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Silica Concentrations in Dominant Vegetation Species of Wax Lake Delta, Louisiana

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SILICA CONCENTRATIONS IN DOMINANT VEGETATION SPECIES OF WAX LAKE DELTA, LOUISIANA

A Thesis

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Abbreviations

WLD	Wax Lake Delta
SI	Silicon
WBAS	Wetland Biochemistry Analytical Services
NIST	National Institute of Standards and Technology
AA	Autoanalyzer
LF	Leaf
FW	Flower
ST	Stem
RZ	Rhizome
RT	Root
DDW	Double Deionized Water

Abstract

Coastal deltaic wetlands colonize dynamic environments and experience fluctuations of salinity, river connectivity, sediment dredging or deposition, and periods of inundation resulting in unique vegetation and soils. Louisiana’s coastline is experiencing enhanced loss of coastal deltaic wetlands in coastal basins where the Mississippi River no longer is connected to waterways and channels that would normally provide freshwater and sediments representing the abandonment phase of the delta cycle. There is a coastal basin in the central regions of the state, the Atchafalaya Bay, that is still in the active phase of delta cycle resulting in the growth of the Wax Lake, and Atchafalaya Deltas at the mouth of the Atchafalaya River. The Atchafalaya River receive 30% of the combined flows of the Mississippi and Red Rivers that supply freshwater, nutrients, and sediments that enable land building. Observations of silicate concentrations in river water ~60-200 μM in lower Mississippi River stations in St. Francisville and New Orleans are found to be usually stable with occasional variation. We can use the finding of relatively constant silicate concentration to assume that the Mississippi River is a sable stock of silica to Wax Lake Delta (WLD). a major component of sediment and an important micronutrient for vegetation. Vegetation will take up bioavailable silica for protection and growth. Silica is beneficial for plants’ resiliency against toxins, molds, heavy metals etc. Flowers and other structures such as leaves, need silica to have optimal shape and support for growth and photosynthesis. There is minimal existing research on the silica concentrations of the vegetation of WLD. We are interested to see where in the plant most silica is stored and which species out of those in this study contain the most silica on average. The results of this study will begin to fill in the lack of data on the Si concentrations of plants and potentially highlight possible locations of silica fluctuations in deltaic environments.

Observations of what plant or plants take up most silica may imply a sink of silica such that it will not reach the water column around it and potentially improving water quality. We found that species in the higher and intermediate zones take up more silica than those in the channel.

Introduction

Wax Lake Delta is a subtropical active delta at the mouth of Wax Lake Outlet that was constructed as a flood control feature in 1942 to divert water flowing by Morgan City along the Atchafalaya River. The delta began forming subaerially in the Atchafalaya Bay following the 1973 flood and has been growing at about 1-2 km^2/yr since then (Roberts et al. 1997). This delta allows for the study of an active deltaic environment, with its emerging ecosystems connected to delta morphology (Paola et al. 2011; Twilley et al. 2019). WLD has a stable supply of Si from the Mississippi River while Atchafalaya River due to its more natural flow through landscape without levees and flood patterns, is found to have significantly higher concentration than Mississippi River (Reiman et al. 2018). We chose WLD as the site of this study for these reasons as well as previous research on vegetation species, abundance, and elevation range associated with the ecogeomorphology of Wax Lake Delta (Twilley et al. 2019). The work of Bevington et al. (2022) describes species selection to specific elevation ranges, informing which dominant species occupy hydrogeomorphic zones following disturbance. A range of eleven dominant species were chosen to have a robust analysis of species from different elevations such as including two aquatic species one of which remains submerged, species that can tolerate flooded and dry conditions, and upland species that rarely experience flooding to have a through overview of silica allocation. The older regions of Mike Island and the natural levees of the coastal edges support many upland species. Intermittent flooding ranges can be found past the levees and towards the middle of the island. Frequently flooded sites are located surrounding the fronts of the levees or southern portions of the islands where there is less retained sediment deposition.

Silica is the second most abundant element in soil. Large quantities of dissolved silica are

taken from soil by land plants annually, but it is not well studied on a global scale (Carey & Fulweiler 2016). Silica is most soluble at pH of 9 or less, making soils in this range a stable supply of bioavailable Si (Ma & Yamaji 2006). Silicic acid (Si(OH)4) is taken up by roots and is later incorporated into cell walls as amorphous silica ($SiO_2 \cdot nH_2O$) (Epstein 1999) (Carey & Fulweiler 2012). Plants can be classified as Si accumulators or non-accumulators based on the amount of Si that they take up and incorporate into their structures. Plants can be grouped by absorption of Si: high accumulation (more than 5% Si on dry weight basis), intermediate (accumulators (1%)), and low accumulators (<0.1%). Once inside the plants, Si can be incorporated into epidermal cells especially in leaves as amorphous silica ($SiOH_2$) (Carey & Fulweiler 2012). Amorphous silica or silica gel ($SiO_2 \cdot nH_2O$) is incorporated into cell walls or epidermal cells of most plant species but most notably in Equisetaceae (horse tail family), Cyperaceae (sedges), Gramineae or Poaceae (grasses), and Urticaceae (nettle family). Morphological parts of plants such as spines, spicules, and needles tend to have high concentrations of amorphous silica (Lewin & Reimann 1969).

The focus of this study is the placement of silica in the form of silicon (Si) in the within vegetation and its implications. Si is considered a micronutrient thus expressed customarily in mg/kg but can be found in concentrations as high as macronutrients (Epstein 1994). Si is required by certain accumulator species and not harmful in abundance (Epstein 1994; Frantz et al. 2011). Plants can benefit from the mitigating effect of Si when experiencing stress from heavy metals or salinity (Epstein 1999; Matoh et al. 1986; Daoud et al. 2018). Non-accumulator species also take up Si when stressed and show signs of benefiting from Si's effects (Frantz et al. 2011; Epstein 1999). Silica is found in high concentrations in earth's crust second to oxygen. Silica must be in its soluble form silicic acid $Si(OH)_4$ or the iodized form of silicic acid $Si(OH)_3O_2^-$ to

be taken up and utilized for structural support and immunity defense by vegetation (Currie & Perry 2007). Concentrations of $Si(OH)_4$ in soils change seasonally as shown in the Carey & Fulweiler 2014 study of Spartina. Silica has been found to create barriers against toxins and injury as well as structural support (Currie & Perry 2007). Si accumulating species will consistently have higher Si concentration in their structures than non-accumulating, but both will take up Si when experiencing stress from fungal infections, herbivory, salinity, and or heavy metals (Lewin & Reimann 1969; Epstein 1999). Lack of sufficient silica can result in misshapen plant structures and decrease in fitness (Frantz et al. 2011).The vegetation of WLD should have an optimal silica supply and as the Atchafalaya and Mississippi Rivers both supply Si to the delta as reasonably stable stocks (Rabalais et al. 1996; Reiman et al. 2018). In WLD is possible to study species of older higher elevation, middle age intermediate elevation, and newer low elevation submerged aquatic vegetation. An initial hypothesis is that out of the 11 species in this study, the most silica would be found in *Nelumbo lutea* and *Justicia ovata*. This expectation comes from the idea that silica provides extra structural support necessary for a plant to grow and support itself upward in an aquatic environment compared to a plant at higher elevations less likely to be submerged or impacted by flooding. *N. lutea* is an aquatic perennial that emerges above the water column for its leaves and flowers to open (Hall et al. 1944). *J. ovata* in the site in WLD was found at an elevation where there was dry land mixed with shallow puddles and had adventitious stems that potentially supports the plant from being displaced from flooding. *J. ovata* was found to efficiently trap sediments (Shaffer et al. 1992), which is possibly attributed to its adventitious stems with many prop roots. Past research has iterated that grasses, with rice, wheat, and sugar cane studied extensively, have been found to be Si accumulators. With this in mind, the grass species in this study should also have high Si concentrations since high

concentrations of Si have been observed in aerial parts of plants (leaf sheath, leave blade, and stem nodes (Lewis & Reimann 1969).

