Metal Deficiencies and Imbalances in Wetland Plants from a Manganese-Enriched Wetland in Southern Guam: A Preliminary Investigation

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Abstract: Trace metal levels were determined in surface waters, soil, soil pore-waters and plants from a perennial wetland in southern Guam. This wetland is unusual in that it has formed in an area geologically enriched with pyrolusite, a manganese bearing ore found primarily in fractures and fissures of the soft volcanic rocks that dominate the upland terrain. Iron and manganese levels in surface waters draining the wetland were at least an order of magnitude higher than those in other Guam rivers and streams draining watersheds without wetlands, while levels in sediment pore-waters were up to three orders of magnitude higher. The metal status of the common sedge, *Fimbristylis tristachya,* suggested the species was deficient in iron, copper and zinc. In contrast, manganese concentrations in this species and in the climbing fern, *Lygodium microphyllum,* sometimes approached levels considered phytotoxic to most other plants. Extremely high levels of manganese were encountered in the leaves of the monocot tree, *Pandanus tectorius,* with values exceeding 10,000 µg/g dry weight in certain wetland representatives. Historically, dried Pandanus leaves were used extensively as a source of domestic fiber in the local Chamorro culture. A possible link between the use of this plant and a neurodegenerative disease complex that once plagued the island and is symptomatically similar to the occupational disease, 'manganism', is considered.

Keywords: Guam, wetland, trace elements, soil, soil pore-waters, wetland plants, manganism

1 Introduction

Perennial and seasonal wetlands are scattered throughout watersheds in southern Guam and collectively occupy about 5,000 acres, or slightly less than 4% of the island's total land area[1]. Their location, configuration, day-to-day functions, and long-term evolution result from several hundred years of diverse land-use activities superimposed on bedrock geology, weathering and erosional processes, plant coverage and natural drainage development. Their ultimate value depends on a suit of physical, chemical and biological parameters that contribute to any given wetland functioning effectively as a wildlife habitat, chemical or physical buffer to water quality and stream flooding, temporary sediment sink, recreational or research site, recharge basin, or even a small scale aquifer. Although it is doubtful that any two wetlands in southern Guam are identical in this respect, certain physical and chemical conditions are common to all. A complete analysis of these conditions for any one wetland can, therefore, provide a better framework for understanding and managing it effectively.

One such study focused on a perennial riverine wetland on the western side of southern Guam (Fig. 1). The wetland sits astride the Taelayag River that winds its way through 'badlands' terrain that has developed in the soft volcanic rock (saprolite) in this part of the island. While the river is little more than a small stream, it is unusual in that it drains an area that is particularly rich in manganese (Mn) and carries so much of this element in solution that it precipitates MnO_2 varnishes on pebbles downstream of the wetland and on reef carbonate debris at the coast. The Mn deposits are heterogeneously distributed throughout the watershed as pyrolusite, a Mn^{4+} oxide mineral that occurs as metallic-black lustrous coatings and layers sandwiched between cracks, faults and other cavities in the saprolite. In this form, the element is essentially insoluble, and thus is unavailable for uptake and use by biotic components in the area. However, slope failures and sheet wash erosion during wet seasons continuously transports Mn bearing soil from adjacent uplands into the wetland below, where redox conditions favor the reduction of solid Mn^{4+} to soluble and biologically available Mn^{2+}. The impact of this Mn enrichment on plant and animal communities within the wetland continues to be of interest to us, especially for those resources of traditional importance to the Chamorro people of Guam. This paper presents preliminary trace metal data (specifically iron [Fe], Mn, copper [Cu] and zinc [Zn]) for biotic and abiotic components in the wetland study area and discusses the significance of the findings from an ecological and human health perspective. Details of all other parameters measured were presented earlier[2].

