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Beneficial Effects of Humic Acid on Micronutrient Availability to Wheat

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ABSTRACT

Humic acid (HA) is a relatively stable product of organic matter decomposition and thus accumulates in environmental systems. Humic acid might benefit plant growth by chelating unavailable nutrients and buffering pH. We examined the effect of HA on growth and micronutrient uptake in wheat (*Triticum aestivum* L.) grown hydroponically. Four root-zone treatments were compared: (i) 25 μ*M* synthetic chelate *N*-(4-hydroxyethyl)ethylenediaminetriacetic acid ($C_{10}H_{18}N_2O_7$) (HEDTA at 0.25 m*M* C); (ii) 25 μ*M* synthetic chelate with 4-morpholineethanesulfonic acid ($C_6H_{13}N_4S$) (MES at 5 m*M* C) pH buffer; (iii) HA at 1 m*M* C without synthetic chelate or buffer; and (iv) no synthetic chelate or buffer. Ample inorganic Fe (35 μ*M* Fe^{3+}) was supplied in all treatments. There was no statistically significant difference in total biomass or seed yield among treatments, but HA was effective at ameliorating the leaf interveinal chlorosis that occurred during early growth of the nonchelated treatment. Leaf tissue Cu and Zn concentrations were lower in the HEDTA treatment relative to no chelate (NC), indicating HEDTA strongly complexed these nutrients, thus reducing their free ion activities and hence, bioavailability. Humic acid did not complex Zn as strongly and chemical equilibrium modeling supported these results. Titration tests indicated that HA was not an effective pH buffer at 1 m*M* C, and higher levels resulted in HA-Ca and HA-Mg flocculation in the nutrient solution.

Humic substances are the result of organic decomposition (Stott and Martin, 1990). Humic substances are readily found in soils, aquatic systems, and in biologically based human life support systems, such as those being evaluated by NASA's Advanced Life Support (ALS) program. These systems will use higher plants for a portion of food production, oxygen revitalization, and water treatment. Humic substances exist in recycled nutrient streams used for crop production and so their effects on plant growth and nutrient chemistry need to be evaluated for ALS. For example, HA from wheat straw leachate can inhibit the formation of insoluble Ca phosphates and thus may enhance P bioavailability (Grossl and Inskeep, 1991).

A benefit of HA in agricultural systems is its ability to complex metal ions (Stevenson, 1982). Humic acid can form aqueous complexes with micronutrients, though not to the same extent as many synthetic chelating agents (Aiken et al., 1985). Since HA binds to soil colloidal surfaces, it is not easily leached (Jardine et al., 1989; Spark et al., 1997a) and soil HA promotes heavy metal (i.e., Cu and Zn) sorption to soil minerals, such as goethite and silica (Spark et al., 1997b). Synthetic chelate availability can decrease by 50% through soil sorption processes (Norvell, 1991), making field application costly. In contrast, HA can be inexpensively incorporated into soils via biowastes (such as manures) and the organic matter has the added benefit of improving soil physical properties.

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Abbreviations: ALS, advanced life support; HA, humic acid; DAT, days after transplanting; FA, fulvic acid; HEDTA, *N*-(2-hydroxyethyl) ethylenediaminetriacetic acid; ICP, inductive coupled plasma; MES, 4-morpholineethanesulfonic acid; NC, no chelate; PS, phytosiderophores; TOC, total organic C; WUE, water use efficiency.

Table 1. Chemical equilibrium constants (log K^0) used by the GEOCHEM speciation model for the HEDTA and humic acid (HA) treatments.

Metal	HEDTA†	HA‡	HA reference
Ca^{2+}	9.5	5.4	Takahashi et al. (1997)
Mg^{2+}	8.3	5.3	Estimate based on data from Van Dijk (1971)
Fe^{3+}	21.8	13.0	Takahashi et al. (1997)
Zn^{2+}	15.9	5.2	Takahashi et al. (1997)
Mn^{2+}	12.7	5.4	Takahashi et al. (1997)
Cu^{2+}	18.7	11.0	Hering and Morel (1988)

† HEDTA log K^0 values were obtained from GEOCHEM-PC, version 2.0 (Parker et al., 1995).
‡ Humic acid (HA) log K^0 values were obtained from references in column four (HA reference).