Bevington et al. (2022) found that *Colocasia esculenta*, *Polygonum punctatum*, *Alternanthera philoxeroides*, *Schoenoplectus americanus*, *Sagattaria latifolia, Sagattaria lancifolia*, *Nelumbo lutea*, and *Potamogeton nodosus* are the dominant species in transects of varying elevation across the deltaic islands of WLD. Flooding and elevation were found to have a significant impact on growth. Along with elevation, grain size is thought to impact ecological ranges (Johnson et al. 1985). The impact of hydrology of WLD is likely a combination of elevation, seasonality, and grain size. These factors should have the biggest roles in hydroperiod and freshwater inundation in WLD.

Material and Methods

Study Sites

Plant samples were collected from WLD (Figure 1) among monospecific stands of species of interest. Species were located by walking around Mike Island of WLD or searching from the boat while in the adjacent channel. Once we found monospecific patches, 10 plants of each species were collected. The aboveground steams, leaves, flowers etc. were put into marked paper bags before harvesting the below ground roots and rhizomes. Sites were logged in GPS and/or Google Earth (Figure 2). Occasionally two or more species were found in patches in close proximity to each other resulting in overlapping points on the map. Sites were located mostly in the older regions of Mike Island where the island is dominated by supratidal hydrogeomorphic zones. All samples were harvested in late summer.

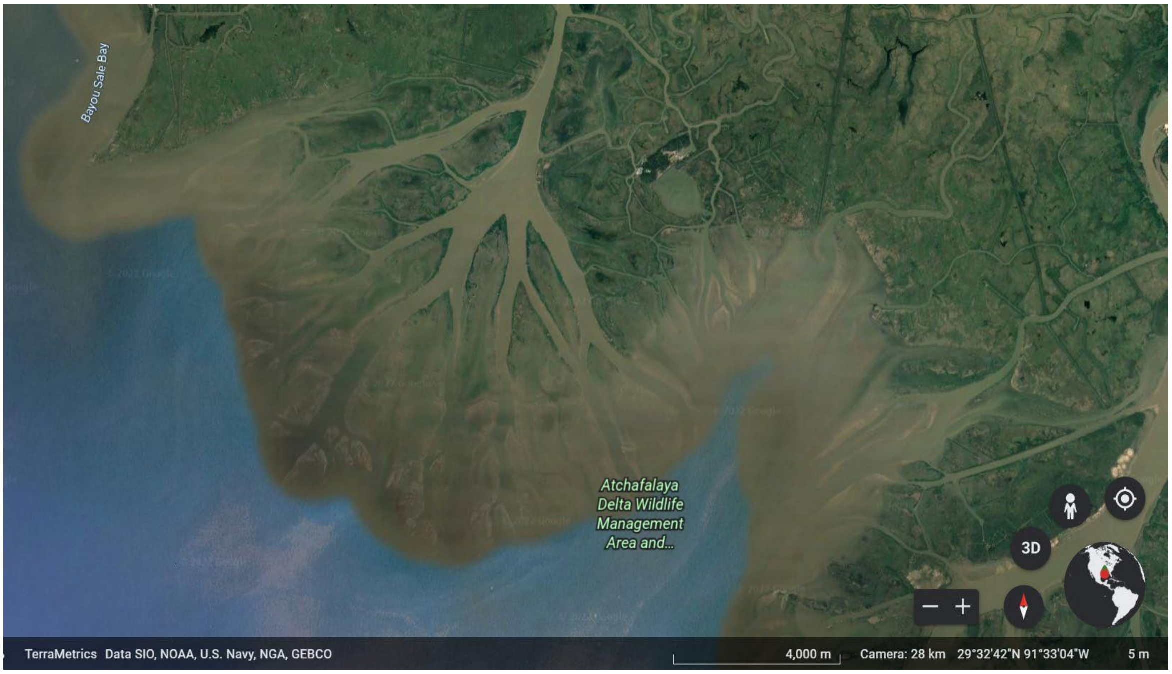

Figure 1. Google Earth image of Wax Lake Delta and surrounding area at 4,000 m scale.