2 Materials and Methods

Sample Collection

Surface waters were collected on four separate occasions from two sites on the Taelayag River, in the center of the wetland, and on one occasion from eight sites along the length of an adjoining drainage ditch that delineated the wetland edge. The samples were taken during mid-wet season, August-October, 1996, when the study area was heavily water-logged and all adjacent streams were flowing freely. Sediments, and leaf/stem clippings of two dominant plants, the relatively deep-rooted common sedge, *Fimbristylis tristachya,* and the shallow rooted climbing fern, *Lygodium microphyllum,* were collected only once over the same time frame while vegetative samples of a third common representative, the monocot tree, *Pandanus tectorius,* were taken on two occasions at the end of the dry season in May, 2001 and April, 2002.

The surface water samples were withdrawn into 50 ml polypropylene syringes held ~5cm below the water surface. The syringes were filled with nitrogen gas for several days beforehand to minimize oxidation of soluble Fe^{2+} and Mn^{2+} species. Filtered (0.45 µm) and non-filtered ~5-ml subsamples were subsequently dispensed into separate polypropylene vials containing 20 µl of concentrated HNO_3 as a preservative. Soil pore-waters were collected from eight sites scattered throughout the wetland using ceramic-cup lysimeters (mean pore size ~5 µm) mounted on rigid PVC tubes (4.4 cm i.d.) of various lengths. The lysimeters were installed at depths ranging from 30-90 cm at each site. They were filled with nitrogen gas at the time of deployment and left to equilibrate to field conditions for several weeks prior to pore-water removal. Upon collection, samples were withdrawn by syringe and treated as described above for surface water samples. The process was assisted by pressurizing the interior of each lysimeter with oxygen-free nitrogen from a small, portable gas cylinder.