Although numerous tests have been conducted with humic substances added to complete nutrient solutions or solutions with varying metal concentrations, information is limited on the effect of replacing synthetic chelates entirely with HA. As early as 1951, synthetic chelates were being tested in plant systems as a means for chelating Fe (Jacobson, 1951). Today, chelates are a standard part of the nutrient solution recipe in hydroponic systems, but there are some inherent problems regarding their use. Unlike soil systems where synthetic chelates tend to enhance micronutrient availability by increasing solubility, excess chelate in hydroponic solutions can induce micronutrient deficiencies by lowering metal activities (Norvell, 1991). Soluble organics, such as HA have lower stability constants (log K^0) than synthetic chelates for metals, thus providing greater metal activity in solution (Table 1). In addition, plant species differ in their ability to extract some elements from synthetic chelates. Most grass species can adequately reduce Fe-HEDTA to acquire Fe but they have more difficulty with the highly stable Fe-EDDHA chelate (Marschner and Römheld, 1994). Since HA has a lower log K^0 than even HEDTA, HA may be particularly suitable for grass species.

Hydroponic solutions have low pH buffering capacities consequently organic buffers, e.g., MES are sometimes used (Bugbee and Salisbury, 1985). Humic acid might provide pH buffering because of a large number of weakly acidic functional groups (carboxylic acid and phenolic) that make up the molecule. In fact, carboxylic acid groups comprise ~90% of all dissolved organic C found in the environment (Thurman, 1986). Based on typical acidity values of ~5 mol kg^{-1} (Aiken et al., 1985), HA theoretically could buffer pH as well as more commonly used compounds, such as MES.

The objective of this study was to compare the effects of NC, HA, HEDTA, or HEDTA + MES, on micronutrient bioavailability and solution pH buffering using hydroponically grown wheat.

MATERIALS AND METHODS

Plant Materials and Cultural Conditions

Wheat, 'USU-Apogee', was germinated in a 40-mm layer of moist inert medium (Isolite, Sumitomo Corp., Denver, CO). Four days after emergence, the seedlings were transplanted (0 d after transplanting [DAT]) to polyethylene tubs (10 plants per tub) containing 50 L of nutrient starter solution (Tables 2 and 3). The nutrient solution level was checked daily with a portable glass manometer bearing a mark showing the liquid level setting. Each tub received ~76 L of refill solution (Tables 2 and 3) during growth to replace water and nutrients loss from evapotranspiration. The nutrient solutions in each tub were vigorously mixed and aerated using polyvinyl chloride manifolds fed from an inhouse air supply. Nutrient solution pH (5.3 ± 0.3), electrical conductivity (0.75 ± 0.1 dS m^{-1}), and air temperature (21 ± 3°C), were manually measured within the greenhouse unit each day.

Table 2. Organic components of treatment solutions. The starter solution was used to fill the system and the refill solution was used to replace transpired water. Inorganic components of these treatments are listed in Table 3.

Treatment, symbol	Starter solution	Refill solution
No chelate, NC	–	–
HEDTA + $FeCl_3$, HEDTA	25.0 μ*M* Fe-HEDTA	5.0 μ*M* Fe-HEDTA
MES + HEDTA + $FeCl_3$, MES	5.0 m*M* MES (as C) 25 μ*M* Fe-HEDTA	0.0 m*M* MES (as C) 5.0 μ*M* Fe-HEDTA
Humic acid + $FeCl_3$, HA	1.0 m*M* HA (as C)	0.2 m*M* HA (as C)

The nutrient solution compositions were similar to that used by Grotenhuis and Bugbee (1997); however, 15% of the total N in the refill solution was NH_4-N to minimize pH shifts. All treatments contained starter solution but varied by the type of chelate and buffer used. The four treatments were: (i) 25 μ*M* synthetic chelate HEDTA (Fe-HEDTA at 0.25 m*M* C) equals the HEDTA treatment; (ii) 25 μ*M* Fe-HEDTA with MES (5 m*M* C) pH buffer equals the MES treatment; (ii) HA at 1 m*M* C without HEDTA or MES equals the HA treatment; and (iv) no HEDTA or MES equals the NC treatment.

Each treatment had its own refill nutrient stock, where Fe was replenished at 7.5 μ*M* Fe, with the source of the Fe ($FeCl_3$ vs. Fe-HEDTA) dictated by treatment (Table 2). In addition, the HEDTA and MES treatments were resupplied, HEDTA at 5 μ*M* Fe-HEDTA, and HA at 250 μ*M* HA (20% of starter concentrations). The MES was added to the starter solution but it was not part of the refill solution since it was previously determined that it had a minimal degradation rate in nutrient solutions (Bugbee and Salisbury, 1985). The HA (Aldrich Chemical Co., Milwaukee, WI) used in the study was dissolved with KOH and desalted by placing it in 15 000 molecular

Table 3. Inorganic components of treatment solutions. The starter solution was used to fill the system and refill solution was used to replace transpired water. Organic components of these treatments are listed in Table 2.

