtropical very wet lowland mountain forest. Cayey had only less than 20% of forest coverage by 1937, which was then recovered naturally by secondary forest growth up to 62% by 1995 (Pascarella et al., 2000). Secondary succession changed the recovery of up to 62% of forest coverage by 1995, but did not change much the characterization of soil, except for the areas built with concrete and impacted by removal of soil cortex and drainage construction.

Very little has been studied about Cayey's environmental issues. There has been a series of projects that changed the face of urbanization and economic growth in Cayey, Sadly, the emphasis on urban enhancement and restoration to the human structures speak little about problems coping and resolve in terms of perturbations to environment. There are huge differences between the authorities' initiative agility about environmental decisions versus the urbanization and industrial development decisions. Shrinking of the speedways, transit roads and human frequent attendances have been experienced by population inflation. Centralization of social, economical and political activities towards the urban centre has been degrading forests sustainability and resiliency, facing a very different challenge when compared to past human activities.

In the case of our area of study, both the impacted zone and the nursery are being taken care of at the University of Puerto Rico, Cayey University Campus (CUC), established in 1967. The CUC was previously a Spanish military camp until 1897, serving as one of the internal military fronts against US Armed Forces. Once the Spanish-American War exploded beneficially for U.S., it was occupied by US Military Forces in 1898 for three years, and then turned into the Henry Barracks military unit (its name honoring the third military governor since the occupation), then into the National Guard base until it was designated the new University of Puerto Rico (UPR) Campus in 1967. All of the activities created a peculiar impact of the current College area to be studied in terms of nutrients fluxes and nutrient conservation. When the property title was conceded to the University of Puerto Rico at Cayey in 1967, there was a contract stipulation that stated it is forbidden to build human structures in green areas and that the forests are for recreation, conservation and public use. There is very limited space to make building extensions within the UPR-CUC. The Park of the Green Shadows (PGS) is a remnant of what it used to be a riparian ecosystem. Evidence of this statement is the characteristic creek that houses some of the transplanted trees as part of the reforestation program promoted by the DNER at the beginning of the 2000's, and the big river that surrounds the human-originated structures that belong to the Miguel Meléndez Muñoz High School and the UPR-C.

Theoretical Framework

Nutrient use efficiency (NUE), a parameter positively correlated to retranslocation percentage (%RT) is a biochemical (nutrient fluxes within organisms) in which each unit of reabsorbed nutrient becomes the raw material of biomass generation (Vitousek, 1982; 1984; Galicia, L., García-Oliva, F., Murillo, R. & Oliva, M., 2002). The route of nutrient retranslocation starts in the foliar tissue of the soon-to-be-abcissed, senescent old leaf and ends at the phloem of the shoot (Chapin et al., 2002), though this is not the only one (Attiwill & Leeper, 1987; Millard & Proe, 1993). This mechanism decreases the ratio of nutrient loss to litterfall (Hobbie, 1992; Galicia et al., 2002). The mechanism, considered a physiological trait (Allison & Vitousek, 2004), gives certain species the ability to alter the soil conditions by increasing pH, nitrification rates, and litterfall deposition (Ehrenfield, Kourtev & Huang, 2001; Allison & Vitousek, 2004). According to Chapin et al. (2002), the mean %RT, though variable, is approximately 50%, which means that half of the nutrients stay longer within the plants species and the other 50% percent is an inversion to the litterfall bank. In summary, this mechanism is considered as a plant austerity measure.

Despite of the frequent efforts to fully understand the NUE mechanisms and their relations to plants nutritional status and nutrients availability in soil (Aerts, 1996; Birk & Vitousek, 1986; Piatek & Allen, 2000), there are still certain conditions that induce a decreasing retranslocation percentage, such as major litterfall contribution from invasive plants (Allison & Vitousek, 2004) and use of fertilizers that favor nutrient availability (Näsholm, 1994). This mechanism is useful in terms of self-sustainability, while contributing to reducing dependence on current soil nutrient status (Aerts & Chapin, 2000) while characterizing ecosystems processes (Covelo, F., A. Rodríguez & A. Gallardo, 2008). Nutrient resorption proficiency (Killingbeck, 1996), or nutrient retranslocation proficiency (Aerts, 1996), which is the percentage left on the senescing leaf, is a physiological index of maximum reabsorbed quantity that stays longer internally and a minimum to be added into the litterfall (Covelo et al., 2008).

This research topic has an array of quantitative variables such as resorption efficiency and resorption proficiency. Resorption efficiency is the percentage of reabsorbed nutrients in terms of the maximum amount of those nutrients in green leaves (Eckstein et al., 1999; Milla et al., 2005; Building & Lansing, 2005; Covelo, Rodríguez & Gallardo, 2008; Oleksyn, Reich, Zytkowiak, Karolewski & Tjoelker, 2003; Rivera, 2011; Westoby, 2003). Resorption proficiency

is the final nutrient concentration within leaves prior to their abscission and sum into the litterfall deposits (Covelo et al., 2008; Killingbeck, 1996).

Productivity, Biomass and Nutrient Dynamics

An effective way studies made an approach to distinguish different phases of the mature leaves by relating the total content of organic matter versus concentration of nutrients in the foliar tissue is by studying the relation between nutrient retranslocation and specific foliar area (SFA) (Allison & Vitousek, 2004; Vera, Cavalier & Santamaría, 1999; Pérez-Amaro et al., 2004). This way, the correlation of nutrient reabsorption and SFA occurs, especially around nutrients such as N and P. Vera et al. (1999) found the negative correlation between poor soil fertility and low concentration of N, P, and K versus high rates of nutrient resorption. Others have quantified in many tree species high NUE and %RT indexes due to scarce nutrient conditions (Montagnini et al., 2000; Silver, 1994; Bridham et al., 1995; Escudero et al., 1992).

The concept of productivity is best explained when looking at its unit: it means the quantity of biomass is produced per hectare per year (kg/ha/yr). Primary productivity means how much raw organic matter is translated from photosynthesis to its storage and use in an ecosystem (Montagnini & Jordan, 2002; Rivera, 2011). When considering only the organic matter produced by photosynthesis, the process is known as gross primary productivity (GPP). The remaining energy flux stored after cell respiration in all plants is known as net primary productivity (NPP). NPP is important to be functionally maintained because its optimum performance helps restore the detriments caused by natural and anthropogenic impacts and revitalize any damaged ecosystem (Montagnini & Jordan, 2002).

An index that helps measure primary productivity is biomass generation, which is defined as the main reservoir of nutrients along nutrients available in soil (Lugo, 1998). Water irrigation, sunlight, adequate temperature, moist and an appropriate nutrient pool, among other factors, help keep balance in productivity (Murphy & Lugo, 1995).

Nutrient cycling is heavily dependable of primary productivity and biomass generation because it represents the ignition of a continuous mechanism that helps sustain ecological and biological processes (Montagnini & Jordan, 2002; Lugo, 1998). Litter fall decomposition has been very well studied as a way of contribution and diffusion of nutrients in a determined ecosystem. A good manner of avoiding any mistake is by locating and monitoring sources of gain and loss of nutrients, either by quantifying lixiviation or vegetation loss (Montagnini & Jordan, 2002; Grubb, 1995; Lugo, 1990). Nutrient cycling is dependent on the ecosystem and might occur due to endogenous (litter fall chemical features), or by exogenous (environmental factors, climate, natural and anthropogenic perturbations, among others (Read & Lawrence, 2006; Pérez-Salicrup, 2004).

According to Popper et al. (1999) and Lugo (1999), there are diverse methods that can give us information about nutrient exchange because of litter fall. The goal is to turn the biomass variable to a mineral mass variable (Lugo, 1998). In order to do this, it is necessary to obtain the dry weight of the collected litter fall. Once this measure is obtained, we get to understand how plant species generally behave due to either high or low fertility in soil (Lugo, 1998). Litter fall production and decomposition have been well recorded due to its basic but stellar role in nutrient exchange to an ecosystem-level (Grubb, 1995). To a plant-level, it is of vital relevance to sustain an optimum nutrient pool in order to keep functionally an ecosystem. Despite of the need all living organisms have of nutrients, these macromolecules based on elements such as N, P, Ca, K, Mg, Fe, Mn, Co, Zn, among others (Montagnini & Jordan, 2002), scientific literature has focused major efforts in N, P, and K due to their limiting effects in metabolic processes in living tissues. N is present in proteins, nucleic acids (DNA and RNA), enzymes, coenzymes (vitamins, all containing amino groups), and chlorophyll. P, as mentioned earlier, is involved directly to Adenosine tri-phosphate (ATP) formation, which is the way processed reduced sugars such as glucose are transformed chemically to energy in all organisms. K is involved to a rigid structure of shoot (same as human beings depend on Ca in bone and muscle formation), but it is also involved in herbivores and illnesses resistance. Since these nutrients are limited during specific periods influenced by climate, weathering and other environmental factors, plants have developed physiological traits to cope with these stressful events by incurring in nutrient conservation mechanisms.

Retranslocation as a measure of nutrient use efficiency (NUE)

Nutrient use efficiency is a parameter directly related to biomass generation per unit of absorbed nutrient. This is a common nutrient conservation mechanism used by terrestrial plants (Vitousek, 1982; Montagnini et al., 2000). Nutrient conservation is a trait that allows plants the use of a percentage of nutrients to generate biomass in ecosystems where the essential nutrients are scarce in soil or the environment in general (Lugo, 1998; Montagnini & Jordan, 2002). The NUE is mathematically a variable defined as the quantity of organic material in the form of litterfall that is contributed by the plant to the soil, plus the organic material stored per-

manently within the confinement of the plant, divided by the quantity of the lost nutrients (Rivera, 2011). Vitousek calculated the relationship between the litterfall dry weight and the amount of nutrients mass contained in the plants as a mathematical relation that defines NUE. When expressing these two variables as parts of a function of the content of nutrients in litterfall, litterfall nutrient content, or NUE index, is the independent variable (x) versus litterfall dry weight, or the dependent variable (y) (Vitousek, 1982; 1984; Lugo, 1998; Rivera, 2011). Higher NUE index indicates higher nutrient conservation. This index has been widely approved and used by the research community so the variation across other kinds of ecosystems can be compared (Vitousek, 1982; 1984; Lugo et al., 1990; Lugo, 1998; Rivera, 2011).

Nutrient reabsorption must be included when taking into account something as important as resorption (or reabsorption) efficiency, which is a specialized function in the tissues of a plant. Nutrient retranslocation, or nutrient reabsorption efficiency, or retranslocation efficiency (Aerts, 1996; Wright & Westoby, 2003; Killingbeck, 1996; Rivera, 2011) is a physiological nutrient conservation mechanism that most terrestrial plants incur when they do not rely on nutrient uptake due to scarce resources available in soil and when conditions are not favorable (Van Heerwaarden et al., 2003). The percentage of retranslocation, or %RT, is mathematically defined through the following formula: %RT= 1- ([nutrients in senescing leaves]/[nutrients in mature leaves])x100 (Allison & Vitousek, 2004; Pérez et al., 2003; Lin & da Silveira Lobo Sternberg, 2007; Van Heerwaarden et al., 2003).

Study of Cases

Leaf senescence and N uptake parameters as selection traits for nitrogen efficiency of oilseed rape cultivars

Schulte auf'm Erley, Wijaya, Ulas, Becker & Horst et al. (2007) hypothesized that high grain-yielding capacity of oilseed rape in the field at limiting N supply (N efficiency), there are to be considered three key factors: high N accumulation in young leaves due to high efficiency of the N uptake until flowering, delayed induction of leaf senescence, and higher photosynthetic N efficiency under low-light conditions during reproductive growth.

Ectomycorrhizal fungi enhance nitrogen and phosphorus nutrition of Nothofagus dombeyi under drought conditions by regulating assimilative enzyme activities

According to Álvarez et al. (2009), physical and chemical soil variables such as pH, aeration, temperature and moist affect nitrogen and phosphorus biogeochemical processes, causing them to be soaked-off to a sink where they are needed. 'Biogeochemical pathway rates' is a concept that explains extreme drought conditions versus extreme flood conditions, with the first interfering with diffusion of nutrients and the second limiting light from igniting photosynthesis in plants, also limiting plant growth in both cases.

Mycorrhizal colonization, i.e., the symbiosis of fungi mycelium and roots of capable plants, might improve photosynthesis and increase P uptake. One good thing about acidification combined with drought conditions is that phosphorus solubilization is significantly induced by ammonium assimilation. Since drought conditions may limit plants nutrition and photosynthetic rates, ectomycorrhizas' assimilative enzymes have the ability to help sustainability and tolerance in plants.

High geochemical background of potentially harmful elements in soils and sediments: implications for the remediation of contaminated sites

In this case study, Armiento et al. (2011) explain that what drove the scientific community to study and contribute to solve problems about polluted sites listed, without yet taking into consideration those located in developing countries that still need to be identified and studied. One of the greatest concerns is the (apparent) irreversible loss and degradation caused by anthropogenic sources, before and after. Pollution of soil implies major risks to human health due to direct or indirect toxicity found on watersheds.

One concept that is to be important for this study is 'geochemical background', which is the result of exploratory geochemistry and was introduced as a qualitative distinction between standard elemental concentrations and 'anomalies' (or a quantitative dispersion of sampling far away from data that characterizes standard levels). Clearly, this 'geochemical background' has been used formally as a natural availability of an element in a specific material such as sediment, rock or soil.

Effects of nitrogen deposition on growth and relationship of *Robinia pseudoacacia* and *Quercus* acutissima seedlings

According to Ding, W., Wang, R., Yuan, Y., Liang, X. & Liu, J. (2012), in the last century, anthropogenic sources of environmental stress such as agriculture and use of fossil fuels as

energy have brought elevated nitrogen emissions and deposition over land and oceans. In a good way, added availability of nitrogen contributes to greater plant growth rate and higher net primary in N-limited temperate ecosystems. In an adverse way, excess of nitrogen deposition lowers nitrogen retention capacity, soil acidification, eutrophication, interfering in the meantime with carbon geochemical cycling by helping increase emission of atmospheric carbon dioxide. All these factors are key to populations interactions and composition around ill ecosystems.