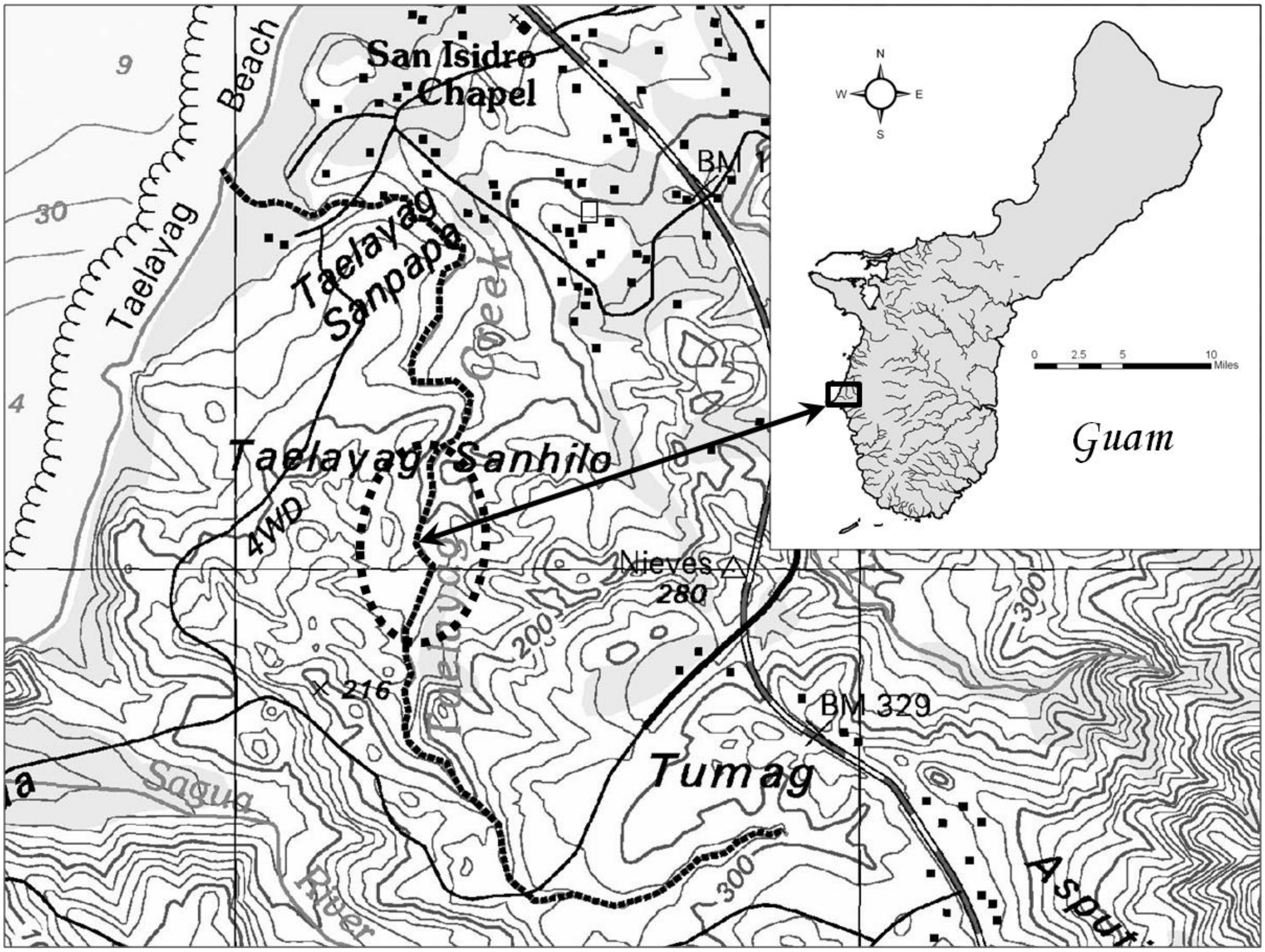

Figure 1: Portion of a 1:24,000 United States Geological Survey (USGS) Topographical Map of Guam. The dashed line and circle, respectively, delineate the location of the Taelayag River and wetland study area in the southern half of the island.

Anaerobic wetland soil samples were obtained at a depth of ~60 cm from freshly augured holes within 1 m of each lysimeters site and placed in Whirl-Pak bags using a plastic spatula. Plant specimens were also collected as close to the lysimeters as possible. Apical leaf and stem samples of *F. tristachya* and *L. microphyllum*, and distal, mesial and proximal portions of mature *P. tectorius* leaves, were clipped from representative plants at each site using stainless steel instruments. Leaf clippings were also collected from two Pandanus trees growing on the adjacent uplands and root, shoot and fruit samples were taken from a single wetland specimen. All biotic and abiotic components sampled were immediately placed on ice and transported to the laboratory within 2 hours of collection. In the laboratory, the plant materials and a subset of soil samples were dried to constant weight in an oven at 65^{o}C. The remaining soil and all aqueous samples were stored at 4 ^{o}C for analysis the following day.

Sample Analysis

The elemental analyses were performed on all samples using conventional flame atomic absorption spectroscopy. All acidified surface and pore-water samples were aspirated directly into the flame without further treatment. Background corrections for non-atomic absorption were made simultaneously by the instrument (deuterium lamp). Wet soils (~50% water) were extracted with 20% HCl for labile metal determinations. The oven dried soil subset was also subjected to a more rigorous digestion procedure with hot concentrated HNO_3 to oxidize organic matter and remove strongly bound metals without completely destroying the mineral matrix of non-carbonate components in the sample. The dried plant materials were mineralized by the same technique. Final solutions of all digests were diluted to volume with either 10% HCl or 10% HNO_3 as appropriate. QA/QC procedures included the use of analytical grade reagents, method blanks and matrix spikes. Approximately 10% of the samples were run in duplicate. All plastic and glassware were acid-washed and de-ionized water rinsed prior to use and standard stock solutions were purchased from a commercial supplier. Metal recoveries from certified standard reference materials (soil, orchard leaves) were all within acceptable limits. A more detailed account of the analytical procedures adopted is described elsewhere[3].

