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Barr Report

Barr Report

with Tom Barr, Greg Watson, and the Plant Guru Team

Copper and Zinc's Role in Aquatic Macrophyte Uptake and Growth

Special points of interest:

- Feature Article "Copper and Zinc's Role in Aquatic Macrophyte Uptake and Growth"
- Copper and Zinc are both Nutrients required by plants.
- Copper has a role in photosynthesis and chlorophyll formation.

Copper has a long history in aquatic management due to its ability to kill algae and aquatic weeds. Zinc has a less prominent history but more research has been in the last several years in roles of each in transport, cycling and their importance. While both are nutrients, higher concentrations they are toxic. Most trace metals fall into this group. Copper is required for mitochondrial electron transport, pathogen defense, cell wall lignification, vitamin C metabolism, ethylene perception, carbohydrate metabolism, nitrogen fixation, fatty acid desaturation/ hydroxylation, and, in the chloroplast, for both electron transport (plastocyanin) and oxidative stress responses (Cu/Zn superoxide dismutase). Copper typically is found only in protein-bound forms in cells, since as a free ion it may generate oxidative stress and cause serious damage to organic molecules. This means the reactivity of copper that makes it so useful in redox reaction and electron transport also makes it toxic. Free copper ions readily oxidize thiol bonds within proteins (the disulfide bridges in proteins that hold the enzyme together), causing a disruption of their secondary structure. The principal mechanism of copper toxicity involves the "Fenton reaction", characterized by metal catalyzed production of hydroxyl radicals from superoxide and hydrogen peroxide (Elstner et al., 1988; Briat and Lebrun, 1999):

Reworking a tank

$$O_2^{\cdot -} + Cu^{2+} \Rightarrow O_2 + Cu^{+}$$
$$H_2O_2 + Cu^{+} \Rightarrow Cu^{2+} + OH^{-} + OH^{\cdot}$$

Peroxide and the singlet oxygen are very destructive compounds and the plant must quench these to grow without damage. This chemical process has been demonstrated in isolated chloroplasts (Sandmann and Böger, 1980), in algal cells (Sandmann and Böger, 1980) and in roots (De Vos et al., 1993). Reactive oxygen species destroy biological macromolecules like proteins, lipids (and forms MDH via peroxidation of lipid membranes which is also very toxic), DNA, and as a consequence cause cell death by necrosis. Generally copper and Zinc are added to a liquid trace formulation at some ratio relative to the other micronutrients. Iron is the proxy for these other trace metals but some adjustments and slight changes for aquatic plants may be helpful.

Ratios:

The discussion of ratios is geared to help the aquarist or researcher use the data to make predictions in horticulture about trace elements that are difficult to measure but are essential nonetheless. This allows the horticulture of aquatic macrophytes without relying heavily to test to ensure

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... Copper has a role in photosynthesis and chlorophyll formation ....

that the macrophytes are not being limited nor inhibited at high ranges of trace metals. As many tests do not measure the bioavailable forms of Cu and Zn, this compounds the testing methods available to hobbyists. Typical research methods use the atomic absorption method(AA). A discussion of the key enzymes is critical to predict what the pathways will be affected and how the plant responds to copper and zinc deficiencies as well as excess concentrations. These enzymes show where and how a plant regulates copper, zinc and the various functions each has in plant metabolism. Below are some typical levels found in six species of submersed plants.

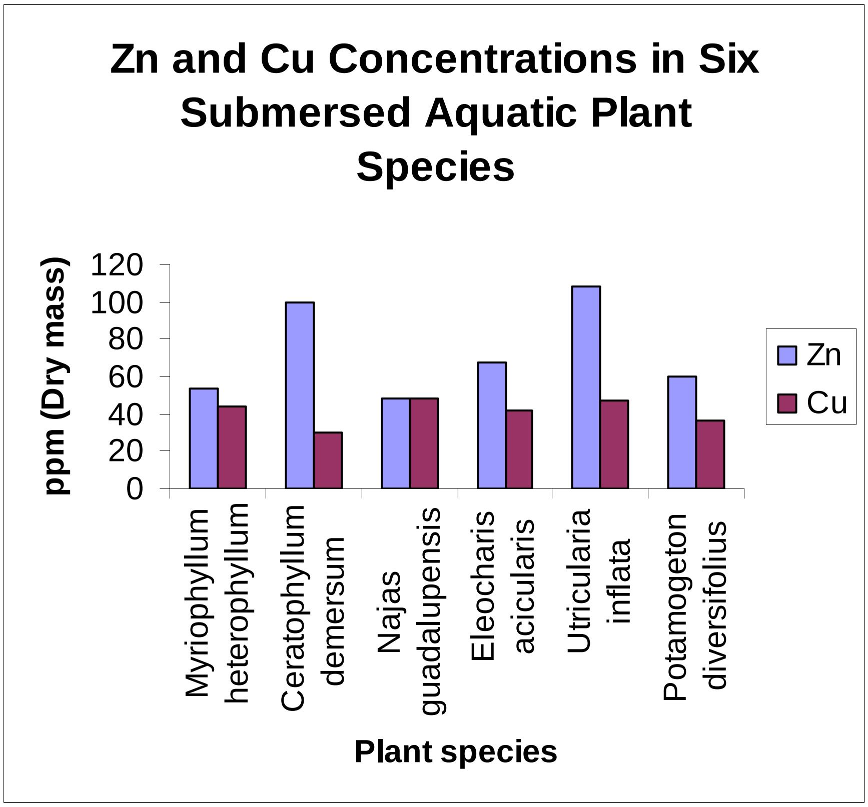

Figure 1. PPM dry weigh of each trace metal for 6 aquatic plant species.
In general for all species surveyed, roughly 35 plant species a 1:1 to 2:1 Zn:Cu ratio was found. Note, this does not imply this is the best ratio range for growth, it's just what is found in plant tissues in natural systems.

"… At higher concentration levels they are toxic. Plants have several methods to address toxicity of trace metals such as Zn and Cu through gene regulation of various enzymes used to assimilate and uptake nutrients."

The functions of Copper and Zinc in plant physiology and growth:

Plant Nutrient Uptake Characteristics
• Selective uptake and transport
• Concentrate (or exclude) ions against the existing gradient
• Homeostatic control of tissue nutrient concentration

Requires:
• Energy (ATP, reducing power=> NADPH)
• Selectively permeable membranes (PM, tonoplast, etc)
• Selective pumps and carriers (membrane localized proteins)

Zinc is an essential catalytic component of over 300 enzymes.