Robinia pseudoacacia is an example of opportunistic and aggressive invasive behavior. This species is the second most widespread deciduous tree in the world. This nitrogen-fixing tree has a warfare strategy, turning low-nutrient environments into over-enriched environments by increasing nitrogen fixation and deposition by mineralization and excessive litterfall.

Inhibition of ammonium assimilation restores elongation of seminal rice roots repressed by high levels of exogenous ammonium

Hirano et al. (2008), reported that when an excess of nutrients is present in soil, primarily nitrogen, shoot-to-root ratio increases as the development of plants centers toward the shoot, decreasing growth of root. Soil deposits of nitrate and ammonium are inorganic nitrogen sources for vegetation. Reports have demonstrated that great doses of uniformly applied nitrate to the whole of the primary root has a remarkable inhibitory effect on lateral and seminal roots, and that potassium nitrate has no inhibitory effect. Potassium sulfate, however, has a restorative effect in high ammonium concentrations present. Ammonium alone as a nitrogen source in soil contributes greatly to acidification of harvester medium.

The difference between breeding for nutrient use efficiency and for nutrient stress tolerance

Maia et al. (2011) talk about the correlation between low crop yields and scarcity of water and soil nutrients, also contributing to limited plant metabolism and underdevelopment of plant organs. They also stated that there are current breeding programs and projects that study how are plants adapted to abiotic stress in terms of these limitations and how efficient they are in their use. In their project, they focused their research in both tolerances to availability below the optimum versus the efficiency in their use. They found no correlation on the stress tolerance and nutrient use efficiency parameters, by which is presumed these traits are controlled by different gene groups. On the other hand, they theorize that simultaneous selection between these parameters is possible if there is no antagonistic mechanisms gestating the expression of the traits.

Relationships between leaf lifespan and structural defences in a low-nutrient, sclerophyll flora

Wright & Cannon (2001), consider factors such as leaves construction cost, net photosynthetic rate and lifespan are important to determine the net carbon gain from a leaf over its lifetime. A wide variety of research studies demonstrated the relationship between species with greater leaf lifespan, high leaf mass per leaf area (LMA), and slow net photosynthetic rate versus those with shorter leaf lifespan, low LMA and fast photosynthetic rate. The article also explains that leaf mass per leaf area, or LMA, is the product of leaf thickness and density (mass per volume), indicating that the intrinsic relationship between leaf lifespan and LMA among species that reinforced construction of leaves is most likely to increase their lifespan by increasing their structural defenses against herbivory and other physical threats. The article exhibits the different ways a leaf might die: this is due to herbivory as well as temperature or water stress, damage by wind, rain or the thrashing action of neighboring branches, all of these reasons being external to the plant. Leaf death can also be metabolically

mediated by the plant, and it might be due to some phenological cue, to self-shading or to shading from neighboring plants. It is explained that the difference between the metabolically-mediated loss of leaf tissue and the unexpected loss due to external facts is the amount of nutrients withdrawn from the resorption or retranslocation process versus the ones that are stolen from the impacted nutrient pool within the plant, about 50% of N and P lost before leaf abscission could occur. Wright and Cannon also explain some defenses these plants have toward threats to nutrient storage in leaves and explain the concept "force of fracture", which is important to measure the resistance and strength of leaf construction.

Legal Framework

State Laws

Constitution of the Commonwealth of Puerto Rico: Art. VI, Sec. 19. Indicates that it will be public policy of the Commonwealth the most efficient conservation of its natural resources, and the major profit and development of the same for the sake of the community.

Law No. 416 of September 22, 2004: Environmental Public Policy Act of the Commonwealth of Puerto Rico. This State Law amends the Law No. 9 of July 18, 1970. The same creates and confers authority the Environmental Quality Board (Junta de Calidad Ambiental, or JCA) to adopt, promulgate, amend or derogate regulations for the protection of the environment in Puerto Rico. As the law clearly states: "that the JCA is to find all media for the protection of the environment and the well-being of man". The same got into effect on March 22, 2005. EQB if the organism entitled to regulate and implement controls over the pollution, environmental degradation and the protection of air, water and soil. As simple and poetic it sounds, it is important to reconcile man with nature through the existence of this law (López-Feliciano, D., 1999).

Law No. 23 of June 20, 1972, Organic Act of the PR Department of Natural and Environmental Resources, as amended. This law creates the DNER and makes responsible of planning, protecting and managing all of the natural resources of Puerto Rico. The amendments to this law have included the protection of the wetlands as shown in the Law No. 31 from March, 14, 2000. Also through this law is the DNER able to grant permissions, franchises and licenses to the citizens in order to regulate and control the impact over the resources (López-Feliciano, D., 1999).

Law No. 133 of June 1, 1975, Puerto Rico Forests Act, as amended. This law establishes that the forestal public policy of the Commonwealth, which is addressed to the preservation, conservation, protection and expansion of the public forests and to stimulate the private initiative to-wards the same goals. Through this, there is an attempt to achieve the profit of our forest resources for the sake of this and future generations. Mangrove forests, declared as State Forests, are protected by this law. Also, the DNER is conferred authority by the law to acquire lands with the goal of designated them as forests (López-Feliciano, D., 1999).

Organic Act of the Board of Planning. This law creates the BP, which is going to be in charge of driving the integral development of Puerto Rico in an economic, but measurable and moderate way, the proper use of lands for the enrichment of the well-being, safety, health, defense of culture, economic stability, order, and coexistence of nature and people alike (López-Feliciano, D., 1999).

Law No. 81 of August 30, 1991, Autonomous Municipalities Act, as amended. This law gives the municipalities the faculty to organize, use and develop infrastructure in consonancy to the environment in a way the people make good use of the resources while they are satisfying their needs (López-Feliciano, D., 1999).

Law No. 241 of August 15, 1999: Commonwealth of Puerto Rico New Wildlife Act. This law with the goal of protecting wildlife species, but most important yet, their habitats. Public agencies are obliged to consult the DNER to avoid direct impact by establishing their projects. The law also forbids perturbation and damage to critical habitats where wildlife species tend to grow, feed and reproduce (López-Feliciano, D., 1999).

Law No. 150 of August 24, 1988: Puerto Rico Natural Heritage Program Act. As a synergic conjunction of federal, state and local regulations, this law gives the DNER the power to acquire areas of high natural and ecological value in order to be protected and conserved for the use and joy of the present and future generations (López-Feliciano, D., 1999).

State rules

Rule to administrate vulnerable and endangered species in Puerto Rico. This rule is made to help identify, preserve and conserve vulnerable and endangered species. It also favors propagation and survival of both vulnerable and endangered species. Identification and promotion of critical natural habitats and essential critical natural habitats. Regulation of import and export of vulnerable and endangered species. Regulates designation criteria used by the global scientific community for species with population trend could become critical or extinct in a brief amount of time (López-Feliciano, D., 1999). Rule to administrate conservation and management of wildlife, exotic species and hunting in Puerto Rico. This contributes to the protection, conservation and management of wildlife species. Regulates control of hunting licenses, inscription of hunting arms and the suspension of arms and licenses in case of penalty. It also regulates introduction of exotic species (López-Feliciano, D., 1999).

Rule of water quality standards of Puerto Rico. This rule favors preservation, conservation and improvement of water quality in Puerto Rico. It sets water quality standards, qualifications of watersheds, and physical, chemical and biological characteristics such as dissolved oxygen, chemical oxygen demand, biological oxygen demand, and turbidity, among others (López-Feliciano, D., 1999).

Rule for erosion control and prevention of sedimentation (December 30, 1997). The purpose of this rule is to exert control over the erosion while controlling and preventing watershed pollution and its resources. It also prohibits and regulates removal of terrestrial cortex. It prevents or controls sedimentation and pollution by heavy metals can be refrained and damage to human health, flora and fauna can be avoided (López-Feliciano, D., 1999).

Federal Laws

The National Environmental Policy Act (NEPA), 1969. With the expedition of this law, there is an establishment of public policies such as Environmental Impact Assessments (EIA's) and Declarations of Environmental Impact (DEI's), which are documents required for the consideration of authorities with political power that might help them pass a judgment in terms of environmental planning and construction. With the NEPA comes the birth of the Environmental Protection Agency (EPA) and the Council on Environmental Quality (CEQ), both important for the federal decisions about the environmental management and regulation (López-Feliciano, D., 1999).

Clean Water Act (CWA), 1970. The ultimate goal of this legislation is to favor the restoration and maintenance of the chemical, physical and biological properties of the national watersheds. It demands the development of a comprehensive plan that requires the cooperation of both federal and state agencies. It is important to focus the preservation and enhancement of water quality in order to protect the life beneath the surface, fish and wildlife, to keep the drinking water safe, for agricultural and industrial use, among other purposes. Through this law there are countless programs that affect directly the problems of dispersed and direct sources of water pollution, runoff waters, treatment plants, dredging and refill of aquifers, oil control and hazardous substances control, among others (López-Feliciano, D., 1999).

Safe Drinking Water Act (SDWA), 1986. The main purpose was to provide safe drinking water to all citizens. There are two types of regulations about water treatment systems: primary, which is focused on public health-related use of water, and secondary, which include all the non-relative to public health. SDWA also offers protection to underground water. In Puerto Rico, the Board of Environmental Quality has the power to oversee the underground injection control (UIC), which is relevant in terms of dispose of hazardous wastes without compromising the quality of underground water. Amendments made in 1986 include: (1) proclamation of regulation for 83 water pollutants, (2) prohibition of lead use in public water systems, (3) civil and criminal penalties to each and every person that affects the water public systems, and (4) strict

supervision of state programs, with the full injection of the legislation, if necessary (López-Feliciano, D., 1999).

Endangered Species Act (ESA), 1973. Promotes the conservation of species and their habitats due to ecological, educational, historic, recreative, and scientific value. This applies to both governmental and private entities. It is powered by the Secretary of the Interior, by which the US Fish & Wildlife Service (USFWS) is attached to, and by the Secretary of Commerce. ESA protects the endangered and threatened flora and fauna. Through this law there are specifications of species, threat qualifications and threats. If a species is counted as threatened or endangered, it is posted on a list for its protection. This includes a procedure by which a species is classified in order to be protected or to be included on the list (López-Feliciano, D., 1999).

CHAPTER III

METHODOLOGY

Introduction

In order to analyze and quantify the NUE, exclusively for N, P and K by measuring retranslocation percentage (%RT) in two native tree species, *Thespesia grandiflora & Guarea guidonia* - deciduous with senescent leaves - at the UPR-C campus, we need to obtain the concentrations of Nitrogen, Phosphorus and Potassium ([NPK]) from the foliar tissues. This way, we can apply the calculations based on their total concentrations, and the total concentrations in the soil samples. The purposes we will do this is to take into consideration these species in order to monitor the efficiency of these nutrients' fluxes within an ecosystem where past impacts and present ecological consciousness collide in a piece of land that have experimented peculiar changes. Once we are able to prove this, right or wrong, we will proceed to recommend further studies these species to be added in a list of suggested species for mitigation in soil recovery and reforestation projects. Area of study

The Park of the Green Shadows at the University of Puerto Rico, Cayey Campus (UPR-C) is located in the Cayey urban zone, in the Central-South-East area of Puerto Rico. As its Arahuacan-Tainian name suggests, the "land of waters" covers up for a subsoil that formed by volcanic activity during the Cretacic Period. According to the United States Geological Service (USGS), there are 13 types of soil in the watershed that intrinsically closes to the Cayey area. Its forestal classification is from humid subtropical rain forest on volcanic substrate to very humid subtropical rain forest on volcanic substrate. The approximate coordinates of our area of study, in UTM, are 18.114576, -66.151921.

The study is to be made in the forest area of "Park of the Green Shadows", area ascribed to the UPR-CUC administration, and a physically fragmented area that is part of the forest located in the main campus area, but interrupted by roads of car transit, schools, and another facility of the University, an athletic complex that includes the gym and its race and jogging track. For the purpose of this study, we will examine the two native tree species at the Park, which serves as a rehabilitation and revitalization for the native tree species.

Objectives

- 1. To quantify in an exploratory manner which process, retranslocation efficiency (%RT) and bioaccumulation factor (%BCF) is being benefited, and thereby measure nutrient exchange in the ecosystem.
- 2. To quantify the difference in %RT between *Thespesia grandiflora* (*Tg*) & *Guarea guidonia* (*Gg*), and their %BCF in terms of nutrient concentrations in the soil.
- 3. To quantify which nutrient is benefited the most and in which process.

Tree Species profiles and selection criteria

The following species have been selected because they are two of the most abundant species at the Park of the Green Shadows and because of their mutual proximity that could be a causal to study competence between them. Though these variables are not going to be considered, further studies can be very helpful in terms of relationship between various native species within a vicinity. This is also an exploratory study of the area since there is extremely limited or nonexistent scientific literature regarding the objectives of this research in the Cayey area.

Thespesia grandiflora (common name: Maga) from the Malvaceae Family, is an attractive tree that does not reach 20m of height (Appendix 1). The Maga tree is endemic to Puerto Rico, being established in the latitude 18°N, considered by the Puerto Ricans as the national tree with its pompous trumpet-like flowers with their colors ranging from red to dark pink. The Maga tree grows in slightly alkaline to very acidic soils, and can grow in clayey to sandy soils alike. It prefers to grow in lands with good drainage, but can also grows almost every other types of drainage. The optimum condition for these trees to grow is at alluvial slopes inferior to limestone hills and in the alluvial dips between hills. Their leaves reminisce the shape of the poker spades, turning senescent in a brief amount of time. The Maga tree is of natural occurrence in the subtropical moist forest zone, with mean annual precipitation from 1250 to 2500mm, and a mean annual temperature from 20 to 27°C (Francis, 1989).