3 Results and Discussion

The data for all abiotic and biotic components analyzed are summarized in Tables 1 and 2 respectively. Clearly, Fe and Mn are very abundant throughout the area reflecting high levels of both elements in soil and parent rock materials. Aqueous levels of each varied appreciably between sites in the oxygen depleted surface waters of the small ditch draining the wetland and likely reflect substrate heterogeneity and differential flow rates within the main body of the wetland itself (Table 1). In contrast, levels recorded in the main Taelayag River were lower and more consistent with no significant spatial or temporal differences emerging from the data. A comparative analysis between filtered and unfiltered samples revealed the soluble fractions of Fe and Mn to account for >95% of their respective totals in the majority of samples analyzed. The values recorded for both elements are significantly higher than those normally encountered in local rivers that drain watersheds without wetlands. In such environments, dissolved Fe and Mn levels rarely exceed 0.1 mg/L and are mostly below 0.05 mg/L[4]. A broad concentration range that encompasses much of the published data for filterable Fe and Mn in river waters throughout the world is in the order of 0.003-0.3 mg/l and 0.001-0.1 mg/L for each metal respectively[5]. Zinc was barely measurable in most surface water samples analyzed, while Cu was consistently below the analytical detection limit of the instrument. Levels of both of these metals in other Guam rivers seldom rise above 0.003 mg/L[4].

The highly anoxic pore waters taken from the wetland were significantly enriched with Fe and Mn compared with Taelayag River surface (Table 1). Levels of both elements varied considerably between and within lysimeters over the study period, presumably in response to localized differences in the composition of the underlying soil and to discrete spatial and short-term temporal variations in the flushing rates of water through the system, as inferred above. At one lysimeter, for example, Fe levels varied from 0.05-95.7 mg/L in samples collected only two weeks apart. Within lysimeter concentrations of Mn were considerably less variable than that observed for Fe and rarely differed by more than a factor of two over time. Overall, no significant depth-dependant difference were apparent for either metal and the marked similarities noted between filtered and unfiltered sample values suggested both elements were present almost exclusively in the dissolved form.

Table 1: Trace Metals in Surface Waters, Pore Waters (mg/L) and Sediments (µg/g) from Taelayag River Wetland in Southern Guam

Sample	Statistic[a]	Fe	Mn	Cu	Zn
Surface Waters[b]					
Taelayag River	Range (n = 8)	0.20 - 0.57	0.49 - 0.71	all <0.03	<0.01 - 0.04
	Mean[a] (± 95% conf. limits)	0.39 (0.32 - 0.47)	0.55 (0.50 - 0.61)	nc	0.02 (0.01 - 0.03)
Wetland Ditch	Range (n = 8)	0.31 - 9.93	1.50 - 6.35	all <0.03	0.01 - 0.03
	Mean[a] (± 95% conf. limits)	1.70 (0.63 - 4.60)	2.67 (1.82 - 3.91)	nc	0.02 (0.01 - 0.02)
Soil Pore Waters[b]					
Depth: 30 cm	Range (n = 22)	0.04 - 140	1.34 - 30.1	all <0.03	0.01 - 0.04
	Mean[a] (± 95% conf. limits)	3.68 (1.20 - 11.3)	7.32 (5.18 -10.4)	nc	0.02 (0.02 - 0.03)
Depth: 60 cm	Range (n = 16)	0.05 - 95.7	2.82 - 66.5	all <0.03	<0.01 - 0.03
	Mean[a] (± 95% conf. limits)	11.6 (4.18 - 32.4)	6.41 (4.46 - 9.22)	nc	0.02 (0.01 - 0.03)
Depth: 90 cm	Range (n = 12)	0.40 - 33.3	0.82 - 11.5	all <0.03	<0.01 - 0.04
	Mean[a] (± 95% conf. limits)	14.4 (6.86 - 30.2)	3.24 (1.95 -5.38)	nc	0.02 (0.01 - 0.03)
Soil					
Depth: 60 cm	Range (n = 8)	34,371 - 60,735	599 - 3,036	135 - 183	97.0 - 151
(20% HCl)	Mean[a] (± 95% conf. limits)	47,083 (41,265 - 53,720)	1,220 (870 - 1,711)	161 (151 - 173)	123 (113 - 135)
Depth: 60 cm	Range (n = 8)	82,229 - 105,148	829 - 1,798	147 - 188	107 - 143
(Conc. HNO_3)	Mean[a] (± 95% conf. limits)	94,655 (90,030 - 99,517)	1,114 (921 - 1,346)	170 (161 - 181)	125 (118 - 133)