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Copper and Zinc's Role in Aquatic Macrophyte Uptake and Growth

Copper:

Copper has a role in photosynthesis and chlorophyll formation. Some key enzymes are : Plastocyanin, Superoxide dismutase (SOD), Cytochrome oxidase**,** Ascorbate oxidase, Polyamine oxidases.

50% of the copper localized in chloroplasts is bound to plastocyanin. This compound serves to shuttle electrons between the PSII and PS1 light reactions in photosynthesis. If a plant is copper deficient, the activity of photosystem I is reduced and uncoupled. This causes the plant to slow its growth significantly. Important in the utilization of proteins in the growth processes of plants. (The photosynthesis rate of Cu-deficient plants is abnormally low due to low electron transfer reducing ATP and NADPH production that drives CO2 fixation.)

There are various types of SOD (superoxide dismutase) enzymes. The CuZnSOD is located in the cytoplasm in mitochondria and glyoxysomes, but occurs also in the chloroplasts together with the FeSOD. Under copper deficiency, CuZnSOD activity declines drastically in leaves (chloroplastic and cytosolic) with simultaneous corresponding increase in activity of the MnSOD. Plants have to have SOD since the production of superoxide radicals (O^{2-}) will do great damage to the key enzymes and the SOD enzymes serve as protection (antioxidants).

Cytochrome oxidase serves as the terminal oxidase of the mitochondrial electron transport chain. It contains two copper atoms and two iron atoms in the heme configuration. As respiration rates either remain unaffected or are only moderately decreased by copper deficiency, cytochrome oxidase seems to be present in large excess in the mitochondria.

Ascorbate oxidase catalyzes the oxidation of ascorbic acid to L-dehydroascorbic acid. The enzyme occurs in cell walls and in the cytoplasm. There is a close positive correlation in the suboptimal concentration range between the copper content of leaf tissue and its ascorbate oxidase activity(Loneragan et al, 1982). Resupplying copper to deficient plants can restore the activity of ascorbate oxidase only in very young, but not mature leaves(Delhaize et al, 1985).

Polyamine oxidases are flavoproteins which catalyze the degradation of polyamines. Its activity decreases in copper-deficient plants and is confined to very young leaves. Diamine oxidase is located in the apoplasm of the epidermis and the xylem of mature tissues where it presumably functions as an H_2O_2 -delivery system for peroxidase activity in lignification and suberization of cell walls.

Phenol oxidases are enzymes that catalyze oxygenation reactions of plant phenols. Laccase is found in the thylakoid membranes of chloroplasts, where it is presumably required for the synthesis of plastoquinone, a constituent of the photosynthetic e- transport chain as well as another important e-transporter; plastocyanin. Phenolase has two distinct enzyme functions: hydroxylate monophenols to diphenols, resembling tyrosinase activity, and oxidize diphenols to o-quinones such as dihydroxyphenylalamine (DOPA), resembling polyphenol oxidase activity. Both reactions need molecular oxygen. Under conditions of deficiency, the decrease in phenolase is quite severe and is correlated with an accumulation of phenolics and a decrease in the formation of melanotic substances. The decline in phenolase activity may be at least indirectly responsible for the delay in flowering and maturation often observed in copper-deficient plants and shown for the flowering of some plant species. It is known that certain phenols are active inhibitors of IAA oxidase and that ascorbic acid also strongly inhibits peroxidase-catalyzed oxidation of IAA (Davies et al, 1978).

Ohko (Dragon) rock layout in a 5 gal tank with ADA soil

"Nitrogen accentuates copper deficiency and when the nitrogen supply is large, the application of copper fertilizers is required for maximum yield "

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"… Plants use the same transporter for Iron that they use for copper in most plant species ..."

Make sure to trim any plant well and do not be shy about it!

Main pathways affected by copper:

Copper deficient plants possess lower soluble carbohydrates than normal during the vegetative stage (Graham, 1980). Given the role of copper in photosynthesis (PS I), a lower content of soluble carbohydrates is to be expected during vegetative growth. It has been shown repeatedly that nitrogen application accentuates copper deficiency, and when the nitrogen supply is large, the application of copper fertilizers is required for maximum yield. Heavy fertilization with N tends to increase the severity of Cu deficiency (linked to NH4 assimilation and Nitrate reduction). Copper may have an important function in root metabolism. (Cu appears to be concentrated more in the rootlets of plants than in leaves or other tissues. Cu in roots may be 5 to 10 times greater than in leaves.). Impaired lignification of cell walls is the most typical anatomical change induced by copper deficiency in higher plants. This gives rise to the characteristic distortion of young leaves, bending and twisting of stems and twigs ("pendula" forms in trees) (Robson et al, 1981). Lignification responds rapidly to copper supply; transition periods of copper deficiency during the growth period can be readily identified by variations in the degree of lignification in stem sections. The inhibition of lignification in copper-deficient tissue is related to two copper-enzymes in lignin biosynthesis (polyphenol oxidase, diamine oxidase). The mechanisms of copper tolerance in higher plants (see Figure 9.13 below): exclusion or reduction of copper uptake, immobilization binding of copper in cell walls, compartmentalization of copper in insoluble complexes (e.g. binding to organic side chains), and compartmentalization of copper in soluble complexes in the vacuole and enzyme adaptations.

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Uptake in higher plants:

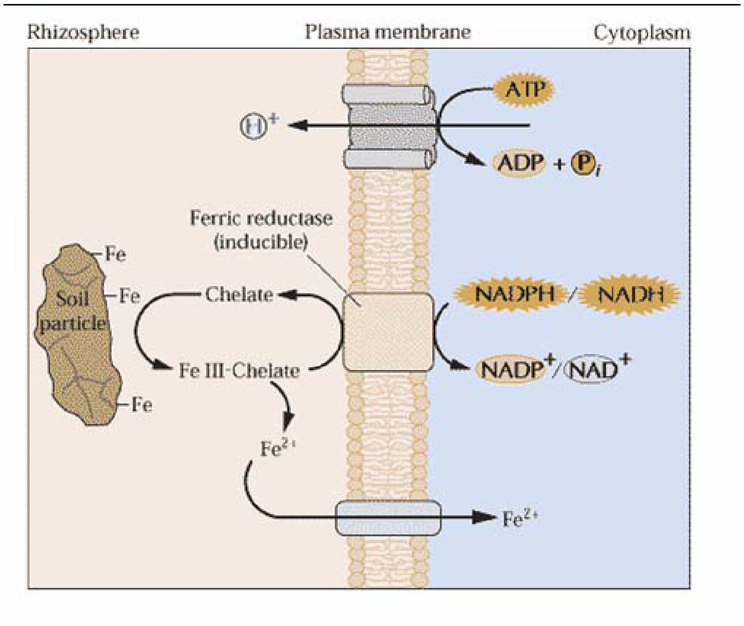


Figure 2

Note: Plants use the same transporter for iron and they do for copper in most plant species (Ferric reductase also has cupric activity). Copper transport in plants likely involves members of the P-type ATPase and copper transporter (CTR) families. You will note that the chelator is not taken into the plant internally. Further, the plant has its own chelator for Fe, but not for Zn2+ as it's not needed once inside the plant. After Cu-uptake into the cell, homeostasis is maintained by copper chaperones involved in intracellular transport (Company and González-Bosch, 2004). These chaperons sequester copper in a non-reactive form and interact with other transport proteins to deliver copper to the sites where it is needed (Himelblau and Amasino, 2000). Two genes have been identified in *Arabidopsis thaliana* (*copper chaperone* and *response to antagonist1* (RAN1)) with high homologies to copper-trafficking genes from yeast and humans involved in sequestering free copper ions in the cytoplasm and delivering it to post-Golgi vesicles (Himelblau and Amasino, 2000). Mutant analysis further showed that RAN1 was involved in ethylene reception because suppression of RAN1 blocked ethylene responses. It was suggested that this plant copper-delivery pathway is required to create functional ethylene receptors (Himelblau and Amasino, 2000). Further details of copper intracellular transport and homeostasis can be found in the review of Polle and Schützendübel (2003).

Figure 3a 3b and 3c.

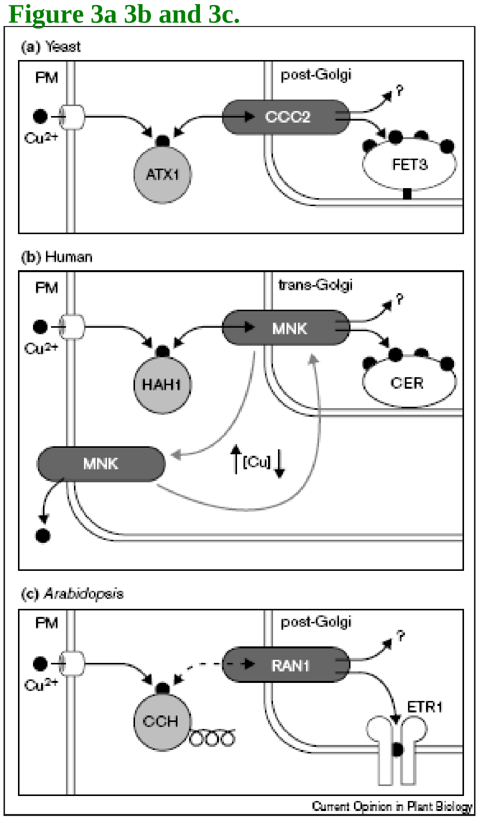
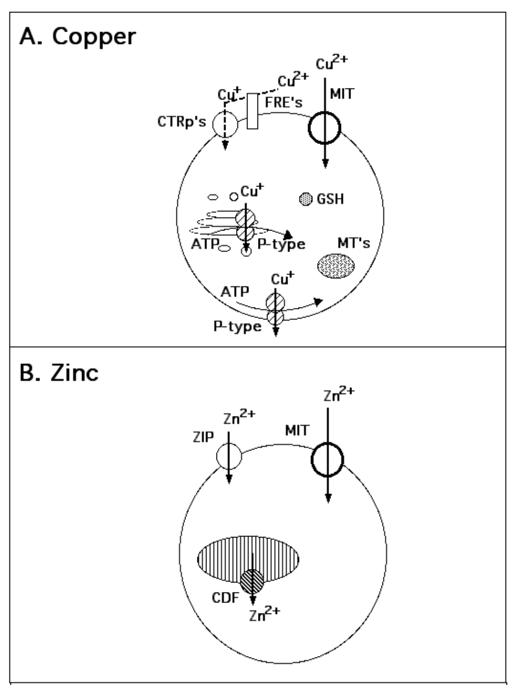


Figure 3a 3b and 3c. 3c shows how Cu enters and is bound to a chaperon and shuttled to the RAN1. You will note the striking similarity between the transport pathways used in plants, fungi and humans.

Figure 4a and 4 b.



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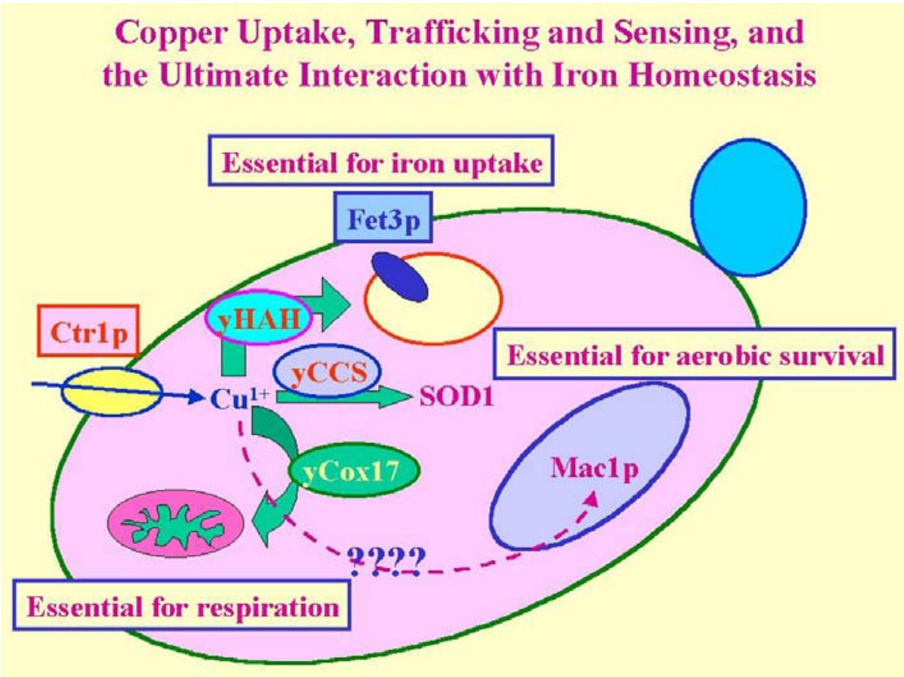