Guarea guidonia (common name: Guaraguao) from the Meliaceae (or Mahogany tree) Family, also known as the American muskwood, is an evergreen species with a dense foliage, with a broad distribution from the latitude 22°N to a little further from latitude 25°S, except from a band in the North Argentinian area. It is reported as a native species in Puerto Rico, La Española, Cuba, St. Croix and Trinidad, and from Nicaragua, Costa Rica, to Panamá, Colombia, reaching south of Brazil, and Argentina, among the islands in the Caribbean and countries in Central and South America. It has a straight stem that usually grows from 25 to 30m (Appendix 2). We can see the Guaraguao tree is the second most common in secondary humid and very humid forests and it well serves as a shadowing tree in coffee farms along the deep, clayey soils of the Central Mountain Chain. The Guaraguao tree is used as an ornamental shadow tree, being one of the most common protective of the coffee harvest lands. The Guaraguao tree grows high enough to reach the high parts of the canopy, though it is not the dominant tree in the Puerto Rican forests. Its wood resembles that from the cedar and the mahogany, and its versatile features makes this species precious for the wood works such as carpentry, furniture confection, and cabinetmaking, among others (Weaver, 1988).

Methodological design

Sampling and time of study (data collection)

In a quadrant of 30m*30m at the Park of the Green Shadows (geographical location: 18.114263, -66.152266), we are going to fetch five individuals for the Maga tree and five individuals for the Guaraguao tree (5 leaves for each individual, and for each leaf status: 5 leaves * 2 status * 2 species * 5 individuals => N = 100 (each collection; see Figure 3). Once the species are counted in, we proceed to obtain samples of the foliage material of the individuals by species from the piece of land at the Park of the Green Shadows area. The number of samples taken are to be identified by species, number of individual, grouped in both mature and senescent popula-

tion samples (Rivera, 2011). We are to collect 5 mature leaves and 5 senescent leaves from each individual in the canopy area (Pérez et. al., 2003; Vann, Joshi & Pérez, 2002; Rivera, 2011). Identification of senescent leaves are generally simple due to color changes and contracted petioles (Wright & Westoby, 2003; Rivera, 2011), but also by the facile detachment from the petioles. The samples are to be packaged and tagged by collection date, individual, species and condition (Sn=Senescent; Mt=Mature). Collection of samples is to be taken in a three-week study period. We are not going to include climate conditions of the Cayey zone because of the non-significant weather variation of NPK %RT as a function of environmental seasoning due to the time of study factor. (Rivera, 2011).

Procedure to obtain total concentrations of NPK in mature and senescent leaves in the species at the area of study

Mature and senescent leave samples to be collected during the data collection time are going to be taken to the Central Analytical Laboratory at the Río Piedras Agricultural Experimental Station where they will be heated up to 70°C until they reach a constant weight (Mejías-Rivera, 2013; Lugo, 1999; Rivera, 2011) and are to be grounded in order to be submitted for chemical analyses. The Kjeldahl method will be applied on the foliar tissue to obtain the total content of N (TKN), and the total content of P (TKP). K will be extracted and obtained by the dry ash method. Soil samples are to be collected in an approximate depth of 15cm by using a stainless steel drill (Lugo, 1999; Bridham, John, Charles & Curtis, 1995; Rivera, 2011) for a collection of the layers of the subsoil. The default drilled holes where the collections are to be made under the soil at the peripherals of the majority of the sampled species in the earlier mentioned areas. The total of samples to be taken is 20 per individual for the foliar tissues and 8 per adjacent area of each individual (28 per collection, 56 total). Once collected, they are to be taken to the laboratory for measurement of dry weight at 70°C (Lugo, 1999; Bridham et. al., 1995) and proceed to measure the dry weight and pH of the soil when exposed to air. All the samples are to be analyzed chemically so the total concentrations of NPK can be obtained. Kjeldahl method is to be used to obtain the total concentration of N and to measure total concentration of P. Ammonium acetate (pH=7) is the chemical analysis that gives us the concentration of K Analysis of NUE in soil by %RT formulas (Allison & Vitousek, 2004; Lugo, 1998, Pérez et. al., 2003; Rivera, 2011).

Once we get results for the concentrations of NPK in both mature and senescent leaves, we make an input of the following formulas to get the %RT of NPK (Allison & Vitousek, 2004; Lugo, 1998; Lin & da Silveira Lobo Sternberg, 2007; Van Heerwaarden, Toet & Aerts, 2003). 1st equation

%RT = [1-(Sn/Mt)]*100

where %RT is retranslocation percentage, Sn is concentration of nutrients in senescent leaves, and Mt is concentration of nutrients in mature leaves.

2nd equation (not to be used for the purpose of this study; typed as a reference)

%RT=[(%RT.Mt / SLA.Mt)] *100 or [(%RT.Sn / SLA.Sn)] *100

where %RT.Mt = retranslocation percentage of mature leaves, SLA.Mt = Specific leaf area of mature leaf, %RT.Sn = retranslocation percentage of senescent leaves, and %SLA.Sn= Specific leaf area of senescent leaf.

3rd equation

%BCF= [NPKleaves] / [NPKsoil]¶

where BCF = accumulation of nitrogen, phosphorus and potassium in relation to soil concentrations, in green and in senescent leaves.

Data Analysis

The average data of total NPK concentrations of mature and senescent leaves are to be identified by week of collection and graphics by species and sample areas are to be made. Once we are done with this phase of the project, we are proceeding to find any significant difference by the statistical test that applies for our research by using T-test statistical analysis. T-test statistical analysis is important due to the observation of the difference between species and date of samples collection, but also due to the small size and the small amount of parameters. Mathematically, NUE is the litter mass divided by the nutrient content in litter (Vitousek, 1982). This research protocol (Protocol No. B01-019-14) was submitted to and approved by the Institutional Bio-safety Committee (IBC) at AGMUS.

Note: this equation is used by dividing the concentration of a sole nutrient per individual per concentration of soil sample in the surrounding of the observed individual. The way the above formula is written is to give a general idea of how the concentration of nutrients is used towards the BCF parameter.

CHAPTER IV

RESULTS AND DISCUSSION

The following results are subjected to cement and promote the Cayey area as an ideal place to develop more scientific research, but also focusing on the concept of the secondary urban forests as a natural machinery of sustainability. This study also recommends the features of the native trees as species that are most conveniently needed to be nursed and used in mitigation, revitalization and soil and forestal restoration, but also as part of landscaping, timber industry and commerce, and joy of Nature-loving visitors, spectators and workers alike. In this chapter we are highlighting the results obtained about partial fertility, total Kjeldahl content for nitrogen and phosphorus both in soil and foliar tissue samples, and available potassium in soil and in foliar tissues, and the %RT of NPK in both *Thespesia grandiflora* and *Guarea guidonia* within the vicinity where these two native tree species coexist.

Foliar Features

Unlike the study from Rivera (2011), the measure for the specific foliar area was not made, so the considerations taken when accounting for foliar features are based on an approximate proportion of leaves per petiole between Maga tree and Guaraguao tree. Maga has just one leaf per petiole, whereas Guaraguao tree has a dense foliage (i.e. it has a huge proportion of leaves per petiole, which gives this species slight advantage in terms of photosynthetic rates, chlorophyll synthesis, higher NUE and higher bioaccumulation). The dense foliage allows this tree species get the most from sunlight and because of its maximum height (which is not enough for this tree to be the dominant species in a canopy) can overshadow most trees in its peripherals, this species has an aggressive way to look for its territory and the resources within. Since the Guaraguao tree has an important role in the timber industry (from the Mahogany tree family, considerably resistant to herbivores and parasites), the demand for resources to build itself is higher than in other species. However, Maga tree, which despite of its endemism, has a solid adaptation to almost every kind of forest ecosystem in Puerto Rico (except coastal forests, subtropical dry forest, and mangrove, among few others). Maga and Guaraguao, in the case of this area of study, have a low competence due to the richness of the biotic and abiotic factors that contribute to their growth and development of the species.

Concentration of N, P & K in the soil and in foliar tissue at PGS

When analyzing the difference in %RT between Maga and Guaraguao, it can be observed a slight advantage of the second for all of the nutrients considered for this study, especially when looking at the %RT for K. The mean values for K %RT's coincide to the low %BCF found in this study, indicating the scarce amount of K in soil. Since the area of study is a subtropical rain forest with three important soil orders (see Figures 3-6), the oxidation rates are higher and the leach of nutrients favors this nutrient reabsorption process due to poor nutrient availability in soil. K is most likely to be the least available macronutrient of the three in soil because of its chemical nature (alkaline metal), so the reabsorption of K is key to compensate for the imbalance in soil. N and P tend to be biologically converted to other chemical compounds that may not be available to plants due to constant, weather-dependent chemical reactions. When comparing the first and second collection, there is a slight difference in %RT, with the first being collected in rainy weather and the second on a sunny day, when moist was less available.

%RT of N, P y K as a function of ecological recovery

The data was analyzed using the T-test on Minitab 17, with the H₀ established as a nodifference between the Maga and Guaraguao tree species and with a confidence interval of 95%, which means a value of 5% (p<0.05) represents a difference and a rejection of the H₀. When looking at the T-test results, there was no significant difference between the first and second collection, nor a difference between Maga and Guaraguao trees (p-value > 0.05). The homogeneity of the plot gives us information on how well both species coexist despite of the proximity between the individuals (see Figures 7-9).

The results also tell that Guaraguao draws more nutrients when comparing the means of %RT (see Tables 8-9). Since Maga is better adapted to grow in rows with high irrigation (more water-dependent), the efficiency in terms of leaf senescence periods (frequent abscission rates) and in terms of leaf construction (one leaf per petiole), makes Maga rely proportionally less in nutrient remobilization than Guaraguao because of its practical ecophysiology. When looking at both species in terms of biomass generation and litter fall contribution to soil, Maga dominates by individual leaf efficiency, but Guaraguao has a number advantage in terms of litter fall and senescence. If not by the numbers in leaves per petiole by Guaraguao, there would not be any virtual difference or there would be a slight advantage of Maga over Guaraguao.

%BCF in Maga and in Guaraguao

When looking at the T-test results for %BCF in senescent leaves versus mature leaves for N, P, and K (see Figures 10-15), the p-values for senescent leaves and for mature leaves in terms of [N], [P] and [K] were less than 5% (p<0.05), which indicates a dramatic difference in nutrient mobilization between the two collections. As mentioned before, the apparent difference between the first and second samples collections was the moist due to rain in the first and the sunny and dry conditions in the second.

When looking at the means of each species and nutrient, it can be observed that in most cases, %BCF in mature leaves almost doubles %BCF in senescent leaves in all nutrients, and that the difference in %BCF between the first and the second collections. It could also be observed that Guaraguao was also benefited proportionally more than Maga, being the species with a relatively major bio-concentration in all nutrients.

Opposite to the %RT mean values, P had the highest %BCF mean in the 1st collection and was the second highest in the 2nd collection, and unlike its N and K counterparts, P was also the macronutrient with a higher mean in both Guaraguao and Maga in the first collection when compared to the second collection (see Tables 10, 11 & 13). It can be suggested that since P is an important macronutrient in the formation of ATP from glucose and important in the dynamics of other metabolic processes, both Maga and Guaraguao tend to rely on P mobilization via bioaccumulation due to interrupted photosynthesis because of precipitation periods. It can also be suggested that rain or shade from sunlight contributes to higher cell respiration than photosynthesis and thereby more P consumption and demand from the plants. On the opposite, N and K showed a slight reduction in their %BCF rates from the 1st to the 2nd collection.

CHAPTER V

CONCLUSIONS, RECOMMENDATIONS, AND LIMITATIONS

The following are a series of final observations related to the findings of this exploratory study, which hopefully will provide a baseline and a commitment to more promising studies and development of management strategies that will be worth the environmental work that needs to be initiated and continued for a sustainable future in Cayey's urban forest ecosystems. There are countless objectives in terms of contributions of science to the community, and this effort is no exception in terms of management measures and conservation of subtropical rain forests over volcanic-derived soils, most importantly in an archipelago rich in terms of natural resources and diversity. Of all the twelve major soil orders in the world's ecosystems, Puerto Rico has six, and three converge around the Cayey zone, which is a great justification to make a conveniently reduced sampling and probing of the plots to study the behavior of nutrient cycling with major soil orders in the same zone.

Conclusions

In summary, there was a consistent result in %RT for N, P, and K, but there was obviously a difference in proportion related to their presence in the metabolic activities within the tree species generally observed in Nature. There was also a characteristic difference in %RT rates between Maga and Guaraguao, with the last having a considerable advantage over the first. When looking to %RT in K in comparison to %BCF, there was a huge difference of K %RT when compared to N and P, which indicates that there could be a natural acidic environment that precipitates K due to its metallic nature. In terms of %BCF, there was a significant difference that marked a reduction in %BCF for N and K, and and increase for P, which of that may be indicative of cell respiration increase due to low photosynthetic activity because of rain.

The results for this study indicate that in conditions of nutrients leaching or any other environmental activity that reduces the amount of nutrients in soil, %RT is higher because poor soil enrichment forces both species to rely in nutrient conservation, especially for K, which is the nutrient with easier oxidation of the three. It was also observed that P was benefited most when in rainy weather, and it is suggested that cell respiration is favored over photosynthesis due to a decrease in daylight and its related activities within the plant. N is the most balanced of the nutrients in study, because it has shown to be withdrawn from leaves in %RT and from soil in %BCF in constant quantities. The observations made in this investigation are consistent with results obtained from other studies where P and K are considerably less when compared with N (Lugo et al., 1990; Montagnini & Jordan, 2002; Rivera, 2011). It is to be noted that physiological traits of the trees are important to consider when recommending the species for revitalization.