[a] Mean = geometric mean; [b] data for filtered samples (not listed) were generally within 95% of unfiltered values; nc = not calculable

Soil within the wetland study area is derived from highly weathered saprolites that characterize the upland topography and belongs to the Akina soil series of ferruginous latisols[6,7]. Soils within this category are typically high in Fe with concentrations frequently exceeding 10% on a dry weight basis[8]. The high Fe levels noted in soil samples analyzed during the present study therefore came as no surprise. Of greater interest were the recovery differences between weak and strong acid extractions which clearly demonstrate that at least 50% of soil-bound Fe in the wetland exists in a non-labile form. No significant differences were observed between the two extractions for Mn, Cu and Zn which suggest these elements have greater mobility within the soil than Fe. The fact that Mn concentrations were far more variable between sites than Fe most likely reflect differences in soil distribution patterns of each element with Fe being much more diffuse and widespread throughout the area. The relatively high concentrations of Cu and Zn found in all soil samples are fairly typical for Fe-rich ferrasols elsewhere[8,9].

Under normal conditions, ferrous metals associated with oxidic soils are frequently limiting to plants[9]. In an earlier study, Motavalli et al.[10] concluded that several soil types on Guam could present a problem to crop growers because of the limited availability of one or more essential trace elements. While such limitations clearly do not exist in the wetland studied here, elemental deficiencies can still occur as a result of competitive interactions between biologically available metals for uptake sites on plant root membranes. It is well known, for example, that high concentrations of soluble Fe and Mn can reduce Cu and Zn absorption from soil solutions and plants growing in waterlogged soils are frequently deficient in both elements[11,12]. The metal status of the sedge, *F. tristachya,* deserves special mention here because it strongly suggests that this species may be growing in the wetland study area under suboptimal conditions (Table 2). The mean Cu and Zn concentrations determined in this species, for example, would almost certainly have resulted in symptoms of Cu deficiency in pasture grasses[8,9,13].

Table 2: Trace Metals in Plants (μg/g dry weight) from Taelayag River Wetland and Upland Areas in Southern Guam

Plant	Statistic[a]	Fe	Mn	Cu	Zn
Wetland					
Fimbristylis tristachya	Range (n = 9)	57.8 - 121	180 - 540	1.30 - 3.30	7.00 - 14.8
Leaf/stem composite	Mean[a] (± 95% conf. limits)	74.7 (62.2 - 89.6)	345 (258 - 460)	1.77 (1.45 - 2.17)	9.77 (8.04 - 11.9)
Lygodium microphyllum	Range (n = 8)	67.5 - 241	92.6 - 483	7.20 - 11.0	45.8 - 128
Leaf/stem composite	Mean[a] (± 95% conf. limits)	123 (84.5 - 178)	219 (144 - 334)	9.20 (8.23 - 10.3)	73.4 (57.3 - 94.1)
Pandanus tectorius	Range (n = 9)	115 - 500	3,995 - 12,251	5.47 - 32.5	192 - 922
Leaf: distal	Mean[a] (± 95% conf. limits)	285 (196 - 415)	8,030 (6,195 - 10,410)	12.5 (8.65 - 18.0)	479 (332 - 691)
Leaf: mesial	Range (n = 9)	69.7 - 543	679 - 2,988	4.90 - 18.7	32.0 - 912
	Mean[a] (± 95% conf. limits)	239 (163 - 351)	1,363 (930 - 1,998)	11.0 (8.07 - 14.9)	449 (341 - 590)
Leaf: proximal	Range (n = 9)	31.2 - 801	280 - 1,011	2.93 - 20.2	13.8 - 296
	Mean[a] (± 95% conf. limits)	165 (93.4 - 290)	419 (322 - 545)	7.77 (4.58 - 13.2)	44.6 (21.9 - 91.0)
Shoot	(n = 1)	11.3	247	3.76	50.6
Fruit	(n = 1)	127	292	2.22	9.8
Root	(n = 1)	176	357	7.11	124
Upland					
Pandanus tectorius					
Leaf: distal	Range (n = 2)	89.8 - 481	430 - 7, 322	5.60 - 8.47	79.6 - 426
Leaf: mesial	Range (n = 2)	63.3 - 601	66.9 - 1,637	3.73 - 18.5	206 - 1,176
Leaf: proximal	Range (n = 2)	373 - 421	41.5 - 334	5.21 - 17.8	45.8 - 390