Figure 5

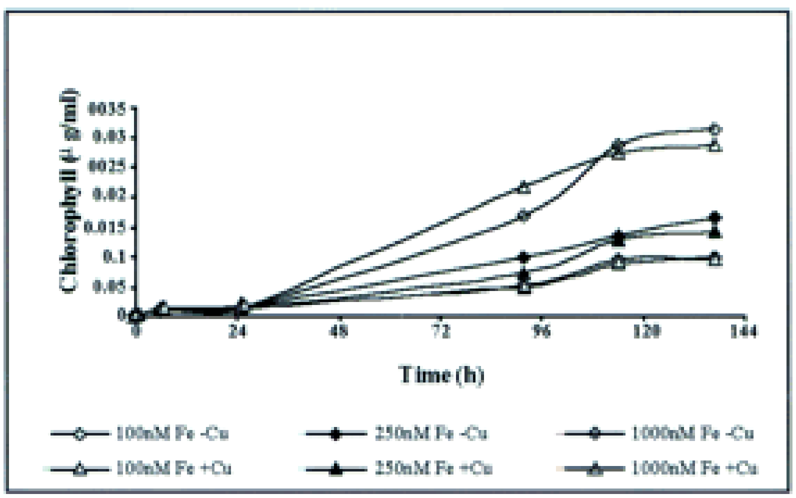

Figure 6 showing Cu + and – treatments in relation to Fe uptake and assimilation in the alga *C. reinhardtii* . While many add Fe alone, many do not add enough copper to allow for the full use of the Fe they add. Additionally, it's has been shown that Nitrogen fertilization can induce Copper deficiency in agricultural crops. Note no effect for 24 hours for the growth and Chlorophyll in Fig 5, this is the window of time typically before a bloom may form for green water and other algae. If a water change is done within this time frame, the blooms are typically avoided. (A) *C. reinhardtii* was grown to exponential phase in TAP medium and then subcultured into TAP medium containing the indicated iron supplement and either 6 µM copper (+Cu) or no copper (-Cu), and growth was monitored over a period of 6 days. (B) *C. reinhardtii* was cultured in TAP medium containing iron concentrations that ranged from 100 nM (-Fe) to 1 µM (+Fe) and either 0 µM (-Cu) or 6 µM copper (+Cu). The cell number of each culture was monitored over a period of 6 days, and chlorophyll content as a measure of iron sufficiency was monitored over the same time period by removing 10 µl of the culture into 1.0 ml of 80% acetone-20% methanol. All measurements were carried out in duplicate. Adapted from La Fontaine *et al* (2002).

For plants to increase uptake capacity, increase supply to root surface and to transport to shoot, they may:

Increase uptake capacity or affinity of active roots and transport to shoot up-regulation of transporters (example for NO3 uptake) transporters with different affinity (LATS(low affinity transporter) for NO3 pulses; iHATS when NO3 in low supply)

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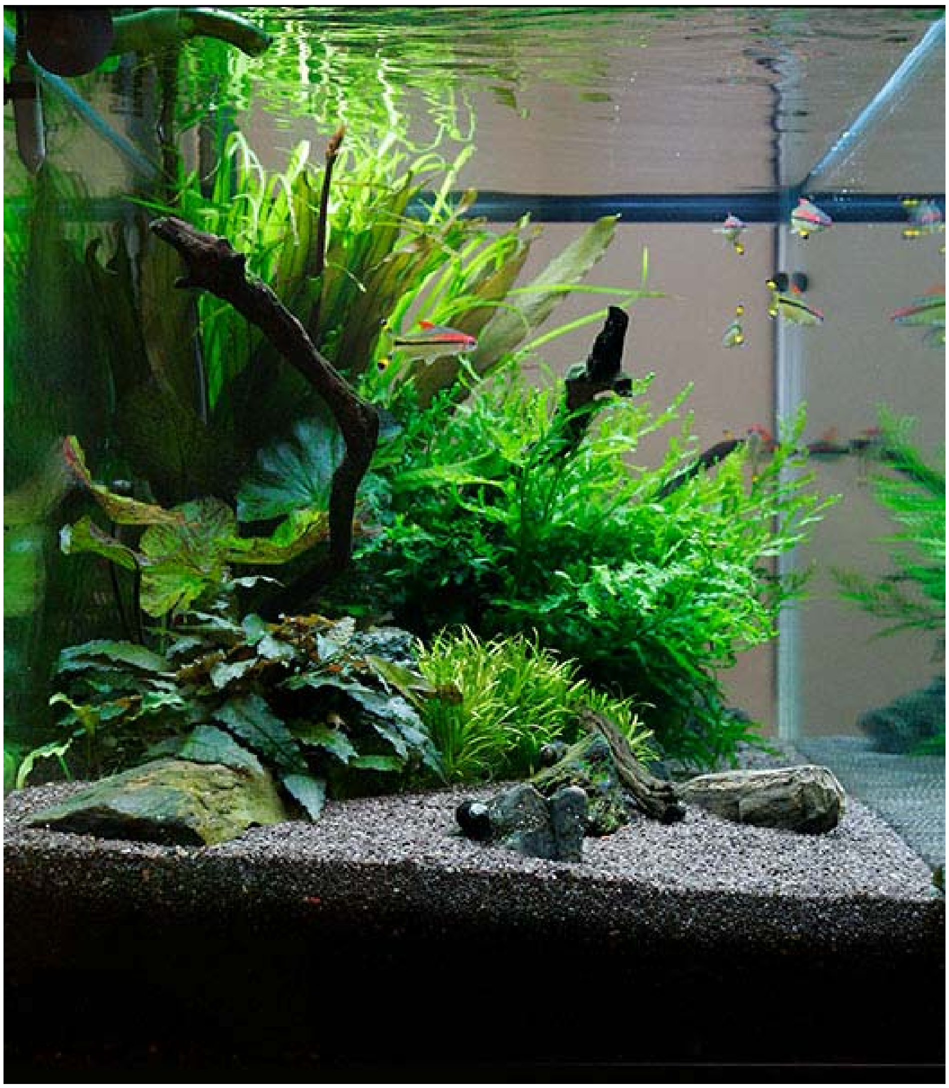
Increase fraction or surface area of fine, active roots increased branching, growth, morphological changes (e.g. cluster roots), root hair growth.

Localize root responses to soil heterogeneity/differences.(Morphological and physiological adjustments: adjust root growth patterns (e.g. more roots in surface soil)

Modify rhizosphere to increase availability or mobility of nutrients (or chelate toxic ions)
pH, organic acids, phytosiderophores (grasses only), enzymes, reductants, etc. Hydraulic lift and water availability

Modify microbial activity in the root/rhizosphere

Form symbioses (mycorrhizae, N-fixing bacteria). Exudate composition (amino acids, carbohydrates, signal molecules, etc.) Root border cells changes. (Physiology: changes in kinetics e.g. Vmax, km) (Morphology: changed root length, radius, density)



This is one of Luis Navarro's tanks. Note: easy to grow, minimal trimming, slower growing plant species, /See how the rocks and wood are interwoven towards the back and the lower light plants are used to blend the rock/wood together. Also, allowing some space, not planting every square inch of real estate!