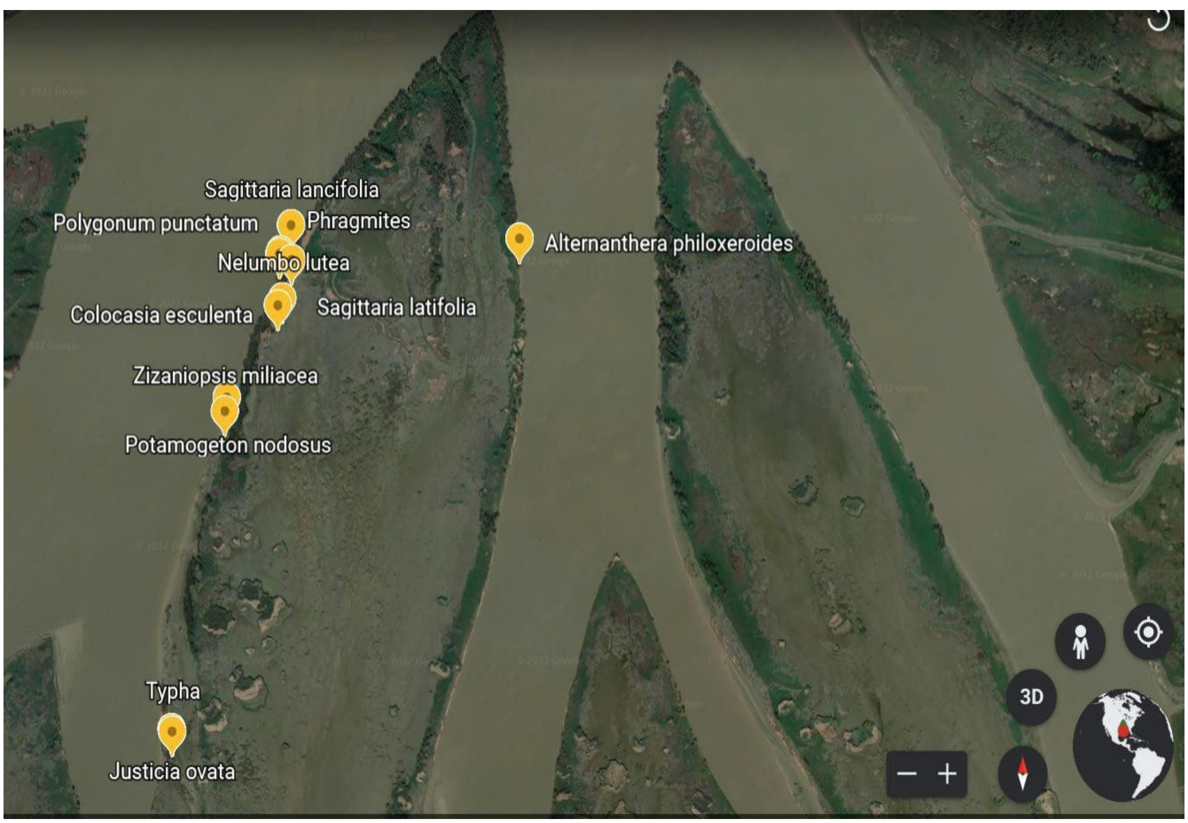

Figure 2. Map of Wax Lake Delta showing sample locations where specific species were collected on Mike Island.

Sample Collection and Preparation

Aboveground portions of vegetation were collected first, cutting the stems right at the soil line. Stem and attached leaves and or flowers were put into labeled paper bags designated by species and number 1-10. Belowground collected next by digging around the stem to gather the associated roots and rhizome then put into labeled zip lock bags then stored in a cooler until reaching the cold room 4^{o} C on LSU campus. Aboveground samples air-dried then washed to remove soil, bugs, algae, etc. Later samples were run through a spice grinder and or mortar and pestle before ground into fine powder using Retsch MM 200 ball mill with two canisters and two balls grinding at a frequency of 23 shakes per second for two minutes. Belowground were treated similarly, mud washed away from roots and rhizome then put in drying oven at 60^{o} C until dry mass was constant (about four days), then ground into fine powder with ball mill for wet digestion. Rhizomes were pre-ground with a spice grinder to reduce bulk size for grinding in ball mill (Figure 3). Flowers, leaves, stems, roots, and rhizomes (Figure 4) were separated by species to determine silica concentration within specific structural components of vegetation. Each plant part was washed with double deionized water to remove non-bioavailable silica in mud that would contaminate samples before drying at 60^{o} C in drying oven. Samples of flowers, leaves, stems, roots, and rhizomes were ground separately with random selection of enough sample to fill a 20 mL scintillation vial for each plant part.

An autoclave wet digestion method was utilized to determine total silica. Past research using autoclave wet digestion or hydrofluoric bomb methods was tested to determine best method based on silica concentrations in standards used in this study. The consensus data of NIST standards was used to validate Si concentrations of this study since there are no certified or uncertified values for NIST 1515 (apple leaves) or NIST 1575 (pine needles).

Figure 3. A. *Typha domingensis* rhizomes finely ground with a Retsch ball mill. B. *Typha domingensis* rhizomes ground with a spice grinder.

Figure 4. Separation of leaves, stems, roots, and rhizomes of *Phragmites australis*. All species of this study were similarly separated after washing and drying samples. Only *Nelumbo lutea* had an additional part (seed pod).

Acid Digestion

Plant material was digested using the method by Elliot & Snyder (1991) with modifications. Polypropylene bottles, 125 mL, were acid leached with HCl and washed with double deionized water (DDW). Once the bottles were dried labeled and weighted. Larger bottles allow for more space for samples to be digested without or with less overflow (Bell & Simmons 1997). First 100 mg sample added in each bottle, then 124 μL (approximately five drops) of octyl alcohol was added to prevent 2 mL 30% peroxide reacting with sample from foaming out of the bottles (Saihua et al. 2017). The peroxide breaks down the organic material of the sample aiding digestion. 4.5 mL of 50% NaOH to digest out Si and 1 mL of 5mM NH4F to keep Si in an easily detectable form (Kraska & Breitenbeck 2009), pipetted into samples before autoclaving at 121^{o} C for 1 hour. After autoclaving pH brought down to 12 with 16-19 mL 20% sulfuric acid and approximately 80-85 mL of DDI water. Weights of polypropylene bottles with digested samples recorded then bottle weights subtracted from bottle and solution weights. Samples were run through the Auto Analyzer for colorimetric analysis of Si concentrations. Results from AA converted into mg/kg as conventional for micronutrients (Epstein 1994).

Silicon Standards

Standards are meant to be run with samples since standards have a list of certified and sometimes uncertified concentrations of elements. There are no certified or uncertified Si values for National Institute of Standards and Technology (NIST) 1515 and NIST 1575. We verified our method using consensus values of past studies using wet digestion of NIST 1515 and NIST 1575 to values found from the results of the digestions of this study (Figures 5 and 6).

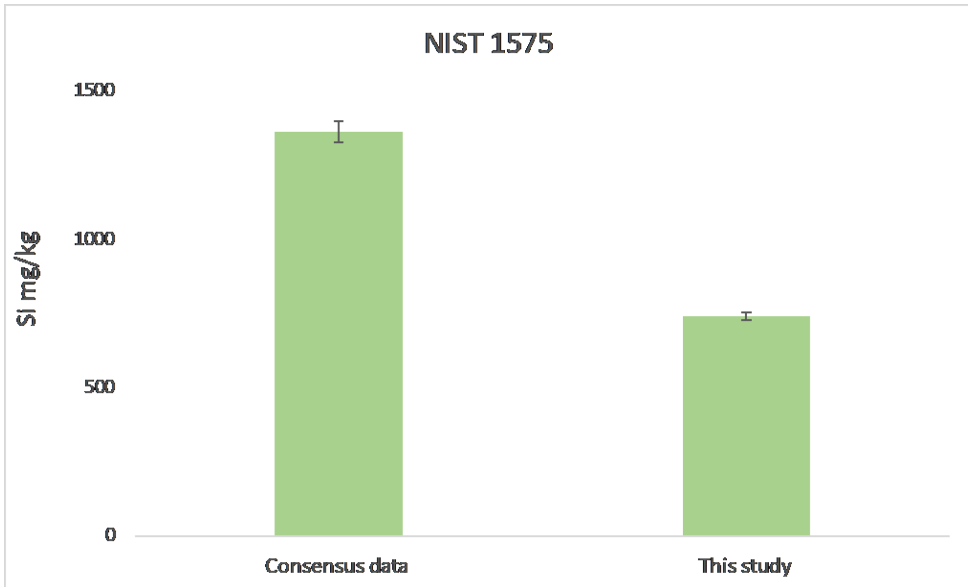

Figure 5. Comparison of Si concentration in NIST 1575 pine needles found in two papers referenced in Table 1 to the concentrations found from the digestions of this study.