Recommendations

Since this is an exploratory study and the scientific literature is scarce in terms of the Cayey zone, the following recommendations will be provided to stimulate further studies:

Further studies

- 1. To measure difference in nutrient dynamics between plots with direct impact versus surrounding plots where Guaraguao and Maga trees are planted or grown.
- 2. To study %BCF in presence of trace elements and heavy metals due to discharge or misuse of lands where poor drainage or flood conditions affect plantations or crops.
- 3. To make comparison between Maga and Guaraguao with other species of interest when taking into account their cultivation to revert weathering and damage to watershed.
- 4. To study if due to past impacts at PGS there are sedimentation, nutrients leaching, or any other disruption in nutrient exchange.
- 5. To compare the concentrations of N, P, and K, along other macronutrients, between different plots, in order to suggest the planting and monitoring of the trees where the availability of nutrients is the poorest.
- To analyze if due to longer climate periods there are greater variations of %RT and %BCF, analyzing in the meantime changes in biomass generation and productivity.

Limitations

- 1. Extreme fragmentation of the ecosystem might not allow an open exchange of the nutrients between this ecosystem and any other external ecosystems.
- 2. The safety measures for the conservation of PGS, such as the cyclone fence or lack of work and personnel to use it as an open Botany laboratory and nursery are not enough.
- 3. Time and cost of the analyses for a study of this Nature are expensive.

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TABLES

Individual	Tag number	Geographical Location
Tg1	730	18.11395526/-66.15258693
Tg2	731	18.11456662/-66.15199151
Tg3	740	18.11447994/-66.15199077
Tg4	729	18.11462786/-66.15181807
Tg5	728	18.11462105/-66.15193913
Gg1	N/A	18.11449539/-66.15192415
Gg2	N/A	18.11457499/-66.15196929
Gg3	2069	18.11458168/-66.15173743
Gg4	N/A	18.11448947/-66.15189188
Gg5	2070	18.11443057/-66.15176684

Geographical location of the species from Group 1 (Tg=*Thespesia grandiflora*), and Group 2 (Gg=*Guarea guidonia*) at the Park of the Green Shadows area of study

PI-ID	Lab ID 1st Sn	Lab ID 1st Mt	Lab ID 2nd Sn	Lab ID 2nd Mt	Tag number
Tgl	134	135	166	171	730
Tg2	136	137	165	170	731
Tg3	138	139	169	174	740
Tg4	140	141	168	173	729
Tg5	142	143	167	172	728
Gg1	144	145	175	180	N/A
Gg2	146	147	176	181	N/A
Gg3	148	149	177	182	2069
Gg4	150	151	178	183	N/A
Gg5	152	152	179	184	2070

Laboratory Identifications of the species from Group Tg and Group Gg at the Park of the Green Shadows area of study for foliar tissues first collection and second collections

PI-ID	Lab ID 1st Collection	Lab ID 2nd Collection	Tag number
Tg1	299	308	730
Tg2	301	307	731
Tg3	303	311	740
Tg4	300	310	729
Tg5	302	309	728
Gg1	304	312	N/A
Gg2	304	312	N/A
Gg3	305	313	2069
Gg4	305	313	N/A
Gg5	306	314	2070

Laboratory Identifications of the species soil samples from Group Tg and Group Gg at the Park of the Green Shadows area of study for the first and second collections

· · · · · · · · · · · · · · · · · · ·					
PI-ID Lab II 1st Co	D ollection	Weight (g) TKN/TKP	Lab ID 2nd Collection	Weight (g) TKN/TKP	Tag number
Tg1-Sn/Mt	134/135	0.127/0.106	166/171	0.135/0.108	730
Tg2-Sn/Mt	136/137	0.143/0.100	165/170	0.161/0.104	731
Tg3-Sn/Mt	138/139	0.106/0.101	169/174	0.100/0.216	740
Tg4-Sn/Mt	140/141	0.146/0.101	168/173	0.104/0.106	729
Tg5-Sn/Mt	142/143	0.101/0.113	167/172	0.103/0.102	728
Gg1-Sn/Mt	144/145	0.100/0.106	175/180	0.109/0.116	N/A
Gg2-Sn/Mt	146/147	0.101/0.102	176/181	0.119/0.172	N/A
Gg3-Sn/Mt	148/149	0.105/0.104	177/182	0.133/0.107	2069
Gg4-Sn/Mt	150/151	0.101/0.115	178/183	0.107/0.104	N/A
Gg5-Sn/Mt	152/153	0.257/0.103	179/184	0.109/0.101	2070

Corresponding weights of the species foliar tissue samples from Group Tg and Group Gg at the Park of the Green Shadows area of study for the first and second collections

PI-ID Lab II 1st Co	D ollection	Weight (g) Dry ash K	Lab ID 2nd Collection	Weight (g) Dry ash K	Tag number
Tg1-Sn/Mt	134/135	1.011/1.000	166/171	1.000/1.030	730
Tg2-Sn/Mt	136/137	1.000/1.003	165/170	1.000/1.020	731
Tg3-Sn/Mt	138/139	1.014/1.002	169/174	1.000/1.010	740
Tg4-Sn/Mt	140/141	1.002/1.000	168/173	1.030/1.010	729
Tg5-Sn/Mt	142/143	1.001/1.005	167/172	1.010/1.300	728
Gg1-Sn/Mt	144/145	1.015/1.002	175/180	1.000/1.000	N/A
Gg2-Sn/Mt	146/147	1.004/1.002	176/181	1.010/1.030	N/A
Gg3-Sn/Mt	148/149	1.161/1.003	177/182	1.020/1.020	2069
Gg4-Sn/Mt	150/151	1.001/1.002	178/183	1.000/1.030	N/A
Gg5-Sn/Mt	152/153	1.007/1.005	179/184	1.020/1.040	2070

Corresponding weights of the species foliar tissue samples from Group Tg and Group Gg at the Park of the Green Shadows area of study for the first and second collections

PI-ID	Lab ID 1st Collection	Weight(g) TKN/TKP	Lab ID 2nd Collection	Weight (g) TKN/TKP	Tag number
Tg1	299	0.412	308	0.458	730
Tg2	301	0.403	307	0.560	731
Tg3	303	0.656	311	0.411	740
Tg4	300	0.411	310	0.636	729
Tg5	302	0.450	309	0.447	728
Gg1	304	0.480	312	0.636	N/A
Gg2	304	0.480	312	0.636	N/A
Gg3	305	0.402	313	0.509	2069
Gg4	305	0.402	313	0.509	N/A
Gg5	306	0.410	314	0.403	2070

Corresponding weights of the species soil samples from Group Tg and Group Gg at the Park of the Green Shadows area of study for the first and second collections

PI-ID	Lab ID 1st Collection	Weight(g) K	Lab ID 2nd Collection	Weight (g) K	Tag number
Tg1	299	5.07	308	5.04	730
Tg2	301	5.03	307	5.13	731
Tg3	303	5.01	311	5.01	740
Tg4	300	5.01	310	5.06	729
Tg5	302	5.02	309	5.08	728
Ggl	304	5.01	312	5.01	N/A
Gg2	304	5.01	312	5.01	N/A
Gg3	305	5.01	313	5.13	2069
Gg4	305	5.01	313	5.13	N/A
Gg5	306	5.02	314	5.16	2070

Corresponding weights of the species soil samples from Group Tg and Group Gg at the Park of the Green Shadows area of study for the first and second collections

PI ID	%RT N	%RT P	%RT K
Maga 1	38.5	37.1	60.9
Maga 2	53.7	89.3	74.6
Maga 3	54.3	6.67	69.3
Maga 4	54.6	50.0	76.6
Maga 5	49.1	42.9	56.9
Guaraguao 1	45.9	30.4	48.6
Guaraguao 2	40.4	41.4	62.8
Guaraguao 3	48.2	42.3	47.3
Guaraguao 4	42.9	40.7	52.4
Guaraguao 5	39.3	34.8	45.9

Corresponding %RT's of Tg and Gg at the Park of the Green Shadows area of study for the 1^{st} collection

Corresponding %RT's of Tg and Gg at the Park of the Green Shadows area of study for the 2^{nd} collection

PI ID	%RT N	%RT P	%RT K
Maga 1	58.6	67.7	79.1
Maga 2	39.7	15.4	37.4
Maga 3	24.6	17.6	20.5
Maga 4	57.3	50.0	84.4
Maga 5	36.2	42.9	74.8
Guaraguao 1	40.2	40.0	58.5
Guaraguao 2	33.3	43.8	42.6
Guaraguao 3	46.0	50.0	49.5
Guaraguao 4	49.2	4.76	30.5
Guaraguao 5	34.0	44.4	46.7

PI ID	N Sn	N Mt	P Sn	P Mt	Sn K	Mt K
Maga 1	2.30	3.74	4.40	7.00	.195	.500
Maga 2	2.02	4.37	0.60	5.60	.189	.742
Maga 3	2.33	5.11	3.50	3.75	.136	.442
Maga 4	1.89	4.16	3.00	6.00	.130	.555
Maga 5	2.08	4.08	3.00	5.25	.126	.291
Gg 1	2.68	4.96	4.00	5.75	.215	.418
Gg 2	2.91	4.89	4.25	7.25	.271	.729
Gg 3	2.89	5.57	3.75	6.50	.410	.777
Gg 4	3.00	5.25	4.00	6.75	.367	.771
Gg 5	2.77	4.56	3.75	5.75	.420	.777

Corresponding %BCF's of Tg and Gg at the Park of the Green Shadows area of study for the 1^{st} collection for Sn and Mt leaves

PI ID	N Sn	N Mt	P Sn	P Mt	Sn K	Mt K
Maga 1	2.94	7.11	2.00	6.20	.237	.147
Maga 2	3.32	5.50	4.40	5.20	.434	.125
Maga 3	1.69	2.51	2.80	3.40	.297	.263
Maga 4	2.32	5.44	1.60	3.20	.068	.166
Maga 5	2.20	3.48	2.40	4.20	.318	.289
Gg 1	3.40	5.69	3.00	5.00	.188	.193
Gg 2	3.43	5.14	2.25	4.00	.298	.193
Gg 3	3.07	5.69	2.50	5.00	.560	.250
Gg 4	3.10	6.10	5.00	4.20	.488	.250
Gg 5	2.60	3.94	2.00	3.60	.485	.303

Corresponding %BCF's of Tg and Gg at the Park of the Green Shadows area of study for the 2^{nd} collection for Sn and Mt leaves

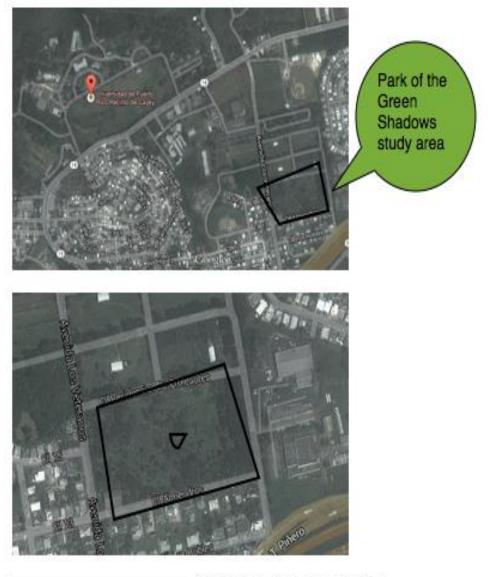
Variable	PI ID	Ν	Mean	SE Mean	Std. Dev.
Nft% Sn	Guaraguao	5	1.3100	0.0202	0.0453
	Maga	5	0.9860	0.0904	0.2021
Pft% Sn	Guaraguao	5	0.15800	0.00374	0.00837
	Maga	5	0.1320	0.0306	0.0683
Kft% Sn	Guaraguao	5	0.6220	0.0817	0.1827
	Maga	5	0.4020	0.0997	0.2229
Nft% Mt	Guaraguao	5	2.3160	0.0414	0.0926
	Maga	5	1.972	0.121	0.270
Pft% Mt	Guaraguao	5	0.2560	0.0117	0.0261
	Maga	5	0.2580	0.0351	0.0785
Kft% Mt	Guaraguao	5	1.280	0.139	0.310
	Maga	5	1.292	0.287	0.642
2 nd collection					
Nft% Sn	Guaraguao	5	1.2560	0.0250	0.0559
	Maga	5	1.0520	0.0760	0.1699
Pft% Sn	Guaraguao	5	0.1220	0.0201	0.0449
	Maga	5	0.1320	0.0306	0.0683
Kft% Sn	Guaraguao	5	0.688	0.109	0.243
	Maga	5	0.642	0.190	0.426
Nft% Mt	Guaraguao	5	2.142	0.142	0.319
	Maga	5	1.924	0.196	0.438
Pft% Mt	Guaraguao	5	0.19000	0.00894	0.02000
	Maga	5	0.2220	0.0282	0.0630
Kft% Mt	Guaraguao	5	1.260	0.190	0.424
	Maga	5	1.750	0.390	0.872

Descriptive Statistics of RT of Tg and Gg at the PGS area of study for all collections

Variable	PI ID	Ν	Mean	SE Mean	Std. Dev.
%BCFSn N	Guaraguao	5	2.8500	0.0561	0.1255
	Maga	5	2.1240	0.0839	0.1877
%BCFSn P	Guaraguao	5	3.9500	0.0935	0.2092
	Maga	5	2.900	0.629	1.407
%BCFSn K	Guaraguao	5	0.3365	0.0402	0.0900
	Maga	5	0.1551	0.0152	0.0340
%BCFMt N	Guaraguao	5	5.046	0.171	0.382
	Maga	5	4.292	0.228	0.510
%BCFMt P	Guaraguao	5	6.400	0.292	0.652
	Maga	5	5.520	0.531	1.187
%BCFMt K	Guaraguao	5	0.6943	0.0696	0.1557
	Maga	5	0.5061	0.0736	0.1645
2 nd collection					
%BCFSn N	Guaraguao	5	3.120	0.150	0.335
	Maga	5	2.534	0.260	0.582
%BCFSn P	Guaraguao	5	2.950	0.539	1.204
	Maga	5	2.640	0.483	1.081
%BCFSn K	Guaraguao	5	0.4037	0.0692	0.1547
	Maga	5	0.2708	0.0599	0.1339
%BCFMt N	Guaraguao	5	5.312	0.375	0.839
	Maga	5	4.808	0.813	1.818
%BCFMt P	Guaraguao	5	4.360	0.279	0.623
	Maga	5	4.440	0.564	1.260
%BCFMt K	Guaraguao	5	0.2380	0.0206	0.0461
	Maga	5	0.1980	0.0328	0.0733

Descriptive Statistics of BCF of Tg and Gg at the PGS area of study for all collections

FIGURES



Confinement of area of study

Figure 1- University of Puerto Rico, Cayey Campus, and its associated area of study, the Park of the Green Shadows.