Nutrient deficiency responses:
1. Decrease nutrient demand
2. Increase nutrient retention & recycling
3. Increase nutrient uptake capacity &/or supply to root surface

Factors Affecting Availability:

- **Root Growth:** Copper is the most immobile micronutrient, therefore anything that inhibits new root growth will inhibit Cu uptake.
- **Soil pH:** Acid soils increase Cu uptake and High pH inhibits uptake.
- **Organic Matter:** Copper is readily and tightly complexed by organic matter, therefore high soil organic matter levels reduce Cu availability.
- **Flooding:** Waterlogged soils can reduce Cu availability while they are saturated, however after they are drained the Cu will become available again.
- **Cu:Zn Balance:** High Zn levels will reduce Cu availability.
- **Cu:N Balance:** High N uptake in the presence of marginal Cu levels can lead to a reduction of Cu transport into the growing tips of plants.
- **Cu:P Balance:** High soil and plant P levels can reduce Cu uptake due to reduced soil exploration by mycorrhizas associated with plant roots.
- **N Stress:** Low N availability decreases the vigor of plants to an extent that it may fail to take up adequate amounts of many other nutrients. Copper uptake can be affected in this way.

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Mechanism for Tolerance:

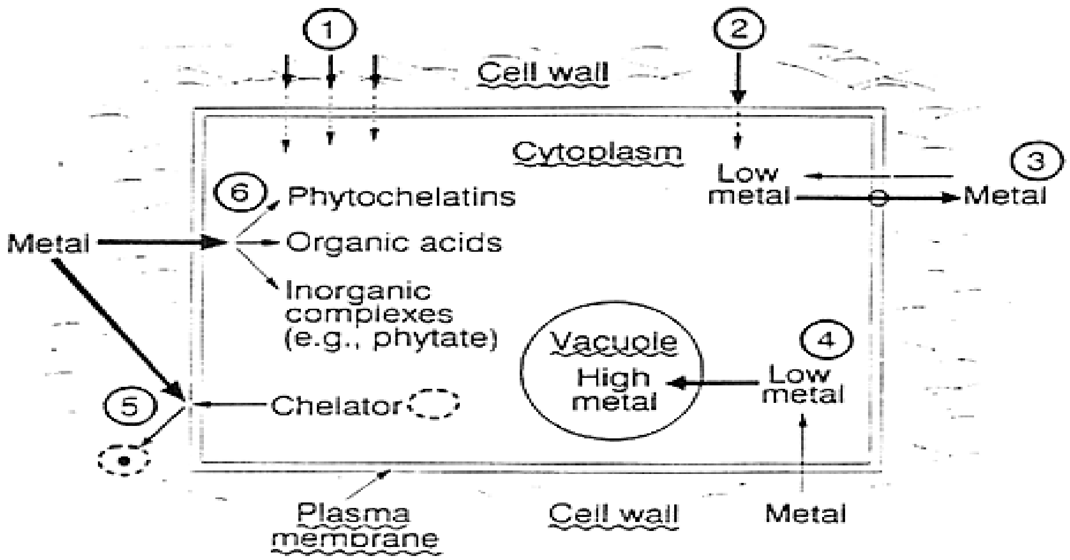

Fig. 9.13 Possible mechanisms of heavy metal tolerance of plants. (Modified from Tomsett and Thurman, 1988.) (1) Binding to cell wall. (2) Restricted influx through plasma membrane. (3) Active efflux. (4) Compartmentation in vacuole. (5) Chelation at the cell wall–plasma membrane interface. (6) Chelation in the cytoplasm.

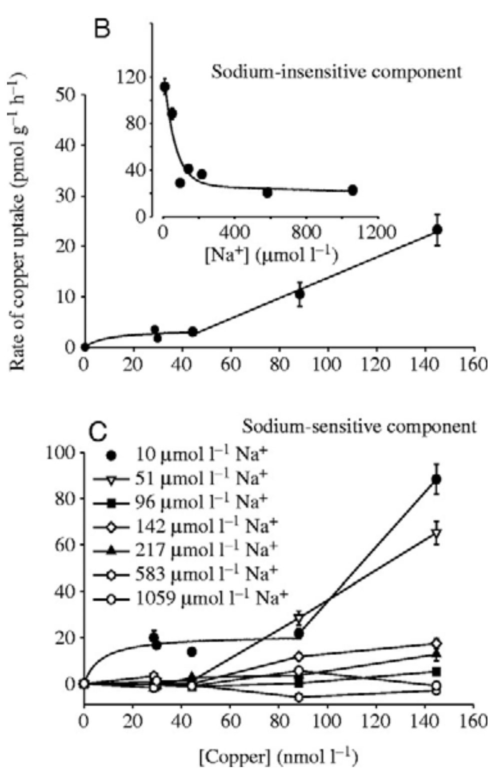

Figure 7

Copper uptake is decrease by salt stress (Fig 7): copper uptake declined rapidly as higher levels of Na+ are present.

How do marine plants address such uptake issues? The difference between plants and their pathways as well as algae's provide the basis for selectivity and usage for aquatic weed control. In general, most aquatic weed killers suggest a range of 0.7-1.0ppm Cu and for algae, 0.4ppm Cu is about the average.