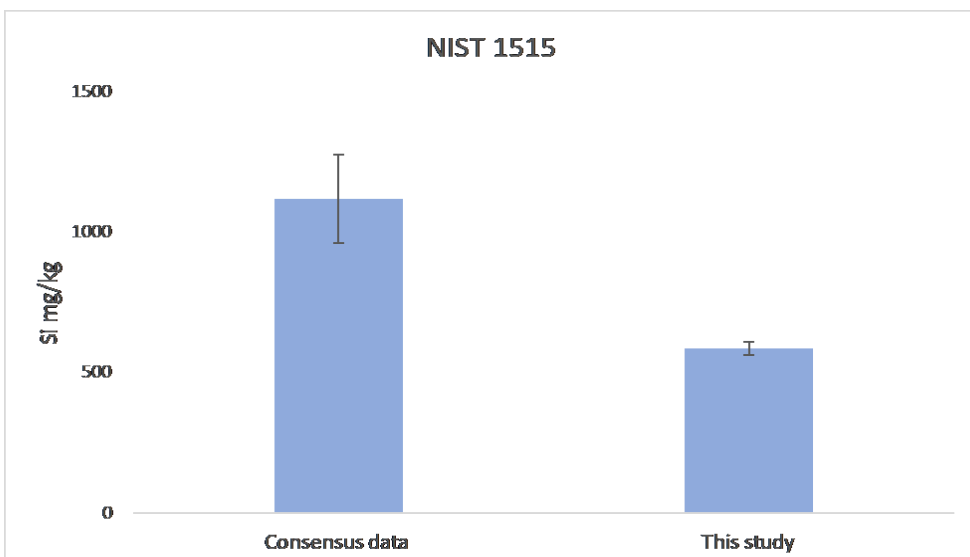

Figure 6. Consensus data of three papers listed in Table 1 for NIST 1515 apple leaves Si concentration compared to the concentrations found in this study.

Table 1. Table of results from past studies and including methods, Si concentration, and standard deviation or standard error.

Citation	Standard	Method	Si mg/kg	Error
Le Blond et al. 2011	NIST 1515 (Apple leaves)	HF closed vessel microwave digestion	909	200
Feng et al. 1999	NIST 1515 (Apple leaves)	HF closed vessel microwave digestion	944	200
Bell and Simmons 1997	NIST 1515 (Apple leaves)	Colorimetric autoclave wet digestion (twice the usual amount of sample and digestants)	1500	200
This study	NIST 1515 (Apple leaves)	Colorimetric autoclave wet digestion	585	25
Bell and Simmons 1997	NIST 1575 (Pine needles)	Colorimetric autoclave wet digestion (twice the usual amount of sample and digestants)	1410	139
Gladney et al. 1989	NIST 1575 (Pine needles)	XRF	1310	200
This study	NIST 1575 (Pine needles)	Colorimetric autoclave wet digestion	738	15

Results and Discussion

In this study *Nelumbo lutea, Colocasia esculenta, Sagattaria latifolia*, *Justicia ovata*, *Polygonum punctatum*, *Alternanthera philoxeroides*, *Typha domingensis*, *Phragmites australis*, *Sagittaria lancifolia*, *Potamogeton nodosus,* and *Zizaniopsis miliacea* are species that Si concentrations were measured. *P. nodosus* is classified as submerged aquatic vegetation and *N. lutea* as floating-leaf aquatic vegetation, while *A. philoxeroides* is semi-aquatic with most of the plant submerged and some leaves and stems on water surface. These species dominate the subtidal hydrogeomorphic zone of Wax Lake Delta. *T. domingensis, P. australis*, and *Z. miliacea* are classified as colony forming emergent wetland vegetation and dominate the supratidal hydrogeomorphic zone. The remaining *C. esculenta, S. latifolia, J. ovata*, *P. punctatum*, and *S. lancifolia* are herbaceous emergent wetland vegetation and are found mostly in the intermediate hydrogeomorphic zone of Wax Lake Delta.

Leaves and roots generally had the highest Si concentration among plant parts of the wetland species sampled at Wax Lake Delta (Figures 7,8,9). Stems and rhizomes had intermediate concentrations of Si (Figures 7, 10,11) and flowers had the least amout of Si (Figures 7, 12). This trend was found in most species, but there was some significant difference across the species. For example, the ranking of leaves>all other parts was evident for *P. australis* and *Z. miliacea*, where concentrations were the highest measured in this study at about 24,000 mg/kg (Figure 7). Roots had the highest concentration of Si among other plant parts in *A. philoxeroides, C. esculenta, J. ovata, N. lutea, P. punctatum, S. lancifolia*, and *T. domingensis* (Figure 7). The stems had highest Si concentration in *P. nodosus*, the submersed aquatic vegetation (Figure 7). It seems where one plant has high concentration of Si stored in leaves it had low concentration in its roots and the other way around. Lewin and Reimann (1969)

summarized that plants that accumulate Si tend to store more in leaves and areial structures whereas non-accumulators store more Si in roots. With this in mind, I would classify the grassey species *Z. miliacea* and *P. australis* as accumulators. *P. nodosus*. *N. lutea, S. lanifiolia, C. esculenta,* and *A. philoxeroides* have non-accumulator tendencies. *N. lutea* collected near the end of its growing season so there were flowers, leaves, and seed pods present.

There seems to be a high degree of variability among the plant parts that had the highest concentration of Si but based on results from Wax Lake Delta the larger and taller colonial wetland plants like *P. australis* and *Z. miliacea* would more likely be accumulators. The Carey and Fulweiler (2014) study of *Spartina* concluded that some grasses are passive (intermediate) or rejective (non-accumulators) of Si, but rice plants are accumulators. Certain aquatic species may be more intermediate based on the findings of *N. lutea*, *P. nodosus*, and *J. ovata.*

Leaves showed the greatest variation among wetland species in this study with *P. australis* and *Z. miliacea* having concentrations nearly 12 times (24,000 mg/kg) than the plants with lower concentrations such as *T. domingensis, S. lafifoia, P. punctatum, N. lutea, C. esculenta,* and *A. philoxeroides* (around 2,000 mg/kg) (Figure 8, Table 2). Intermediate concentrations of Si were found in *J. ovata* and *P. nodosus* at around 6,000 mg/kg. Roots had similar Si concentrations among the species sampled at around 12,000 mg/kg for *A. philoxeroides, N. lutea, P. australis,* and *S. lancifolia* (Figure 9, Table 2). Intermediate Si concentrations of 6,000 mg/kg in roots were observed in *C. esculenta, J. ovata, P nodosus, S. latifolia, T. domingensis,* and *Z. miliacea*. Lowest concentrations of 2,000 mg/kg were observed in *P. punctatum*. Stems had significantly higher concentrations at 10,000 mg/kg in *S. lancifolia* compared to intermediate concentrations of about 5,000 mg/kg in *N. lutea, P. australis,* and *P. nodosus* (Figure 10, Table 2). All the other species had concentrations <2,000 mg/kg, some of

lowest concentrations among the species for a plant part. Rhizomes had highest Si concentration in *S. lancifolia* and *Z. miliacea* at about 8,000 mg/kg compared to <4,000 mg/kg for the other species (Figure 11, Table 2). This is surprising that roots and rhizomes across the species did not have similar trends in Si concentration, suggesting that there is some accumulation processes among species.