Figure 2- View of the area of study where Thespesia grandiflora and Guarea guidonia are being observed

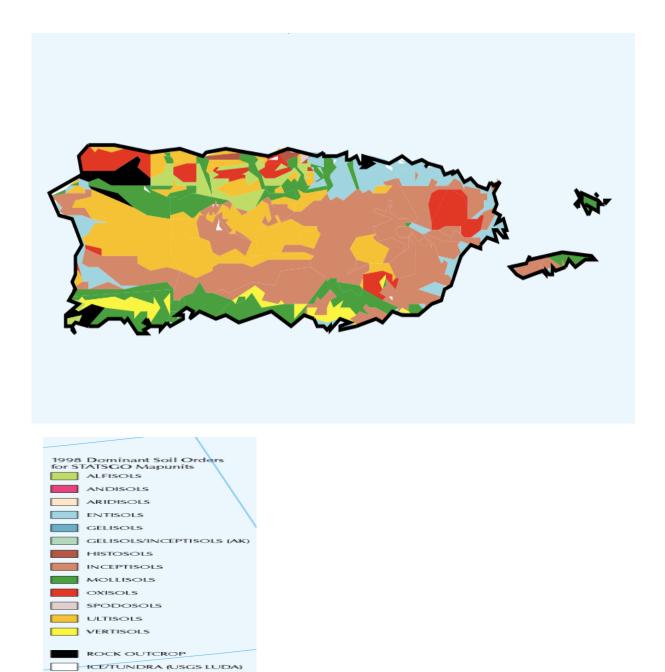
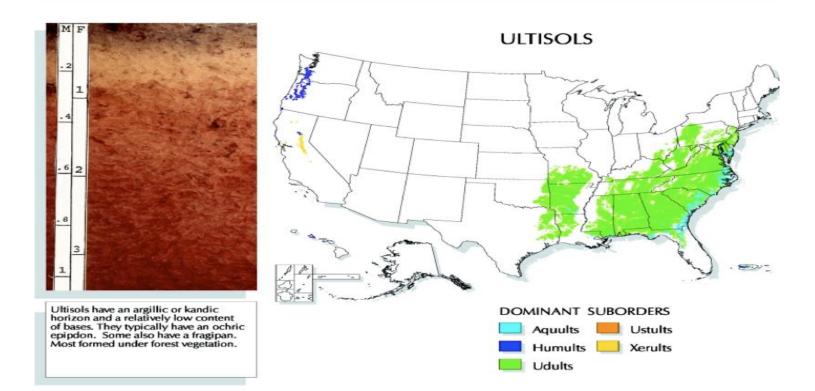
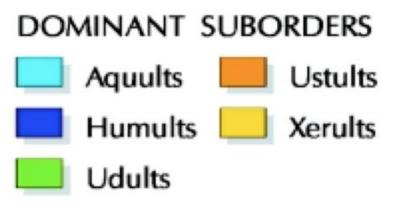


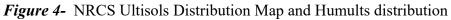
Figure 3- NRCS Dominant Soil Orders Distribution Map (Puerto Rico)

Taken from: NRCS Dominant Soil Orders in the United States.

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Taken from: NRCS Dominant Soil Orders in the United States.