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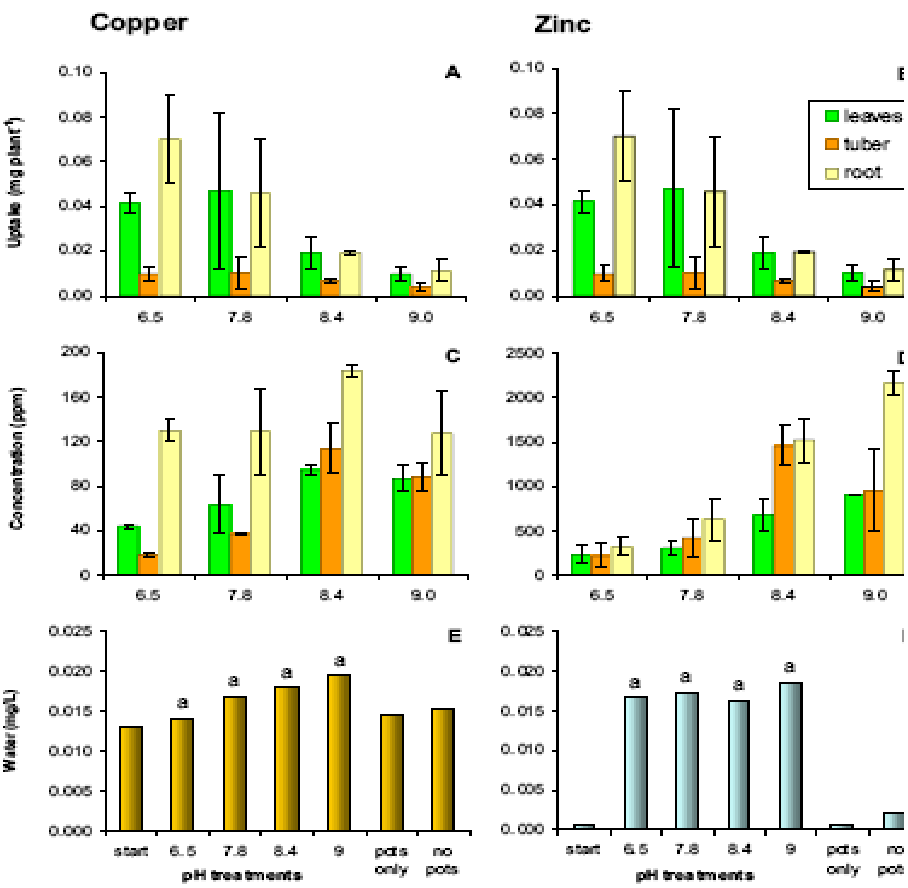


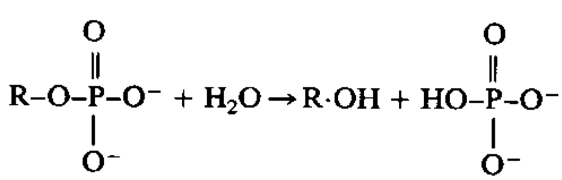
Fig A3.7 The effect of pH on, (**A**) copper and (**B**) zinc uptake (milligrams micronutrient per average dry weight) and (**C**) copper and (**D**) zinc concentration (ppm of micronutrient to dry weight) of foliage, tubers and roots of an average *A. elongatus* plant in run 1. Based on means ± SD (n = 2). Graphs (**E**) and (**F**) show the mean effect of pH on copper and zinc content in the water (mg per litre) of two runs. Treatments with a common letter are not significantly different at 5% level. A mean pH level of 8.4 ± 0.2 (± SD) is the ambient pH (control).

Figure 8a,8b, 8c, 8d, 8e, 8f
Aponogeton elongatus dry weight analysis from Crossely (2002)
Note that there is a very strong correlation between pH and Zn and Cu levels in each plant organ.
Also, you can see the effect of pH on water ppm levels of Cu and Zn.

Zinc:

Zinc is an essential catalytic component of over 300 enzymes. Understanding what inhibits the uptake and translocation of zinc in both soil and plants will also help us to supply this important micronutrient during time of peak demand. CuZnSOD as mentioned prior, alkaline phosphatase, alcohol dehydrogenase (ADH) and carbonic anhydrase (CA) being some of the main types. The role in SOD is important and previously discussed with copper's role in dealing with radical oxidative chemical species.

Phosphatases are general enzymes that catalyze the hydrolysis of esters and anhydrides of H_3PO_4. These enzymes are responsible for organic phosphorus mineralization and the release of inorganic phosphorus needed by both microorganisms and plants.



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Phosphatase enzymes are classified as acid and alkaline phosphatases because their maximum activities occur at low (pH 6.5) and high (pH 11) pH ranges.

ADH's:

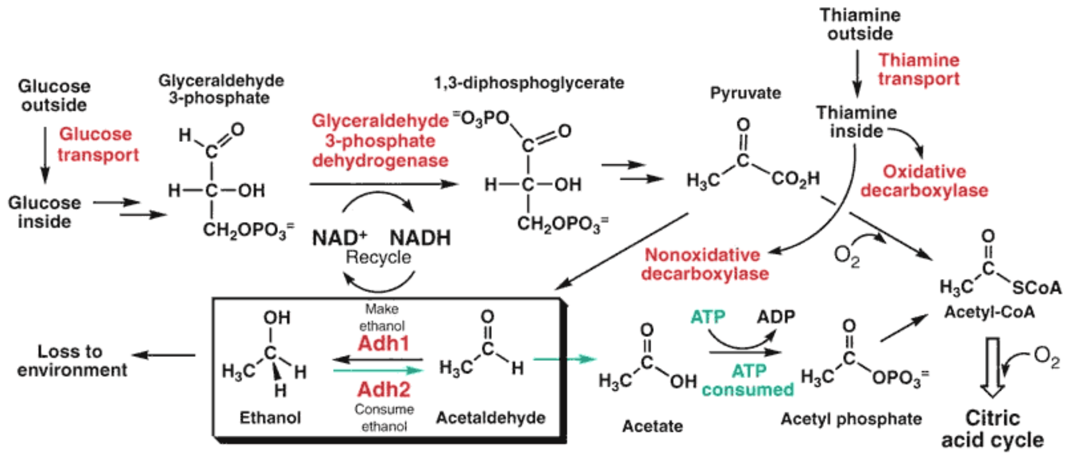

Figure 9

This is a typical plant pathway for catabolism for sugar(Glucose) and you can see the key role ADH has as aquatic plants often deal with anaerobic conditions in the plant parts unlike their terrestrial counter parts. This allow for efficient use of glucose and detoxification of the other by products ethanol and acetaldehyde.

Carbonic anhydrase's (CA) active site generally contains a zinc ion. In plants, CA helps raise the concentration of CO_2 within the chloroplast to increase the CO2 fixing rate of the enzyme Rubisco and as such is the reaction which integrates CO_2 into organic carbon in the form of sugars during photosynthesis and can only use the CO_2 form of carbon, not carbonic acid nor bicarbonate. This enzyme allows both plants and algae to use the HCO3 in the water as a carbon source.

Inhibition of ZIP –Dependant Uptake in Yeast by Other Metals. Why yeast? They are easy to take genes and add them from plants, this allows much more rapid growth to see how these genes respond to stress and measure their uptake rates. So by taking a gene out of a plant, transforming it into fungi, they are better able to study it. ZIP gene facilitate control over Zn uptake in plants/algae. There are several families of such transporters known in a variety of organisms such as *Arabidopsis* and *C. reinhardtii* which are used as models for research along with things like E coli (Bacteria), C elegans (Nemotoda).