There are very few surveys of Si in aquatic plants to compare these results for wetland species in Wax Lake Delta. One of the few comprehensive surveys of lithophile elements in aquatic plants is Linsley Pond (Hutchinson 1975). Si concentrations ranged from 1910 to 19,350 mg/kg (which is ppm reported in Hutchinson 1975, data from Cowgill 1974), which is similar to the range of 198 to 27,136 mg/kg found for plants in Wax Lake Delta. The overall mean was 7920 mg/kg for all species in Linsey Pond compared to 4863 mg/kg in Wax Lake Delta. Hutchinson (1975) remarks about the limited information reported on Si concentration in aquatic plants and the fact that there may be concerns about contamination by silica in diatoms as epiphytes on leaves of aquatic plants, and whether all Si is dissolved in ash as methodology limitation in survey results. It is interesting that *Phragmites australis* and *Oryza sativa* is considered an aquatic grass that stores high concentrations of Si from formation of bulliform cells in leaves that become silicified (Parry & Smithson 1964). *P. australis* leaves in this survey had the highest concentration of Si along with leaves of *Z. miliacea* as reported above. Silicification in leaves of aquatic plants has been noted with formation of opaline phytoliths that have biological significance in providing strength of plant parts but also in reducing herbivory.

The results of this study may have lower values than other studies. It is possible Si evaporated out as there were fumes coming from samples with the addition of NaOH. Or if there was any overflow during autoclaving as the bottles were loosely capped. It is possible that the

AA only detected Si when there were other forms of silica present thus the lower values compared to studies using Inductively Coupled Plasma Mass Spectroscopy which will heat the sample such that all forms of silica become Si.

Conclusion

Future research of coastal wetland species is much needed as there still remains a lack of available data of Si concentrations of many plants. It may be best to test two or more Si digestion methods in future studies so that comparisons and validations are possible. Si concentration may have ecological significance and restoration and or maintaining wetlands may benefit from knowledge of Si concentration in dominant species. This information may help determine if Si additions to the soil may improve productivity, growth, and or sustainability. Si additions to fertilizers have been successful in agriculture, it may also improve constructed or protected wetlands.

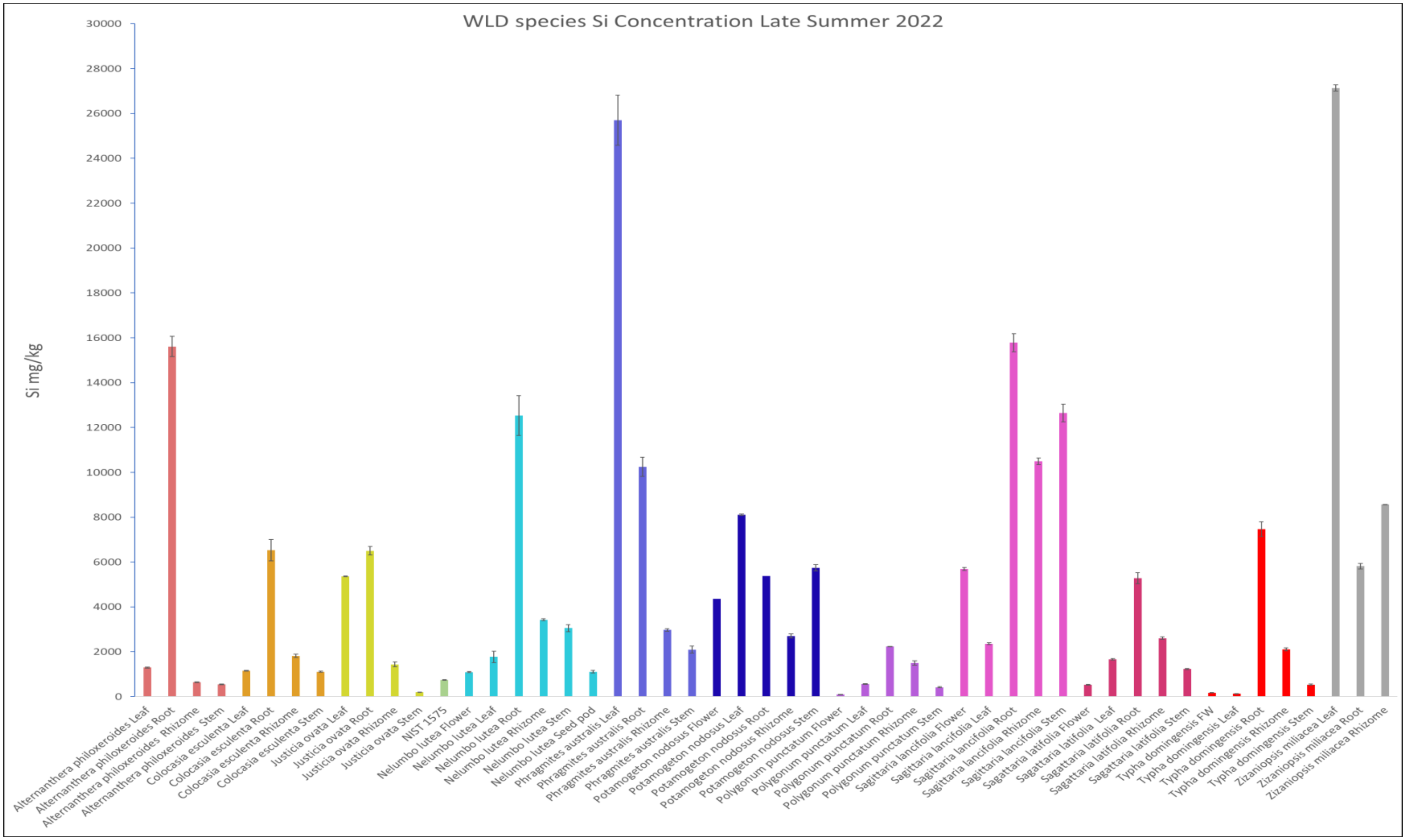

Figure 7. Si concentration (mg/kg) in respective plant parts of *Alternanthera philoxeroides*, *Colocasia esculenta*, *Justicia ovata*, (NIST 1575 (pine needles), *Nelumbo lutea*, *Phragmites australis*, *Potamogeton nodosus*, *Polygonum punctatum*, *Sagittaria lancifolia*, *Sagattaria latifolia*, *Typha domingensis*, and *Zizaniopsis miliacea*.

Table 2. Mean Si concentration (mg/kg) (standard error in parenthesis) in different plant parts in the wetland species sampled in this study.