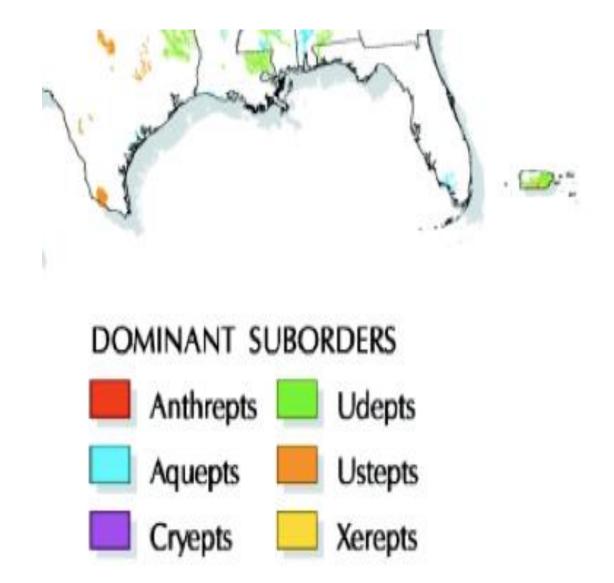


Figure 5- NRCS Inceptisols Distribution Map

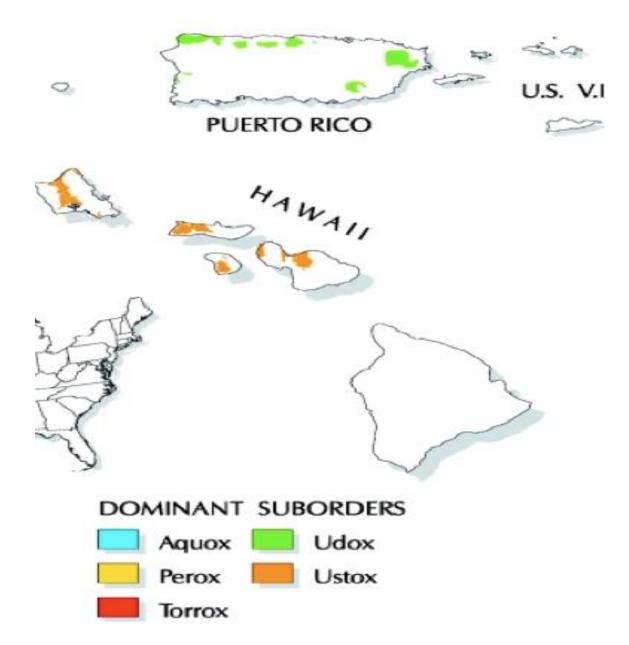


Figure 6- NRCS Oxisols Distribution Map

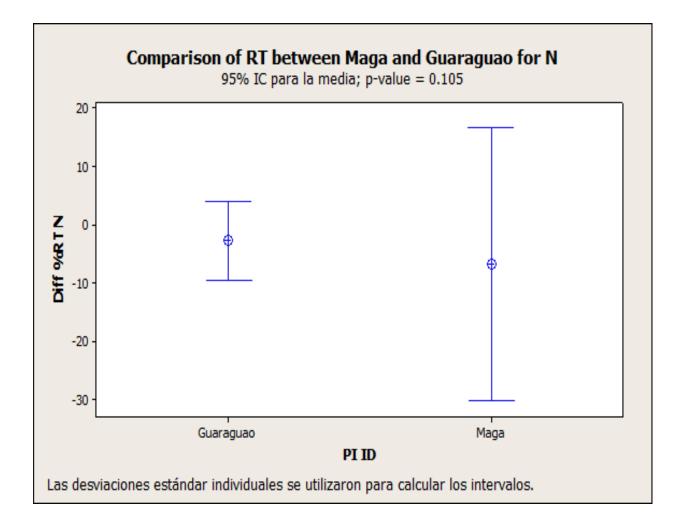


Figure 7- Comparison of RT between Maga and Guaraguao for N

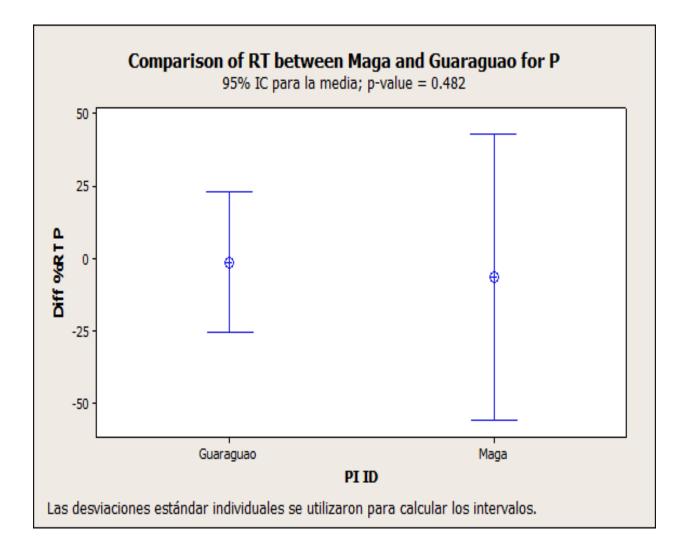


Figure 8- Comparison of RT between Maga and Guaraguao for P

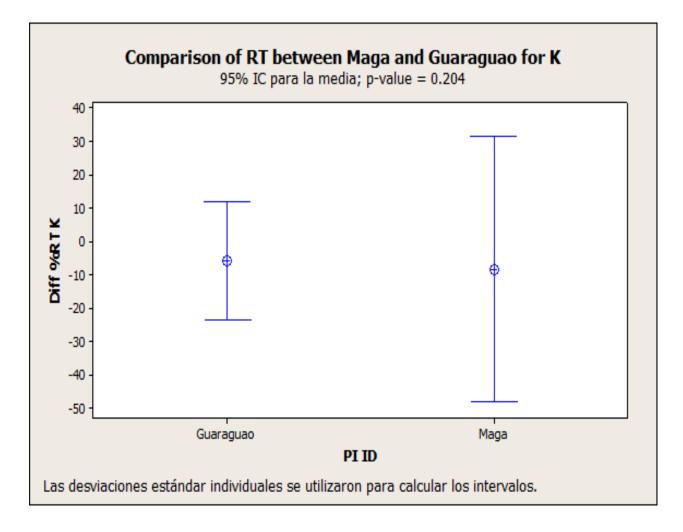


Figure 9- Comparison of RT between Maga and Guaraguao for K

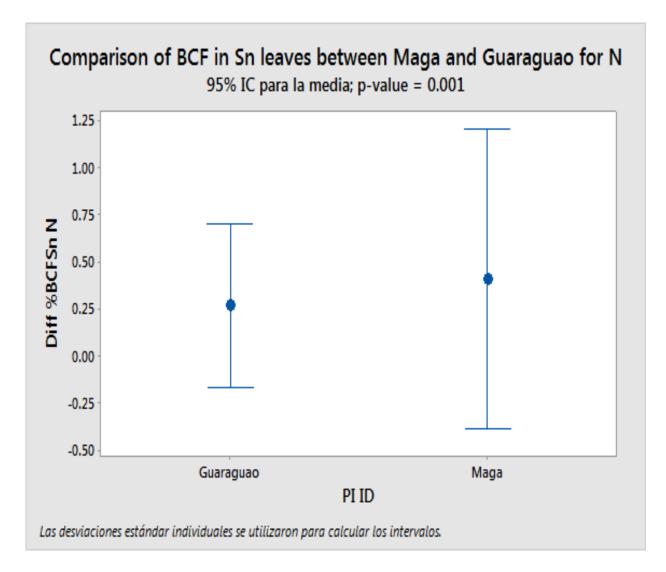


Figure 10- Comparison of BCF in Sn leaves between Maga and Guaraguao for N

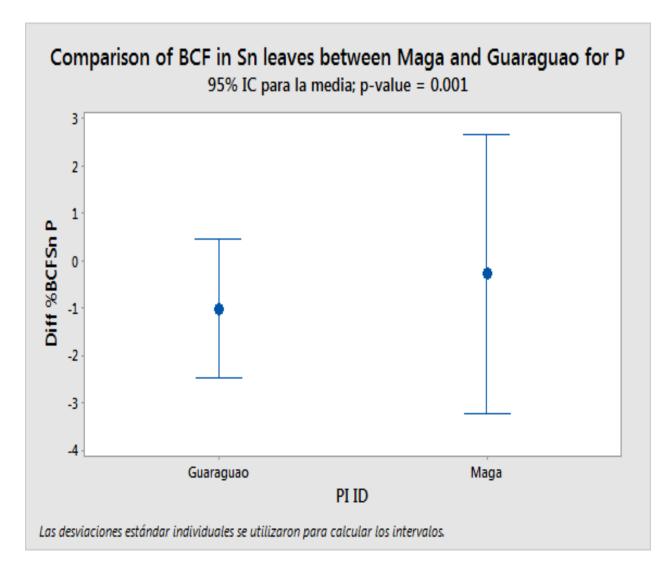


Figure 11- Comparison of BCF in Sn leaves between Maga and Guaraguao for P

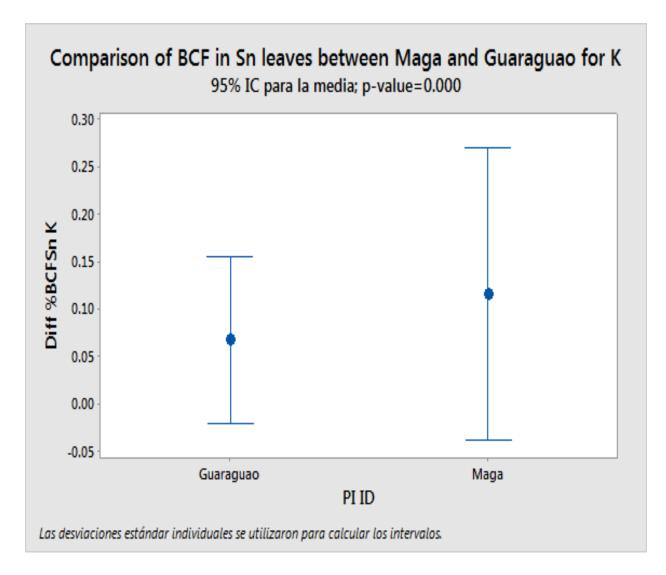


Figure 12- Comparison of BCF in Sn leaves between Maga and Guaraguao for K

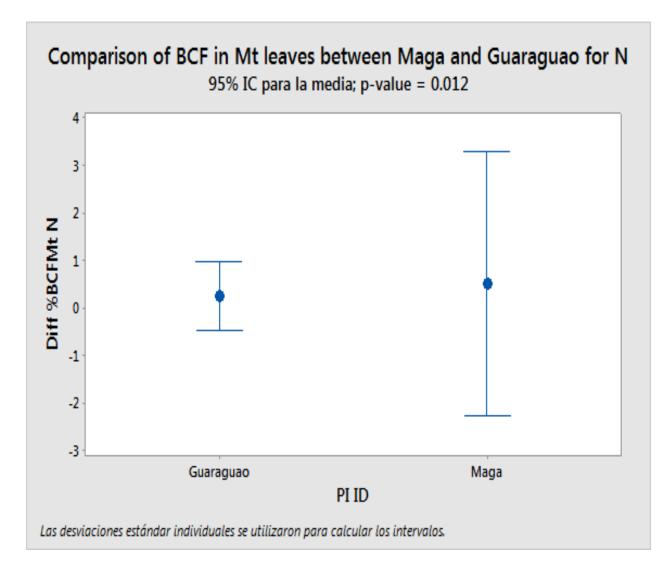


Figure 13- Comparison of BCF in Mt leaves between Maga and Guaraguao for N

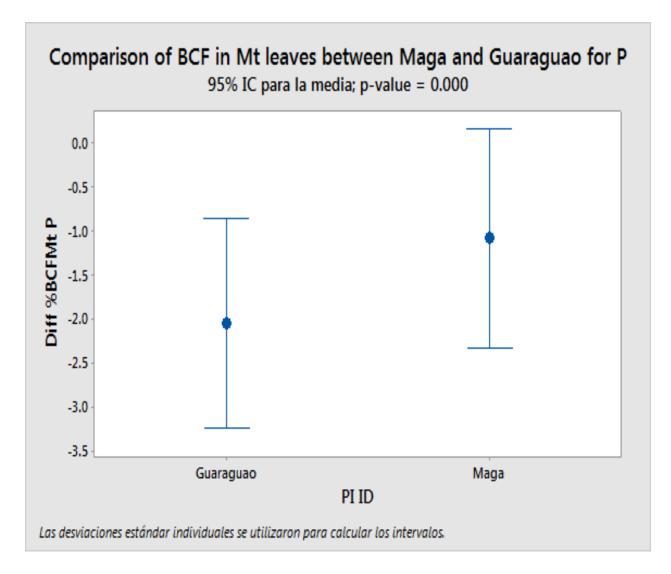


Figure 14- Comparison of BCF in Mt leaves between Maga and Guaraguao for P

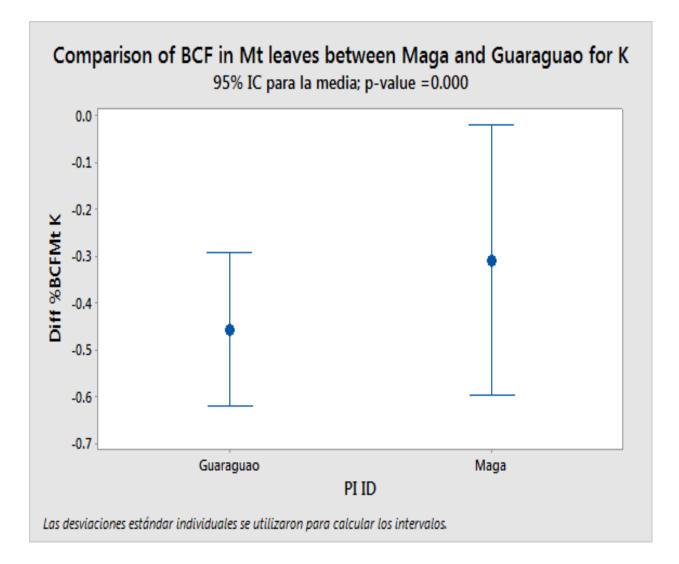


Figure 15- Comparison of BCF in Mt leaves between Maga and Guaraguao for K

APPENDIXES



Thespesia grandiflora

Taken by: David T. Nachi

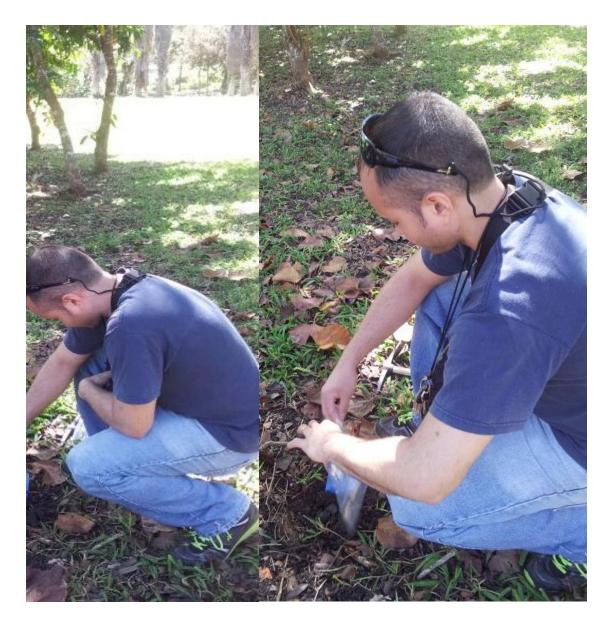
On: February 27, 2014.



Guarea guidonia

Taken by: David T. Nachi

On: February 27, 2014.



Soil samples collected on plastic bags to be later transferred to paper bags Taken by: Jeniffer Rivera On: April 15, 2014.

DEPARTAMENTO DE AGRICULTURA LABORATORIO AGROLOGICO #7 Ctra. 693 Dorado, PR 00646-3445

11 de Febrero de 2014

Estimado Dir. Sr. Miguel Ortiz Colón:

Me dirijo a usted en calidad de estudiante graduado de la Escuela de Asuntos Ambientales (EAA) de la Universidad Metropolitana (UMET), Recinto de Cupey, en virtud de lo que me ha requerido, SOLICITANDO el análisis de muestras de tejido foliar de cuatro especies nativas, maduras y senescentes, para la obtención de porcentajes de nitrógeno, fósforo y potasio, y de muestras posteriores de suelo que estaré llevando a su laboratorio cada dos semanas hasta la semana que termina el 29 de marzo de 2014.

Agradezco por adelantado las gestiones que usted y su equipo habrán de llevar a cabo en torno a mi investigación.

Muy respetuosamente,

David T. Nachi Vázquez EAA UMET Cupey Cons. Man. Rec. Nat.

Agrological Laboratory request submission letter

University of Puerto Rico Cayey College Campus Office of Safety, Occupational Hazard and Environmental Protection

March 24, 2014

Dear Dr. Juan C. Musa,

My name is Félix Velázquez and I am the director of the Office of Safety, Occupational Hazard, and Environmental Protection (OSSOPA) at the University of Puerto Rico, Cayey College Campus. I am also in charge of the Park of the Green Shadows, the forest area ascribed to the UPR-C, which is of interest for study to your Graduate Student from UMET, Cupey Campus, David T. Nachi Vázquez. I am aware and giving him access so he can collect soil samples and foliar tissue samples in order to be taken to the Central Analytical Laboratory and the Experimental Agrological Station at the South Botanical Garden, Río Piedras, San Juan, Puerto Rico.

With no more to add, I sincerely hope I can be of help to your needs and your student's. If you have any questions or concerns, my phone number is (787) 738-2161, ext. 2127.

Best regards,

Félix Velázquez

Fenx venazquez

OSSOPA Director

Felix Velazquez authorization letter for collection



March 25, 2014

Dr. Juan Musa Professor Metropolitan University AGMUS

The Central Analytical Laboratory (CAL) is part of the Agricultural Experimental Station (AES) of the College of Agricultural Sciences of the University of Puerto Rico at Mayaguez. The main objective of the laboratory is to give support to researchers, faculty and students of the University of Puerto Rico in all areas related to soil, water and tissue sample chemical analyses. In addition, for a minimal cost, we receive and analyze soil and water samples from farmers and other business from the private sector. The laboratory also serves as a teaching facility for those students interested in learning specific analytical procedures for their masters and doctoral dissertations. We have received students from University of el Turabo, Inter-American University of Puerto Rico and others. As part of a higher education institution, CAL, is regulated by federal and state laws that places the laboratory as one of the highest regarded analytical laboratory in Puerto Rico.

Student David T. Nachi, a Graduate student at the Metropolitan University, Cupey Campus, is one of our clients. As part of his graduate research he will be collecting foliar tissue and soil samples for analysis at CAL. Mr. Nachi has requested training in analytical techniques regarding soil and tissue samples. At present, he is working as a trainee in our laboratory.

Thank you for the opportunity of correspondence,

Sincerely.

Julia M. O'Hallorans, PhD Director Central Analytical Laboratory Agricultural Experiment Station University of Puerto Rico -Mayaguez

Universidad de Puerto Rico, Recinto Universitario de Mayagüez, Colegio de Ciencias Agricolas Estación Experimental Agrícola, Laboratorio Central Analitico Jardín Botánico Sur, 1193 Calle Guayacán, San Juan, Puerto Rico 00926-1118 787-767-9705

Julia O' Hallorans laboratory authorization letter



11 de marzo de 2014

David T. Nachi Vázquez Calle Lucia Vázquez #5 Norte Cayey, P.R. 00736

Estimado señor Nachi:

El 12 y 18 de febrero de 2014, recibimos en el Laboratorio Agrológico, muestras de Tejido foliar pertenecientes a usted. Adjunto los resultados de las mismas.

Agradecemos el que haya seleccionado nuestros servicios.

Para información adicional, puede comunicarse con la Lcda. Sonia Carrasquillo al (787)796-1735 Ext. 229.

Atentamente,

Agro. Miguel A. Oniz Colón

Director Interino Laboratorio Agrológico

Anejo

MAOC/mem

Printer 1

Ave. Fernández Juncos, 18a. 19 % Sunturce. Apartado 10163, San Juan, PR 00908-1163 Tel (787) 721-2120 Fax (787) 723-8512



DA Agrological Laboratory Director Letter



Martes, 11 de marzo de 2014

David T. Nachi Vázquez Calle Lucía Vázquez #5 Norte Cayey, P.R. 00736

Estimado señor: Nachi

1	Estamos incluyendo los resultado Cavey, recibidas o	s analitice re el Lab	oratorio el	dia	tejido 18 de febre	totiar tro de 2014
el pueblo de stas muestras	pertenecen a:					
						-
NFORME D	E ANALISIS			ANALISIS		
Núm. Lab.	Identificación	96N	%P	%K		
32805	14feb/14- Maga mt-1	1.41	0.11	0.73		
32806	14feb/14- Maga mt-2	1.54	0.40	0.52		
32807	14feb/14- Maga mt-3	1.49	0.13	0.54	-	
32808	14feb/14- Maga mt-4	2.30	0.20	1.21		
32809	14feb/14- Maga sn-1	0.83	0.10	0.19		_
32810	14feb/14- Maga sn-2	1.03	0.33	0.37		-
32811	14feb/14- Maga sn-3	0.98	0.10	0.25		
32812	14feb/14- Maga sn-4	1.30	0.17	0.41		
32813	14feb/14- Guarea Guidonea mt-1	2.85	0.21	1.27		_
32814	14feb/14- Guarea Guidonea mt-2	2.94	0.20	1.39		
32815	14feb/14- Guarea Guidenea mt-3	2.21	0.17	0.81		_
32816	14feb/14- Guarea Guidonea mt-4	2.87	0.31	2.36		_
32817	14feb/14- Guarea Guidonea sn-1	2.30	0.17	0.65		
32818	14feb/14- Guarea Guidonea sn-2	1.97	0.13	0.32		_
32819	14feb/14- Guarea Guidonea sn-3	1.96	0.15	0.90		_
32820	14feb/14- Guarea Guidonea sn-4	1.71	0.15	1.02		
32821	14feb/14-Tebebuia heterophylla mt-1	0.82	0.14	0.28		_
32822	14feb/14-Tebebuia heterophylla mt-2	1.06	0.10	0.75		
32823	14feb/14-Tebebuia heterophylla mt-3	0.83	0.10	0.28		
32824	14feb/14-Tebebuia heterophylla mt-4	1.41	0.14	0.75		_
32825	145eb/14-Tebebuia heterophylla sn-1	0.94	0.10	0.21		-
32826	14feb/14-Tebebuia heterophylla sp-2	0.90	0.10	0.84	at 190 PIA	DO

11 marzo /14 Fecha:

NOTA: Estos resultados representan una porción de la muestra traida al Laboratorio Agrológico y

Senia Carriàquillo Rivera

David T. Nachi Vázquez Calle Lucia Vázquez #5 Norte Cayey, P.R. 00736

Estimado señor: Nachi

	Estamos incluy	tejido foliar	
dei pueblo de	Cayey	, recibidas en el Laboratorio el dia	18 de febrero de 2014
Estas muestras per	rtenecen a:		

FORME D	E ANALISIS		ANALISIS				
Núm. Lab.	Identificación	26N	%P	%K			
32805	14feb/14- Maga mt-1	1.41	0.11	0.73			
32806	14feb/14- Maga mt-2	1.54	0.40	0.52			
32807	14feb/14- Maga mt-3	1.49	0.13	0.54			
32808	14feb/14- Maga mt-4	2.30	0.20	1.21			
32809	146cb/14- Maga sn-1	0.83	0.10	0.19			
32810	14feb/14- Maga sn-2	1.03	0.33	0.37		1	
32811	14feb/14- Maga sn-3	0.98	0.10	0.25			-
32812	14feb/14- Maga sn-4	1.30	0.17	0.41			
32813	14feb/14- Guarea Guidonea mt-1	2.85	0.21	1.27			
32814	14feb/14- Guarea Guidonea mt-2	2.94	0.20	1.39			
32815	14feb/14- Guarea Guidonea mt-3	2.21	0.17	0.81			
32816	14feb/14- Guarea Guidonea mt-4	2.87	0.31	2.36			
32817	14feb/14- Guarea Guidonea sn-1	2.30	0.17	0,65			
32818	14feb/14- Guarea Guidonea sn-2	1.97	0.13	0.32			
32819	14feb/14- Guarea Guidonea sn-3	1.96	0.15	0.90			
32820	14feb/14- Guarea Guidonea sn-4	1.71	0.15	1.02			
32821	14feb/14-Tebebuia heterophylla mt-1	0.82	0.14	0.28			-
32822	145eb/14-Tebebuia heterophylla mt-2	1.06	0.10	0.75			
32823	145eb/14-Tebebuia heterophylla mt-3	0.83	0.10	0.28			
32824	14feb/14-Tebebuia heterophylla mt-4	1.41	0.14	0.75			
32825	14feb/14-Tebebuia heterophylla sn-1	0.94	0.10	0.21			
32826	14feb/14-Tebebuia heterophylla sp-2	0.90	0.10	0.84	1.50	CIADO	1

Fecha: 11 marzo /14

NOTA: Estos resultados representan una porción de la muestra traída al Laboratorio Agrológico y no son consideradas como muestras oficiales.