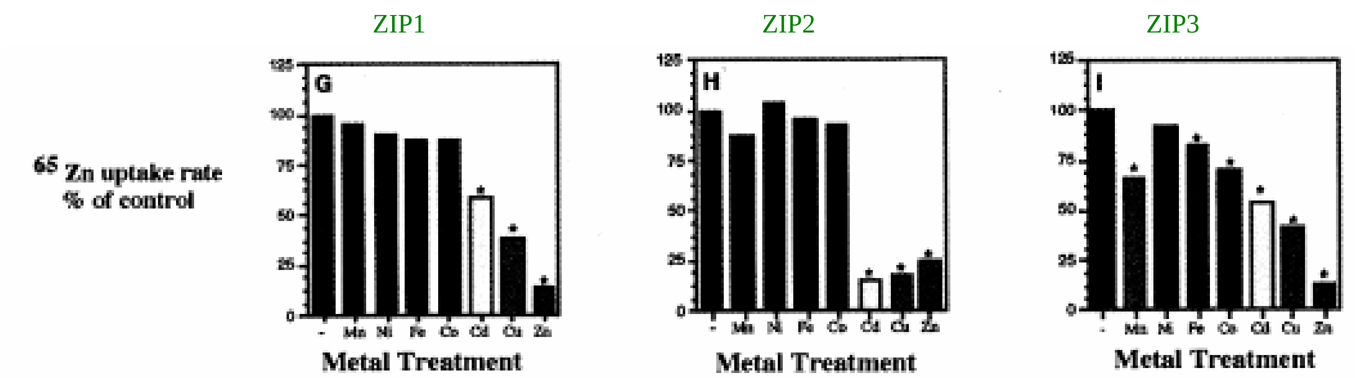

Figure 10a, 10b and 10c

Inhibition of Zn uptake by Mn, Ni, Fe , Co, Cd, Cu, Zn. Adapted from Grotz N. et al. 1998. Figures 10a-c show how other metals influence Zn uptake for individual ZIP genes. In general, most other trace metals do not impose much effect except Cd, Cu and Zn.

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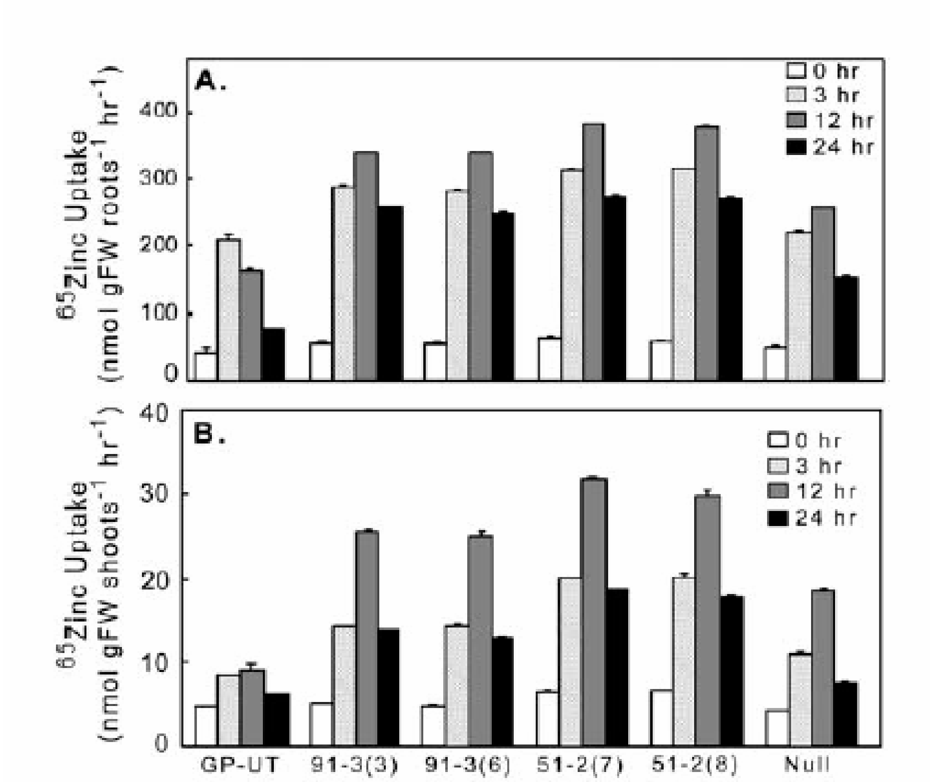

Figure 11.

Keskinkan et al (2003) adsorption kinetics of *Myriophyllum spicatum* (Eurasian water milfoil) for lead, zinc, and copper were investigated and the results were compared with other aquatic submerged plants (Figure 11). Metal biosorption was fast and equilibrium was attained within 20 min. The maximum adsorption capacities (q sub(max)) were 10.37 mg/g for Cu(II) and 15.59 mg/g for Zn(II). This suggest very rapid uptake of trace metals within both the roots and the leaves. Aquatic plants have the ability to take up either location, while the uptake rates in the root zone is higher by a factor of 10-20X, the total leaf area and the immobility of Cu can play a very large role in growth. Uptake in *Chara* was described by Reid et al 1995. . Uptake of ^{65}Zn in *Chara* was linear over several hours, with rapid transfer to the vacuole, but only slow efflux out. Influx occurred in a biphasic manner, which was speculated to possess two separate transport systems, a high-affinity system which is saturated at 0.1 mmol·m^{-3} and a low-affinity system which showed a linear dependence on concentration up to at least 50 mmol·m^{-3}. Zinc is essential for plant growth because it controls the synthesis of indoleacetic acid, which dramatically regulates plant growth. Zinc is also active in many enzymatic reactions and is necessary for chlorophyll synthesis and carbohydrate formation. Because zinc is not readily translocated within the plant, deficiency symptoms first appear on younger leave, Cardswell (2002) suggested that for aquatic macrophytes, Zn was the only readily clear pattern of increasing accumulation in the sediment with increasing concentrations in the macrophytes.

Species	Fe	Mn	Zn	Cu
Submersed plants				
Myriophyllum heterophyllum	2 000	473	54	44
Ceratophyllum demersum	1 053	486	100	30
Najas guadalupensis	712	201	48	48
Eleocharis acicularis	2 920	192	68	42
Utricularia inflata	2 112	480	108	47
Potamogeton diversifolius	1 240	160	60	36
Floating-leafed plants				
Nymphaea odorata	600	128	32	36
Nuphar advena	740	300	50	35
Nelumbo lutea	126	607	50	40
Brasenia schreberi	500	265	267	32
Emergent plants				
Typha latifolia	120	412	30	37
Hydrocotyle sp.	1 245	196	53	53
Panicum hemitonium	133	292	31	26
Eleocharis quadrangulata	560	120	45	20
Sagittaria latifolia	460	355	46	57
Pontederia cordata	200	970	67	60

This table illustrates the relative ratios between Copper, Iron and Manganese in 16 species of aquatic macrophytes.