Species	Leaf	Stem	Rhizome	Root	Flower	Seed pod
Alternathera philoxeroides	1292	543	646	15,604		
	(37)	(17)	(24)	(782)		
Colocasia esculenta	1153	1110	1816	6530		
	(36)	(35)	(131)	(824)		
Justicia ovata	5361	198	1438	6500		
	(25)	(10)	(184)	(335)		
Nelumbo lutea	1773	3054	3424	12,524	1093	1111
	(448)	(263)	(71)	(1539)	(46)	(102)
Phragmites australis	25,693	2095	2973	10,238		
	(2742)	(278)	(102)	(738)		
**Potamogeton nodosus*	8110	5747	2705	5370		
	(64)	(232)	(153)			
Polygonum punctatum	560	420	1497	2232		
	(20)	(45)	(174)	(2)		

(Table cont'd)

Species	Leaf	Stem	Rhizome	Root	Flower	Seed pod
Sagittaria lancifolia	2355	12,639	10,488	15,782	5686	
	(69)	(681)	(264)	(698)	(107)	
Sagattaria latifolia	1662	1226	2607	5283	521	
	(44)	(54)	(112)	(421)	(25)	
Typha domingensis	139	536	2109	7470	167	
	(2)	(33)	(105)	(802)	(12)	
Zizaniopsis miliacea	27,136		8562	5815		
	(232)		(14)	(210)		

**P. nodosus* flower (0.0598 g) and roots (0.0293 g) are exceptions to the averaged triplicates as there was not enough sample for more than one digestion.

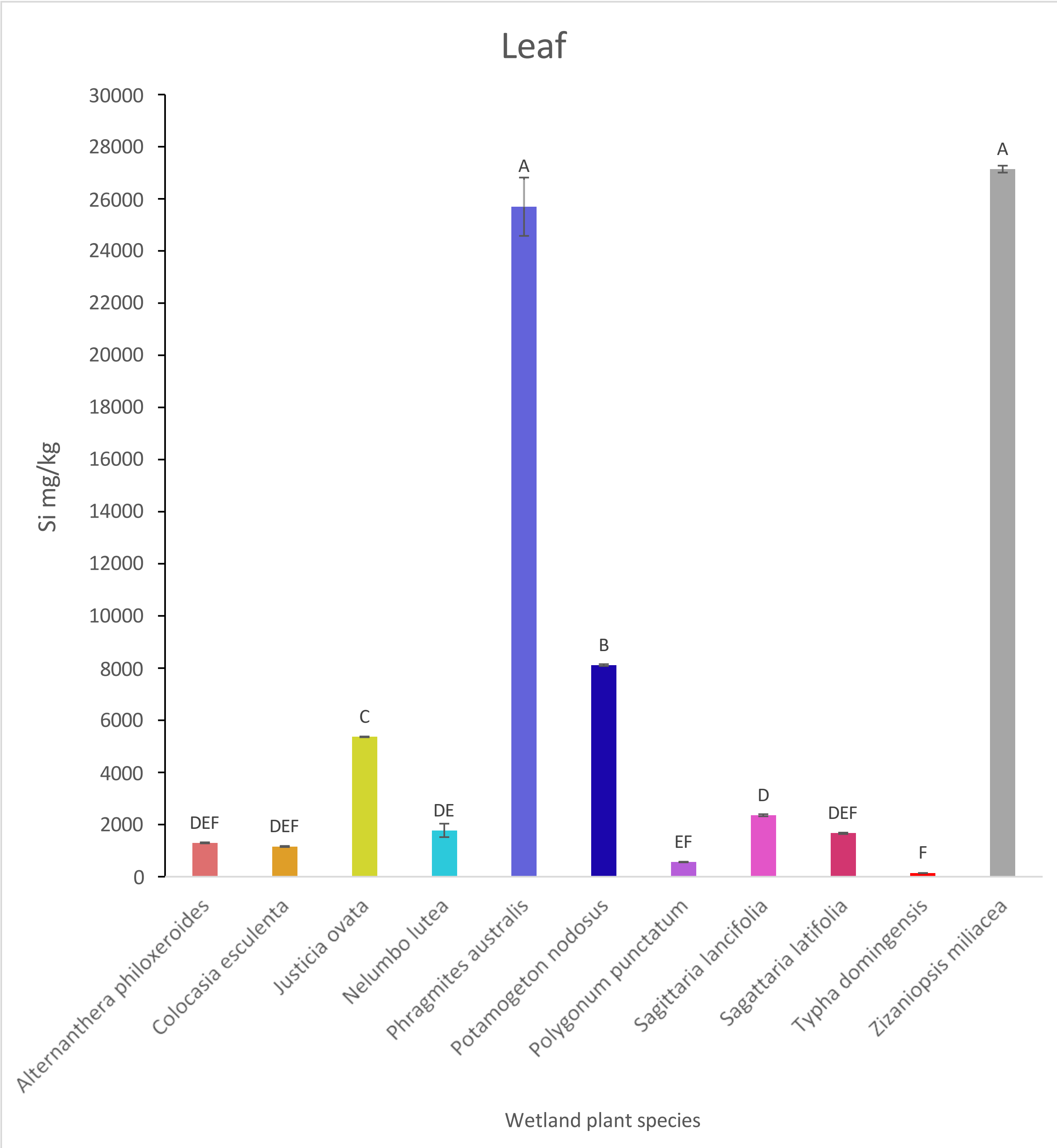

Figure 8. Si concentration (mg/kg) in leaves of all species in this study. Bars with same letters denote no significant difference in Si concentration.