Ave, Fernindez Jancos, Pda. 1935. Santarce, Apartado 1968, San Jaan, PR 9998-86103 Tel (787) 721-2129 Fax (787) 723-8512



Appendix 10	
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Núm. Lab.	Identificación	%N	%P	%K			_
32827	14feb/14-Tebebuia heterophylla sn-3	0.91	0.10	0.20			
32828	4feh/14-Tehebuia heterophylla sn-4	0.90	0.10	0.15			
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Lunes, 10 de marzo de 2014

David T. Nachi Vázquez Calle Lucía Vázquez #5 Norte Cayey, P.R. 00736

Estimado señor: Nachi

		_		ANALISIS	-		
and the second se	E ANALISIS Identificación	96N	%P	%K			
Núm, Lab.	8feb/14- citharexylum fruticosum m	1.41	0.24	0.51			
32781	8feb/14- citharexylum fruticosum sn	0.79	0.24	0.27			
32782	8feb/14- guarea guidonia mt	3.35	0.29	0.78			
32783		1.66	0.35	2.52			
32784	8feb/14- guarea guidonia sn	1.00	0.00				
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Fecha	11 marzo /14			100	-	- Cr	ALLER TO
				AD	Loda, Sonia Carr abig	Carraspuille	2
NOTA: Es	tos resultados representan una porción			-18	Sonia Cam	isquillo Riv	
de la muestr	ra traida al Laboratorio Agrológico y			-	Pulmin	T inenciade	10/
to son cons	ideradas como muestras oficiales.			1	Químico	Licenciad	5/

David T. Nachi Vázquez Calle Lucía Vázquez #5 Norte Cayey, P.R. 00736

TOPME	DE ANALISIS		_	ANALISIS		
Núm. Lab.	Identificación	%N	56P	%K		
32781	8feb/14- citharexylum fruticosum mt	1.41	0.24	0.51		
32782	8feb/14- citharexylum fruticosum sn	0.79	0.24	0.27		
32783	8feb/14- guarea guidonia mt	3.35	0.29	0.78		
32784	8feb/14- guarea guidonia sn	1.66	0.35	2.52		
						_
					-	
				1	ASOCIAL	20 00
Fecha :	11 marzo /14			ADOLIS	da. Sonia Ca Lec Abig-uni	Tesquillo D
e la muestra	s resultados representan una porción traída al Laboratorio Agrológico y leradas como muestras oficiales.			in to	nia Carrasqu	illo Rivera
o sou couse	cristals como mocortas oriciares.			6	Químico Li	NCIADO

TEJIDO FOLIAR

- 1. 🔪 El Nitrógeno fue determinado por el método Kjeldahl.
- 2. () El Fósforo fue determinado usando L Ascorbic Acid + $NH_4MO + H_2SO_4 + Antimony Potasio Tartrate.$
- El potasio, calcio, magnesiofueron extraídos por el método de la ceniza seca.
- El cobre, hierro, manganeso y zinc fueron extraídos por el método de la ceniza seca.
- La fibra determinada por el método del AOAC para alimentos comerciales.
- 6. () Otros

Department of Agriculture list of lab methods used in the chemical analyses



SISTEMA UNIVERSITARIO ANA G MENDEZ Vicepresidencia de Planificación y Asuntos Académicos Vicepresidencia Asociada de Recursos Externos y Cumplimiento Oficina de Cumplimiento

Comité de Bioseguridad (IBC)

Fecha	:	2 de mayo de 2014
Investigador principal	1	David T. Nachi Vázquez
Mentor	1	Beatriz Zayas, PH.D
Título protocolo	1	EXPLORATORY ANALYSIS OF NUTRIENT USE EFFICIENCY VERSUS BIOACCUMULATION ON TWO NATIVE SPECIES IN A COLLEGE CAMPUS
Número de protocolo	1	B01-019-14
Tipo de solicitud	ŧ	Protocolo inicial
Institución/Escuela	1	Universidad Metropolitana / Escuela de Asuntos Ambientales
Tipo de revisión	1	Comité en pleno
Acción tomada	;	Acknowlegdment
Fecha de revisión	2	2 de mayo de 2014

Certificamos que la propuesta/ protocolo de referencia recibida en la Oficina de Cumplimiento fue revisada por el Comité de Bioseguridad (IBC) el 2 de mayo de 2014. La misma fue evaluada y aprobada en un proceso de Comité en Pleno.

Se revisaron los siguientes documentos:

X_Protocolos	X Curriculum Vitae
_X_Formulario de Registro (IBC-01)	X Certificados y adiestramientos
_X_Carta de apoyo	X Propuesta
X Análisis de riesgos (IBC-09)	

Favor de tener presente los siguientes puntos:

- De realizarse algún cambio en los documentos anejados con este estudio, deben ser sometidos nuevamente al IBC para su debida revisión y aprobación utilizando el formulario de solicitud de Cambios/Enmiendas (IBC-08).
- Todo evento adverso o no esperado debe ser informado al IBC utilizando el formulario de Eventos Adversos (IBC-04).

SUAGM_IBC_Approved Revised (06/2013) Página 1 de 2

IBC Approval Letter w/ Protocol Number

- Todos los documentos relacionados con la investigación deben ser guardados por un término de cinco (5) años. Pasado este término los mismos deben ser eliminados/
- Triturados, no quemados.
 De no realizar su investigación en el término aprobado, deberá someter una solicitud de Revisión Continua llenando el formulario de IBC asignado (IBC-07) antes de vencerse el relevantes de vencerse el
- Al finalizar su investigación debe someter una solicitud de cierre utilizando el formulario de IBC Solicitud de terminación de protocolo aprobado (IBC-07)

Usted tiene un periodo de aprobación de tres (3) años que expira el 1 de mayo de 2017. Sin embargo, un informe anual de revisión continua debe ser presentado por el investigador principal.

Para más información, aclarar dudas, notificar algún evento adverso o no anticipado favor de comunicarse con su Coordinadora de Cumplimiento Institucional: Universidad Metropolitana (UMET), Srta. Carmen Crespo al (787) 766-1717 ext. 6366; Universidad del Turabo, Prof. Josefina Meigar al (787) 743-7079 ext.4126; y Universidad del Este, Sra. Natalia Torres al (787) 257-7373 Ext.2279. También puede escribir a:

Oficina de Cumplimiento Vicepresidencia Asociada de Recursos Externos y Cumplimiento Vicepresidencia de Planificación y Asuntos Académicos Sistema Universitario Ana G. Méndez P.O. Box 21345 San Juan, PR 00928-1345 Tel. 787 751-0178 Exts. 7195-7107; Fax 787 751-9517