Table 2

Copper and Zinc's Role in Aquatic Macrophyte Uptake and Growth

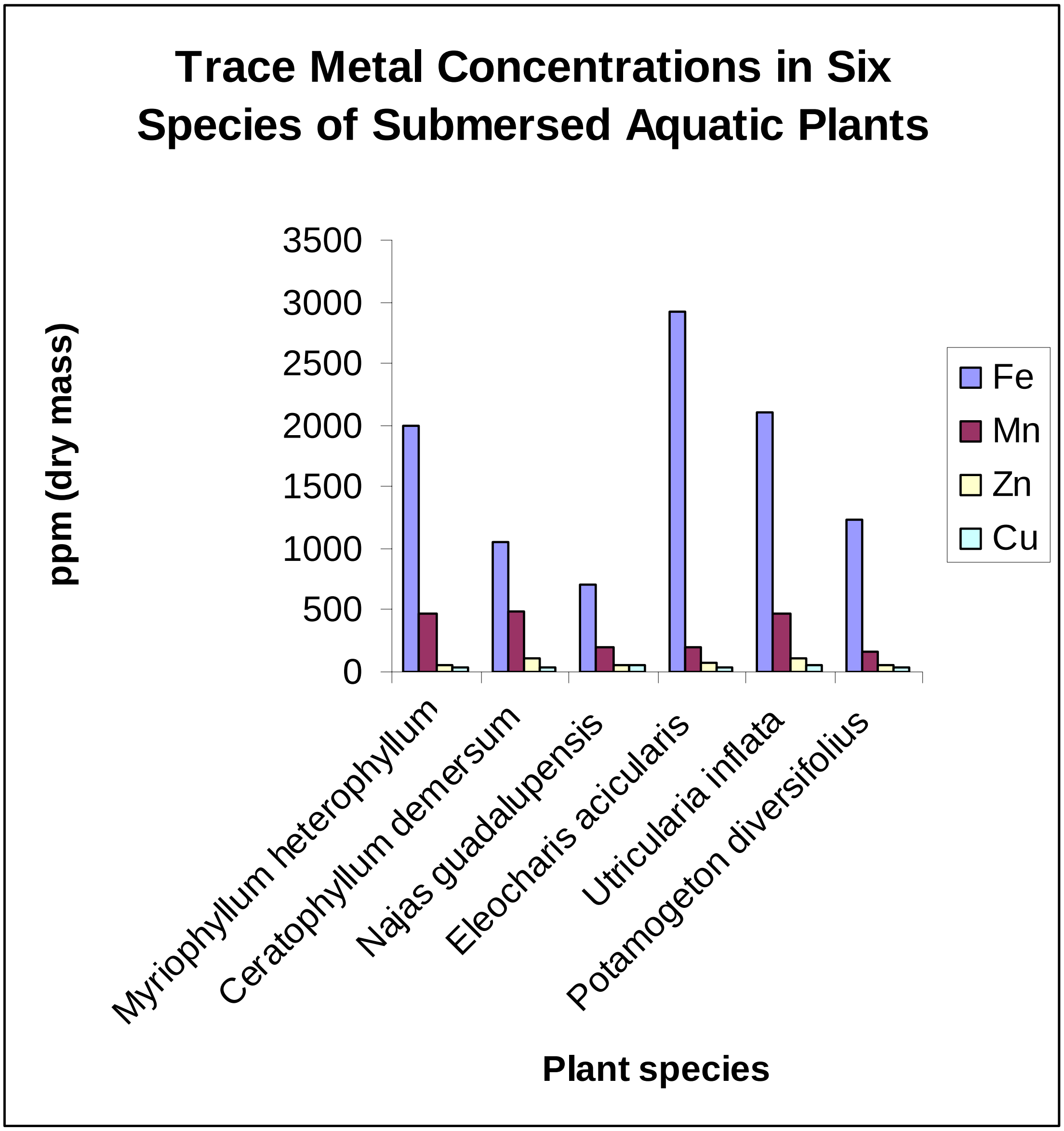

Figure 12
The variation between plant species appears to be mainly a function of Fe and to a lesser degree Mn. There is also some variation in the Cu and Zn as well but when resolved along with Fe and Mn, such trends are not present. Generally a 1:1 to 2:1 ratio range for Zn:Cu.

Table 3. *Juncus americana* Analysis (dry weight) and crop amounts (g/m^2) (Lake Ogletree) over a growing season(%DM= % dry mass):

Metal	mid-May		mid-June		mid-July		mid-August	
	% DM	g/m²	% DM	g/m²	% DM	g/m²	% DM	g/m²
	ppm		ppm		ppm		ppm	
Fe	904	1.0	1 085	2.4	710	1.8	1 644	3.8
Mn	125	0.14	112	0.25	51	0.13	62	0.14
Zn	278	0.3	265	0.58	156	0.38	114	0.26
Cu	27	0.03	26	0.06	33	0.08	29	0.06

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You can see that the Fe, Cu, Zn and Mn all vary seasonally, if the sampling was done in one month, does that give a representative idea of the nutrimental status of the plant or it's average ratio and concentration? When reviewing data and research, when and what are very important considerations. Often is does not apply to all seasons, plant species and applications.

Conclusion:

Aquatic plants have uptake transporters sites on both the leaves and the roots, and generally trace metal accumulation and enzymatic activity is found in the roots more than the leaves. Yet this is speculation to suggest they prefer one over the other locations, a point often overlooked when discussed in horticulture. Generally, the optimal growth rates achieved have nutrients in both locations, not one and the other. The other problem with assuming that the plant prefer one location, these are natural studies and such trace metals are very rarely found and often are limiting in the water column, whereas in an aquarium, the user can add Cu and Zn to any location and with any frequency. Plants have several methods to address toxicity of trace metals such as Zn and Cu and limitations through gene regulation of various enzymes used to assimilate and uptake nutrients. While these are only trace elements, they are still essential and can cause issues in Nitrogen uptake and assimilation as well as CO2 uptake and ATP/NADPH production. General ratios of Zn:Cu in aquatic macrophytes tend to be 2:1 to 1:1 in natural systems. Plant organs such as roots showed high concentrations at lower pHs and relatively similar concentrations at more neutral ranges for Zn:Cu in *Aponogeton elongatus*.

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