[a] Mean = geometric mean

Because of their chemical similarity, Fe and Mn are mutual antagonists and can also influence the uptake of one another depending upon their respective available concentrations in the surrounding soil[14,15]. This could explain why the Fe status of *F. tristachya* was generally lower than that normally encountered in other monotcots[13]. Further evidence that Mn is suppressing the uptake of Fe in *F. tristachya* and possibly *L. microphyllum,* can be seen by examining the Fe/Mn ratios in both species. Under normal conditions the Fe/Mn ratios in plants generally lie between 1.5 and 2.5. Below this range, symptoms of Fe deficiency or Mn toxicity may occur, while above 2.5 the reverse is generally true[9,16]. In the present study, Fe/Mn ratios ranged from 0.15-0.45 (mean: 0.22) in *F. tristachya* and from 0.22-1.62 (mean: 0.56) in *L. microphyllum* (mean: 0.56). Interestingly, the phytotoxic threshold for Mn in most plants is around 500 μg/g dry weight and levels above 400 μg/g dry weight are generally considered to be excessive[9]. Despite our limited samples size, 50% of *F. tristachya* and 25% *L. microphyllum* analyzed yielded Mn concentrations in excess of 450 μg/g dry weight. Thus, Fe, Cu and Zn deficiency and Mn toxicity seem likely to be among the challenges facing plants growing in the wetland study area.

The extraordinarily high Mn concentrations found in the leaves of *P. tectorius* from both wetland and upland sites (Table 2) are particularly noteworthy and undoubtedly reflect a physiological response that permits the species to survive in this Mn-enriched locale. The compartmentation of excessively accumulated metals in foliar tissue is a common strategy employed by plants growing in metalliferous regions[17]. *P. tectorius* is an upland savannah species that has adapted to wetland conditions and its tolerance of high levels of soluble Mn has clearly enabled it to establish itself in the latter environment. However, the fact that upland representatives share the same capability suggests that this adaptive mechanism evolved in response to selection pressures other than those associated with waterlogged soil. One possibility is that it evolved secondarily to another mechanism(s) designed to solubilize essential trace elements from non labile soil sources. In the surrounding upland environment, for example, Fe and Mn occur predominantly as insoluble oxides and oxyhydroxides and are virtually unavailable to plants under normal conditions. Other essential trace metals like Cu, Zn and Co coexist within these amorphous structures and are equally inaccessible. Plants able to survive such conditions frequently do so by secreting metal-reducing and/or metal-chelating substances from their root systems[17]. We speculate that *P. tectorius* has evolved such specialized capabilities and is able to sequester sufficient amounts of essential trace elements from Guam's oxidic upland soils to satisfy its metabolic needs. In the Taelayag River uplands, however, it is frequently faced with oversupply of Mn, a problem it appears to have resolved by shunting the excess metal into subcellular foliar compartments (e.g., cell vacuoles) away from sensitive metabolic activities[18].