Atentamente, Main Miguez Bonago, B.D. Presidenta IBC

SUAGM_IBC_Approved Revised (08/2013) Pagina 2 de 2

IBC Approval Letter w/ Protocol Number



ANALISIS DE TEJIDO VEGETAL

Num. Identificación TON TOP K ICA % % % % 5-14-134 Maga Sn1 1.31 0.22 0.77 5-14-135 Maga MT1 2.13 0.35 1.97 5-14-136 Maga Sn2 0.53 0.034 0.30 5-14-136 Maga Sn2 0.53 0.034 0.30 5-14-138 Maga Sn3 0.84 0.14 0.27 5-14-138 Maga Sn3 0.84 0.15 0.88 5-14-139 Maga NT3 1.84 0.15 0.88 5-14-140 Maga Sn4 1.04 0.15 0.85 5-14-141 Maga NT4 2.29 0.3 1.52 5-14-142 Maga Sn5 0.81 0.12 0.32 5-14-143 Maga NT5 1.59 0.21 0.51 5-14-144 Giaraguao Sn1 1.26 0.16 0.38 5-14-145 Giaraguao Sn2 1.17 0.17 0.48	Aralista Z. Nieto Aralista Revisor Y. Ocasio			Julia M. 8		Los vanile Ocasio Rodrigue, autorio
S14-135 Maga MT1 2.13 0.35 1.97 S14-136 Maga Sn2 0.93 0.034 0.30 S14-137 Maga MT2 2.01 0.28 1.18 S-14-138 Maga Sn3 0.84 0.14 0.27 S-14-139 Maga MT3 1.84 0.15 0.88 S-14-140 Maga Sn4 1.04 0.15 0.45 S-14-141 Maga Sn5 0.81 0.12 0.22 S-14-142 Maga Sn5 0.81 0.12 0.22 S-14-143 Maga MT5 1.59 0.21 0.51 S-14-144 Guaraguao Sn1 1.26 0.16 0.38 S-14-145 Guaraguao Sn2 1.37 0.17 0.48 S-14-145 Guaraguao Sn3 1.27 0.15 0.77		Identificación				
5-14-136 Maga Sn2 0.53 0.034 0.30 5-14-137 Maga MT 2 2.01 0.28 1.18 5-14-138 Maga Sn3 0.84 0.14 0.27 5-14-139 Maga MT 3 1.84 0.15 0.88 5-14-140 Maga Sn4 1.04 0.15 0.45 5-14-141 Maga MT 4 2.29 0.3 1.92 5-14-142 Maga Sn5 0.81 0.12 0.22 5-14-143 Maga MT 5 1.59 0.21 0.51 5-14-143 Maga MT 5 1.59 0.23 0.74 5-14-143 Maga MT 5 1.59 0.21 0.51 5-14-143 Maga MT 5 1.59 0.23 0.74 5-14-145 Guaraguao Sn1 1.26 0.16 0.38 5-14-145 Guaraguao Sn2 1.37 0.17 0.48 5-14-145 Guaraguao Sn2 1.37 0.15 0.77 5-14-148 Guaraguao Sn3 1.27 0.15 0.17 5-14-150 Guaraguao Sn4 1.32 0.16	5-14-134	Maga Sn1	1.31	0.22	0.77	
S 14 137 Maga MT 2 2.01 0.28 1.18 S 14 138 Maga Sn3 0.84 0.14 0.27 S 14 139 Maga MT 3 1.84 0.15 0.88 S 14 140 Maga Sn4 1.04 0.15 0.45 S 14 140 Maga Sn4 1.04 0.15 0.45 S 14 140 Maga Sn5 0.81 0.12 0.22 S 14 142 Maga Sn5 0.81 0.12 0.22 S 14 142 Maga Sn5 0.81 0.12 0.22 S 14 143 Maga MT 5 1.59 0.21 0.51 S 14 144 Guaraguao Sn1 1.26 0.16 0.38 S 14 145 Guaraguao Sn1 1.26 0.17 0.48 S 14 145 Guaraguao Sn2 1.37 0.17 0.48 S 14 146 Guaraguao Sn3 1.27 0.15 0.77 S 14 148 Guaraguao Sn3 1.27 0.16 0.89 S 14 149 Guaraguao Sn4 1.32 0.16 0.89 S 14 150 Guaraguao Sn4 1.32 0.16	\$-14-135	Maga MT 1	2.13	0.35	1.97	
5-14-138 Maga Sr3 0.84 0.14 0.27 5-14-139 Maga MT 3 1.84 0.15 0.88 5-14-140 Maga Sr4 1.04 0.15 0.45 5-14-141 Maga MT 4 2.29 0.3 1.92 5-14-142 Maga Sr5 0.81 0.12 0.22 5-14-143 Maga MT 5 1.59 0.21 0.51 5-14-144 Guaraguao Sr1 1.26 0.16 0.38 5-14-145 Guaraguao Sr1 1.26 0.16 0.38 5-14-145 Guaraguao Sr1 1.26 0.17 0.48 5-14-145 Guaraguao Sr2 1.37 0.17 0.48 5-14-146 Guaraguao Sr3 1.27 0.15 0.77 5-14-148 Guaraguao Sr3 1.27 0.16 0.69 5-14-150 Guaraguao Sr4 1.32 0.16 0.69 5-14-150 Guaraguao Sr4 1.32 0.16 0.69 5-14-150 Guaraguao Sr5 1.33 0.15 0.79	5-14-136	Maga Sn2	0.93	0.034	0.30	
5-14-139 Maga MT 3 1.84 0.15 0.88 5-14-140 Maga Sh4 1.04 0.15 0.45 5-14-141 Maga MT 4 2.29 0.3 1.52 5-14-141 Maga MT 4 2.29 0.3 1.52 5-14-142 Maga MT 5 0.81 0.12 0.22 5-14-143 Maga MT 5 1.59 0.21 0.51 5-14-144 Guaraguao Sh1 1.26 0.16 0.38 5-14-145 Guaraguao Sh1 1.26 0.16 0.38 5-14-145 Guaraguao Sh1 1.26 0.16 0.38 5-14-145 Guaraguao Sh2 1.37 0.17 0.48 5-14-145 Guaraguao Sh2 1.37 0.15 0.77 5-14-146 Guaraguao Sh3 1.27 0.15 0.77 5-14-150 Guaraguao Sh4 1.32 0.16 0.69 5-14-150 Guaraguao Sh4 1.32 0.16 0.69 5-14-150 Guaraguao Sh5 1.33 0.15 0.79	\$-14-137	Maga MT 2	2.01	0.28	1.18	
S 14-140 Maga Sn4 1.04 0.15 0.45 S 14-141 Maga Sn4 2.29 0.3 1.92 S 14-142 Maga Sn5 0.81 0.12 0.32 S 14-143 Maga MT 5 1.59 0.21 0.51 S 14-144 Guaraguao Sn1 1.26 0.16 0.38 S 14-145 Guaraguao Sn1 1.26 0.16 0.38 S 14-145 Guaraguao Sn1 1.26 0.17 0.48 S 14-145 Guaraguao Sn2 1.37 0.17 0.48 S 14-146 Guaraguao Sn3 1.27 0.15 0.77 S 14-148 Guaraguao Sn3 1.27 0.16 0.69 S 14-149 Guaraguao Sn4 1.32 0.16 0.69 S 14-150 Guaraguao Sn4 1.32 0.16 0.69 S 14-151 Guaraguao Sn5 1.33 0.15 0.79	\$-14-138	Maga Sh3	0.84	0.14	0.27	
5-14-141 Maga MT 4 2.29 0.3 1.92 5-14-142 Maga Sh5 0.81 0.12 0.22 5-14-143 Maga MT 5 1.59 0.21 0.51 5-14-144 Guaraguao Sh1 1.26 0.16 0.38 5-14-145 Guaraguao Sh1 1.26 0.16 0.38 5-14-145 Guaraguao Sh1 1.26 0.17 0.48 5-14-145 Guaraguao Sh2 1.37 0.17 0.48 5-14-146 Guaraguao Sh3 1.27 0.15 0.77 5-14-149 Guaraguao Sh3 1.27 0.16 0.59 5-14-150 Guaraguao Sh4 1.32 0.16 0.59 5-14-150 Guaraguao Sh4 1.32 0.16 0.59 5-14-151 Guaraguao Sh5 1.33 0.15 0.79	5-14-139	Maga MT 3	1.84	0.15	0.88	
S 14-142 Maga SN5 0.81 0.12 0.22 S 14-143 Maga MT 5 1.59 0.21 0.51 S 14-144 Guaraguao Sn1 1.26 0.16 0.38 S 14-145 Guaraguao Sn1 1.26 0.16 0.38 S 14-145 Guaraguao Sn1 1.26 0.17 0.48 S 14-146 Guaraguao Sn2 1.37 0.17 0.48 S 14-147 Guaraguao Sn3 1.27 0.15 0.77 S 14-148 Guaraguao Sn3 1.27 0.16 0.69 S 14-150 Guaraguao Sn4 1.32 0.16 0.69 S 14-150 Guaraguao Sn4 1.32 0.16 0.69 S 14-151 Guaraguao Sn5 1.33 0.15 0.79	S-14-140	Maga Sn4	1.04	0.15	0.45	
S-14-143 Maga MT S 1.59 0.21 0.51 S-14-144 Guaraguao Sn1 1.26 0.16 0.38 S-14-145 Guaraguao Sn1 2.33 0.23 0.74 S-14-146 Guaraguao Sn2 1.37 0.17 0.48 S-14-147 Guaraguao Sn3 1.27 0.15 0.77 S-14-148 Guaraguao Sn3 1.27 0.16 0.69 S-14-150 Guaraguao Sn4 1.32 0.16 0.69 S-14-151 Guaraguao Sn4 1.32 0.16 0.69 S-14-152 Guaraguao Sn5 1.33 0.15 0.79	\$-14-141	Maga MT 4	2.29	0.3	1.92	
S-14-144 Guaraguao Sn1 1.26 0.16 0.38 S-14-145 Guaraguao MT1 2.33 0.23 0.74 S-14-145 Guaraguao Sn2 1.37 0.17 0.48 S-14-146 Guaraguao Sn2 1.37 0.17 0.48 S-14-147 Guaraguao Sn3 1.27 0.15 0.77 S-14-148 Guaraguao Sn3 1.27 0.16 0.69 S-14-150 Guaraguao Sn4 1.32 0.16 0.69 S-14-151 Guaraguao Sn4 2.31 0.27 1.45 S-14-152 Guaraguao Sn5 1.33 0.15 0.79	\$-14-142	Maga Sn5	0.81	0.12	0.22	
5-14-145 Guaraguao MT1 2.13 0.23 0.74 5-14-146 Guaraguao Sn2 1.37 0.17 0.48 5-14-147 Guaraguao MT2 2.3 0.29 1.29 5-14-148 Guaraguao Sn3 1.27 0.15 0.77 5-14-149 Guaraguao Sn3 1.27 0.16 0.69 5-14-150 Guaraguao Sn4 1.32 0.16 0.69 5-14-151 Guaraguao Sn5 1.33 0.15 0.79	\$-14-143	Maga MT 5	1.59	0.21	0.51	
S-14-146 Guaraguao Sn2 1.37 0.17 0.48 S-14-147 Guaraguao MT2 2.3 0.29 1.29 S-14-148 Guaraguao Sn3 1.27 0.15 0.77 S-14-149 Guaraguao Sn4 1.22 0.16 1.46 S-14-150 Guaraguao Sn4 1.32 0.16 0.69 S-14-151 Guaraguao MT4 2.31 0.27 1.45 S-14-152 Guaraguao Sn5 1.33 0.15 0.79	\$-14-144	Guaraguao Sn1	1.26	0.16	0.38	
S-14-147 Guaraguao MT2 2.3 0.29 1.29 S-14-148 Guaraguao Sn3 1.27 0.15 0.77 S-14-149 Guaraguao Sn3 2.45 0.26 1.46 S-14-150 Guaraguao Sn4 1.32 0.16 0.69 S-14-151 Guaraguao MT4 2.31 0.27 1.45 S-14-152 Guaraguao Sn5 1.33 0.15 0.79	5-14-145	Guaraguao MT1	2.33	0.23	0.74	
S-14-148 Guaraguao Sn3 1.27 0.15 0.77 S-14-149 Guaraguao MT3 2.45 0.26 1.46 S-14-150 Guaraguao Sn4 1.32 0.16 0.69 S-14-151 Guaraguao MT4 2.31 0.27 1.45 S-14-152 Guaraguao Sn5 1.33 0.15 0.79	\$-14-146	Guaraguao Sn2	1.37	0.17	0.48	
S-14-149 Guaraguao MT3 2.45 0.26 1.46 S-14-150 Guaraguao Sn4 1.32 0.16 0.69 S-14-151 Guaraguao MT4 2.31 0.27 1.45 S-14-152 Guaraguao Sn5 1.33 0.15 0.79	\$-14-147	Guaraguao MT2	2.3	0.29	1.29	
S-14-150 Guaraguao Sn4 1.32 0.16 0.69 S-14-151 Guaraguao MT4 2.31 0.27 1.45 S-14-152 Guaraguao Sn5 1.33 0.15 0.79	5-14-148	Guaraguao Sn3	1.27	0.15	0.77	
S-14-151 Guaraguao MT4 2.31 0.27 1.45 S-14-152 Guaraguao 5n5 1.33 0.15 0.79	\$-14-149	Guaraguao MT3	2.45	0.26	1.46	
5-14-152 Guaraguao 5n5 1.33 0.15 0.79	5-14-150	Guaraguao Sn4	1.32	0.16	0.69	
	\$-14-151	Guaraguao MT4	2.31	0.27	1.45	
5-14-153 Guaraguao MT5 2.19 0.23 1.46	5-14-152	Guaraguao Sn5	1.33	0.15	0.79	
	\$-14-153	Guaraguao MTS	2.19	0.23	1.46	

La información detatlas en este regiones es pars uno exclusivo y presional del solicitante, y no podel ser utilizado para proceso tegales ni como intificación para agencias reglamentacionas. El Laborationis Central Analítico de la Estación Experimental Agricola no se hace responsable por los resultados de los análisis realizados el por su implicaciones legales a tenceros. Los análisis químicos no sen abeolutos y están sujetose veriedires our su instructes no interpor pueden ser controlocios.

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CAL-AES Chemical Analyses Report (1st collection: foliar tissue)

00736

INFORME DE RESULTADOS

SOUCITANTE Nombre David T. Nach Värpuer

Compañía UMET Directión muestra

Dirección postal Calle Lucia Várquez Norte 3 Ducad Cavey, PR Zio Code

Teletoro 787-578-5655

Email datsunai@outlook.com Propisito anà iza fertifidad



ANALISIS DE TEJIDO VEGETAL

Aralista Z. Neto Aralista Revisor Y. Ocasio			gul Il 8	U Anna Carlo Acorgano, anna	
Num.	r t. Ocasio	TKN	TKP	orani, Dilectora K	toda, familia Ordano-Rodriguez, Quimco
LCA	Identificación	56	16		
T-14-165	Tg Sn1	1.26	0.22	1.32	
7-14-165	Tg SN2	1.06	0.10	0.58	
T-14-167	Tg Sn3	1.11	0.12	0.55	
T-14-168	Tg Sn4	0.79	0.08	0.14	
T-14-169	Tg SnS	2.04	0.14	0.62	
1-14-170	7g M9:1	2.09	0.26	2.11	
T-14-171	TE MIC2	2.56	0.31	2.78	
T-14-172	Tg MR3	1.74	0.21	2.18	
T-14-173	Tg Mb4	1.85	0.16	0.90	
T-14-174	78 MPS	1.36	0.17	0.78	
1.14.175	Gg Seit	1.19	0.12	0.34	
1-14-176	Gg Se2	1.20	0.09	0.54	
T-14-177	Gg Sn3	1.29	0.10	0.94	
T-14-178	Gg Se4	1.30	0.11	0.32	
1-14-179	GgSeS	1.30	0.10	0.80	
T-14-180	Gg Mt1	1.99	0.20	0.82	
T-14-181	Gg Mt2	1.80	0.16	0.94	
T-14-182	Gg Mt3	2.39	0.20	1.86	
T-14-183	Gg M14	2.56	0.21	1.18	
T-14-184	Gg Mt5	1.97	0.18	1.50	

La información detallaria en este reporte es para seo escuava par consultados de los analisis tealizados en conso confilmación para apercos regarirentes este consultados de los analisis tealizados en por sus implicaciones lagales a tenderos. En análisis delimicos ne non absolveros y amén sujetose variabilidos por a restundes en por sus implicaciones lagales a tenderos. En análisis delimicos ne non absolveros y amén sujetose variabilidos por a restundes en por sus implicaciones lagales a tenderos. En análisis delimicos ne non absolveros y amén sujetose variabilidos por a restundes en por sus implicaciones lagales a tenderos.

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CAL-AES Chemical Analyses Report (2nd collection: foliar tissue)

SOLICITANT					1-14- 107
	 David T. Nachi Vázov 	92			Fecha de informe 7-May-14
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	Celle Lucia Vileguez N	lorte 5		10	
Guta	Carvey, PR	Zip Code	00736	Laboratorio C	Central Analitico 💁
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	datsunai@out	llook.com			
Proposit	o analisis tertilidad		7	ALC -	a to the second
AN	ALISIS DE S	UELO			and the second se
	IOS EN LCA			and the second	
Analist Analist	s Z. Nieto		Julian M. E	Robber	Yame Dural States
	e Y. Ocasio			orans, birectora	Inda Yamilu Deado-Rodriguez, Quimie
Num.	Identificación	TKN	TKP	ĸ	
LCA	IDENTIFICACION .	5	*	ppm	
5-14-299	5517g1	0.57	0.05	394	
5-14-300	552Tg2	0.55	0.05	346	
5-14-301	EgTE22	0.46	0.05	159	
5-14-302	SS4Tg4	0.39	0.04	175	
5-14-303	SSSTES	0.36	0.04	199	
5-14-304	Gg1-2(556)	0.47	0.04	177	
5-14-305	557Gg 3-4	0.44	0.04	154	
5-14-305	558Gg 4-5	0.48	0.04	188	
5-14-307	559 731	0.38	0.05	304	
5-14-308	5510 730	0.36	0.05	245	
5-14-303	5511 728	0.50	0.05	173	
5-14-310	5512 729	0.34	0.05	205	
5-14-311	5513 740	0.53	0.05	209	
5-14-312	5514 Gg 1-2	0.35	0.04	181	
5-14-313	5515 Gg 3-4	0.42	0.04	168	
5-14-314	5516 Gg 4-5	0.50	0.05	165	

La información detallada en una reporte es para los esclusivo y personal del usionante, a no posisi ser utilizada para procesos lagales si como contribución para agencias reglamentadoras. El usornenia de altitudo y estimación de la Estador logarimental Agricular os los recomposabiles por los recultados de los antilizadoras ripor sus implicaciones lagales a nomenos. Los antilisis que toro este estadora y estim apretides que por au manentes estenços pueden ser contrados.

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CAL-AES Chemical Analyses Report (1st + 2nd collections: Soil)