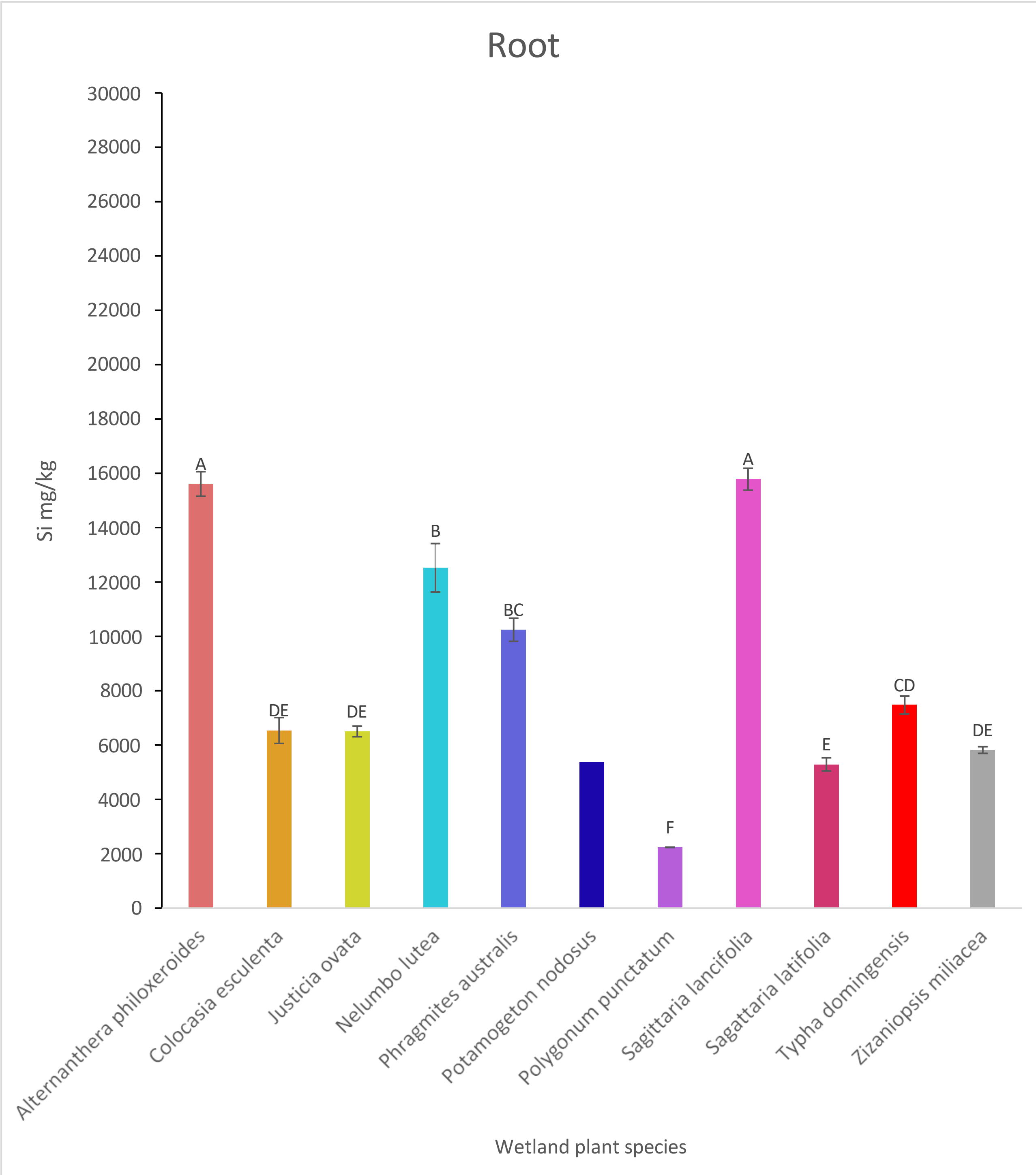

Figure 9. Si concentration (mg/kg) in roots of all species in this study. Bars with same letters denote no significant difference in Si concentration.

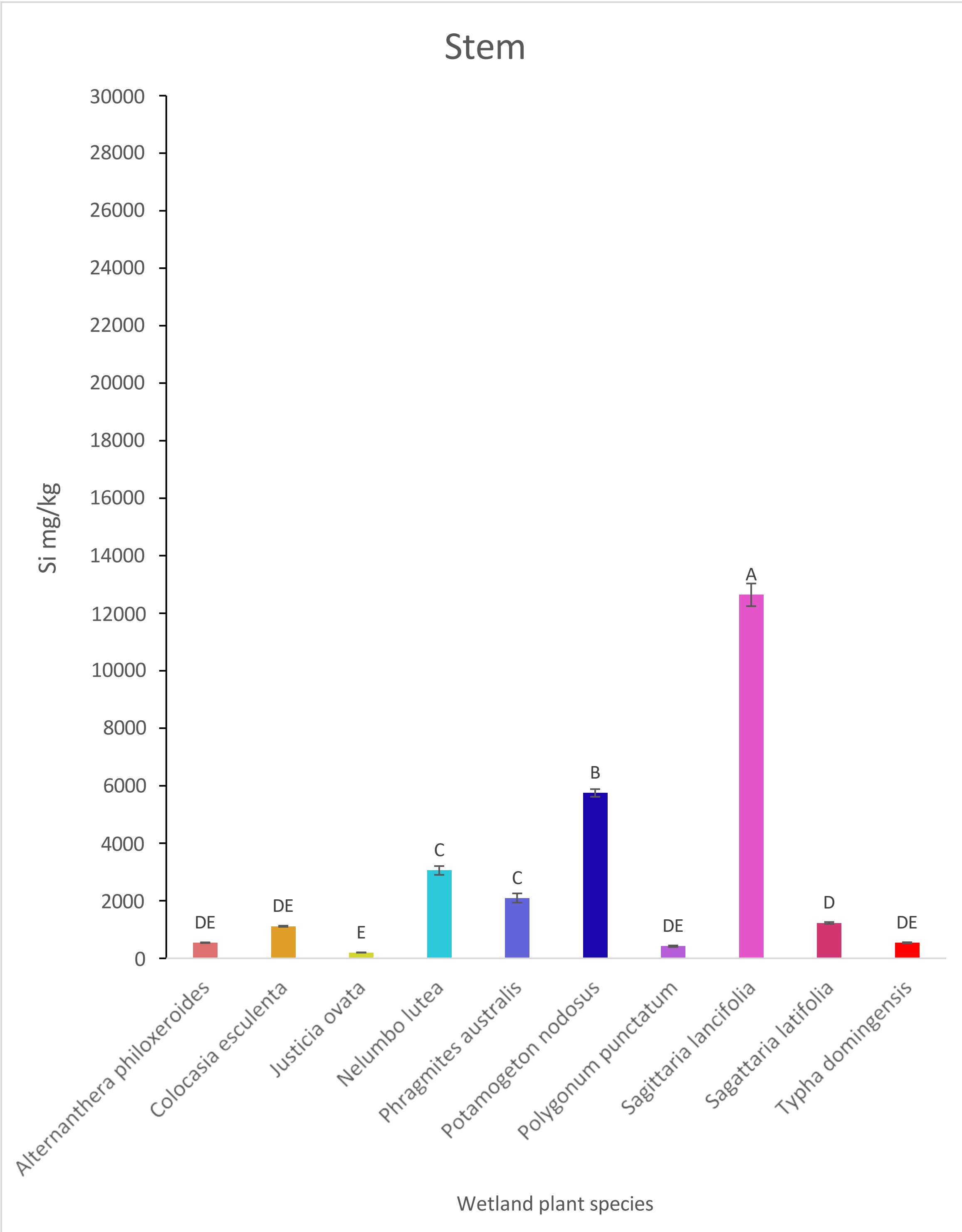

Figure 10. Si concentration (mg/kg) in stems of all species in this study. Bars with same letters denote no significant difference in Si concentration.