Certain Mn resistant species have the ability to precipitate MnO_2 in leaf epidermis[19] and trichomes[20] in close proximity to leaf stoma. This deposition process might also be expected to co-precipitate other elements and, if it occurs at all in *P. tectorius,* could account for the relatively high concentrations of foliar Fe and Zn in several specimens analyzed in addition to the positive correlations ($P<0.05$) that existed between all three metals. In any event, the fact all three elements were generally more concentrated in older leaf sections indicate that deposition is a continuous and irreversible process with leaf fall marking the final elimination step from the plant's system. Other accumulator plants have also been shown to concentrate Mn more so in older leaves and possess higher foliar Fe levels than non accumulator species[9]. Plants generally do not accumulate excessive levels of trace elements in shoots, fruits and seeds and *P. tectorius* would seem to conform to this (Table 2). Such adaptations have presumably arisen in response to selection pressures to protect sensitive meristem and embryonic tissues from potentially toxic metal concentrations.

The marked ability of *P. tectorius* to accumulate Mn is of particular interest from a human health standpoint because of the plant's many traditional uses throughout Micronesia, plus the fact that Mn poisoning ('manganism') is symptomatically similar to a neurodegenerative disease complex that once accounted for one death in five among the native Chamorro people of Guam over the age of 25[21]. This mysterious ailment is locally referred to as 'Lytico-Bodig' and is a Parkinsonism-dementia complex (Bodig) that is sometimes accompanied by amyotrophic lateral sclerosis (Lytico). Today, ALS-PDC, as it is more widely referred to in scientific circles, has all but disappeared from Guam, which has led to speculation that the disease may have been linked to an environmental factor that is no longer threatening[22,23,24,25,26,27,28,29]. Manganese was suspected to have played a role in ALS-PDC as early as 1965[29] although, as yet a connection between the two has never been substantiated. Reported cases of manganism are confined almost exclusively to occupational exposures involving the inhalation of Mn oxide dusts[30]. Since dried Pandanus leaves were traditionally woven into rope, baskets, body ornaments, roofing, sleeping mats, household curtains and screens, food trays, skirts and other miscellaneous items, exposure pathways from plant to human via pulmonary/enteric routes are easy to envisage. For now, however, any connection between the plants predilection for Mn and the prevalence of the neurodegenerative diseases that once plagued the island remains a question of intrigue.

4 Conclusions

The study described herein, although preliminary in nature, clearly demonstrates the existence of chemical and electrochemical processes common to all wetland soils superimposed upon certain physical and chemical characteristics unique to the study area. The resulting aqueous conditions profoundly affect the mobility and biological availability of nutrients and essential trace elements, which in turn, dramatically influences the types of plants that can survive and successfully colonize the area. Gross oversupply of soluble Mn in soil pore waters throughout the wetland, coupled with the potential toxicity and interactive effects of this metal with other essential trace elements, pose additional challenges to pioneer species. Shallow rooting plants like *L. microphyllum* are presumably able to overcome such problems, at least in part, by exploiting surface soil layers of higher redox potential, while deeper rooted species must adopt other strategies to survive. The sedge, *F. tristachya,* for example, appears to have evolved an exclusionary mechanism to cope with excess Mn and maintain tissue concentrations below toxicity thresholds but in so doing may be limiting its uptake of essential Fe. The data also suggest this particular species is struggling to meet its optimum Cu and Zn requirements because of competitive interactions with Fe and Mn for cellular binding sites and transport mechanisms across root membranes. While metal exclusionary processes are common in wetland plants, the evolution of such a mechanism in *P. tectorius* would have most certainly compromised the ability of this facultative wetland species to sequester essential elements from the highly oxidized soils present in its original savannah habitat. Survival of this plant under waterlogged conditions is facilitated by its ability to capitalize on a compartmentation mechanism that we suspect first evolved in upland representatives in response to excessive Mn uptake. Studies are, therefore, continuing to determine: a) whether or not *P tectorius* from other parts of Guam display a similar predilection for this element in their foliar tissues; b) the identification of mechanisms involved in the solubilization and sequestration of soil-bound Mn and other essential elements; c) precisely where the primary Mn deposition sites are in the leaves and in what form the element is translocated and stored, and finally d) whether or not traditional methods of working and weaving of *P. tectorius* leaves could have generated sufficient amounts of Mn dust in ancient Chamorro households to account for the ALS-PD epidemic of the past.

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