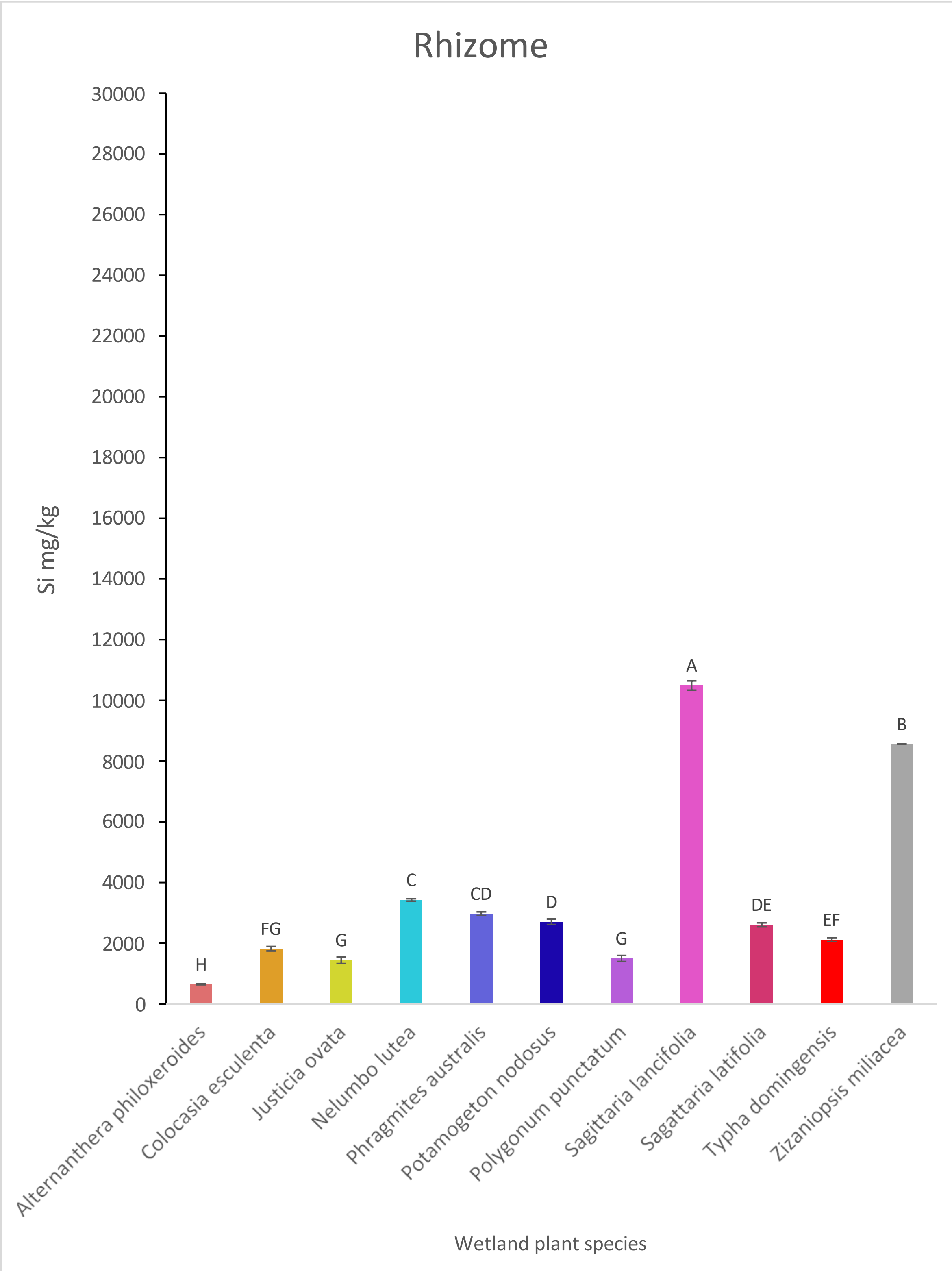

Figure 11. Si concentration (mg/kg) in rhizomes of all species in this study. Bars with same letters denote no significant difference in Si concentration.

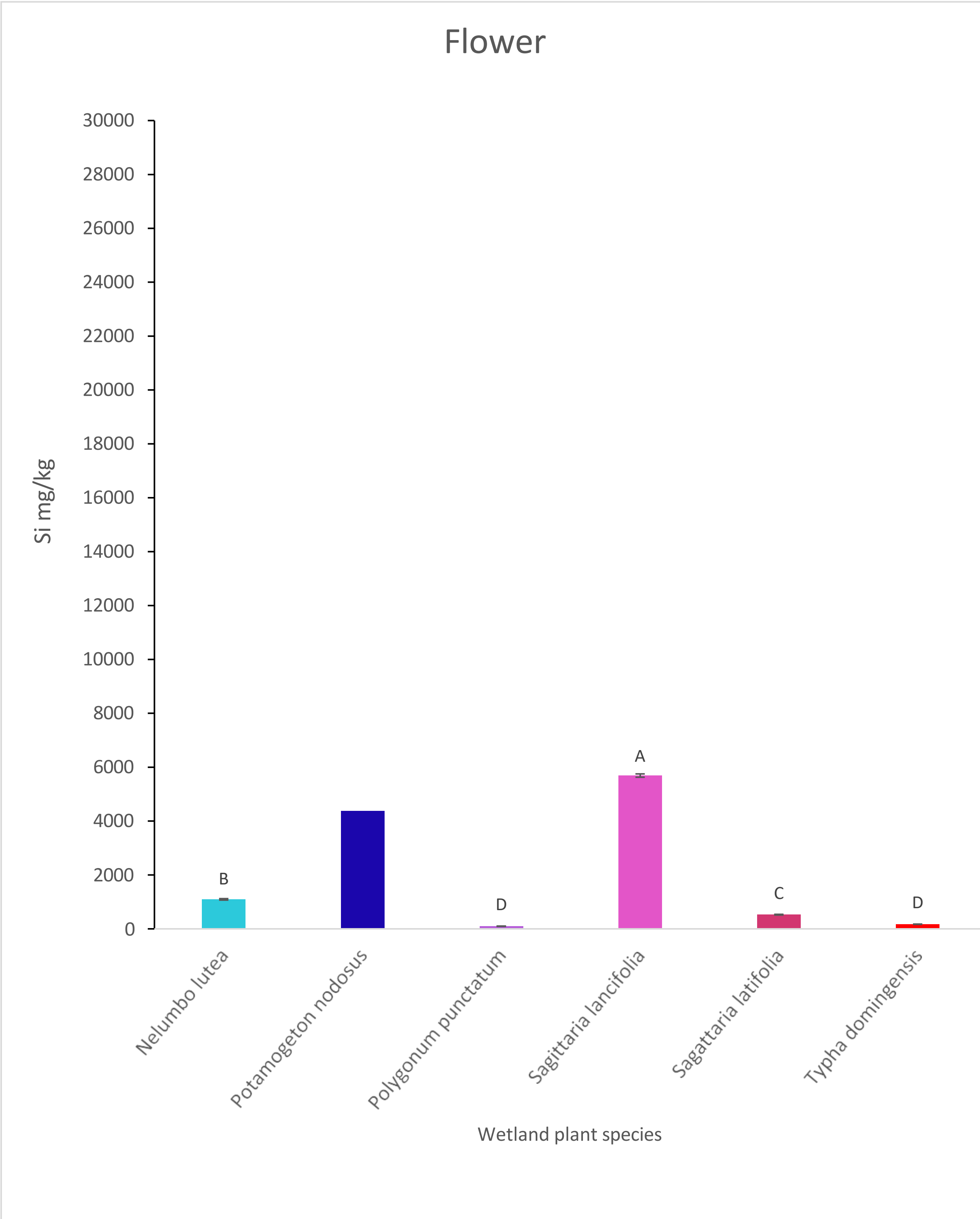

Figure 12. Si concentration (mg/kg) in flowers of species in this study. Bars with same letters denote no significant difference in Si concentration.

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Vita

Danielle Marie Soileau began her undergraduate studies at LSU in August of 2016. After graduating with a Bachelor of Science in Coastal Environmental Science, she began graduate school also at LSU in January of 2021. As an undergraduate she did two salinity studies on seed banks and mature oligohaline species. She later used this work to present a poster at the 2021 CERF conference as a graduate student. Also, in 2021 she won the Joseph Lipsey Sr. Memorial Scholarship. As a graduate student at LSU, she gained experience helping lab members with fieldwork and lab work. She plans on graduating in 2023 and further utilizing her experience to improve environmental quality.