Salt	Starter solution	Refill solution
	m*M*	
KNO_3	1.0	4.0
NH_4Cl	0.0	1.0
$Ca(NO_3)_2$	1.0	1.0
KH_2PO_4	0.5	0.5
$MgSO_4$	0.5	0.5
K_2SiO_3	0.1	0.1
	μ*M*	
Fe^+	35.0	7.5
$ZnSO_4$	4.0	2.0
$MnCl_2$	3.0	6.0
H_3BO_3	2.0	1.0
$CuCl_2$	1.0	1.0
Na_2MoO_4	9.0×10^{-2}	3.0×10^{-2}

† Total Fe starter solution and refill solution concentrations were equivalent among treatments. However, over 70% of Fe in the HEDTA and MES treatments was supplied as Fe-HEDTA, rather than as $FeCl_3$. The total Fe in each system was theoretically ample to supply plant Fe requirements.

weight cut-off dialysis tubing (Spectrum Laboratories, Rancho Dominguez, CA) and soaking it in deionized water until the solution electrical conductivity was <0.1 dS m^{-1} (~2 d). The nutrient solution pH was maintained at 5.3 ± 0.3 by the addition of HNO_3, as needed. A somewhat acidic pH is routinely used in hydroponics and was used in this study because it improved nutrient availability and it may more accurately reflect pH conditions near the root surface. Even with NO_3^- dominated soils, rhizosphere pH may drop below bulk soil pH (Marschner, 1995). Differences between bulk soil and root surface pH can be as much as two pH units (Marschner and Romheld, 1996), but rhizosphere boundary layers are so small in hydroponics that essentially the rhizosphere and bulk solution pH are similar.

Sampling and Analyses

The chemical equilibrium model, GEOCHEM-PC (Parker et al., 1995) was used to predict metal ion activities among nutrient solution treatments. However, GEOCHEM-PC does not contain data for HA, so equilibrium constants were obtained from the literature (Van Dijk, 1971; Takahashi et al., 1997; Hering and Morel, 1988) and entered into the model (Table 1).

Nutrient solution samples (20 mL) were taken weekly and analyzed for total organic C (TOC) using a Phoenix 8000 TOC analyzer (Tekmar-Dohrmann, Cincinnati, OH). The TOC analyzer used persulfate and UV radiation to convert organic C to CO_2, which was measured via the infrared detector. The observed TOC values (Fig. 1a) were those acquired from measuring the solutions directly. The predicted TOC values were the concentrations that should have been in the nutrient solutions, based on what was contributed from either HEDTA or HA in starter and refill solutions. The predicted values were not adjusted for TOC that may have been contributed from plant root exudates or TOC removed via plant uptake or microbial degradation. To test nutrient solution pH buffering capacity, solution samples were taken at 32 DAT and adjusted to pH 7.0 with 0.1 *M* NaOH and then titrated to pH 4.0 with 0.1 *M* HCl. The same procedure was performed with the individual organic components, i.e., HEDTA, HA, and MES, dissolved in distilled water at 1 m*M* C.

A single plant per tub was harvested each week (7 DAT through 56 DAT), and the remaining plants (2 per tub) were left until the final harvest (73 DAT). During each harvest, tissue was separated into leaves, stems, roots, heads, and seeds, as applicable. All tissue was oven dried (80°C for 3 d) prior to weighing. Leaf tissue was ground (2-mm sieve) from the 28, 35, 42, 56, and 73 DAT harvests, digested with HNO_3 and H_2O_2, and inorganic elemental content determined by ICP emission spectrometry. Leaf tissue from all but the MES treatment was analyzed at each sample date. The MES solution composition was comparable with the HEDTA solution (Tables 2 and 3) and since MES is unreactive with metals (Yu, et al., 1997), it should not have affected micronutrient uptake. The GEOCHEM-PC model supported this assumption, calculating that ~95% of MES would form a complex with H^+ and <0.1% would form complexes with metals.

Statistical Analysis

A completely randomized design was used. Three tubs (replicates) represented each treatment. Statistical analysis of data was performed using analysis of variance (ANOVA) and least significant differences (LSD) (Minitab 9.1, Minitab Inc., State College, PA). Water uptake and acid use data had repeated measurements, in which water uptake and acid use were the repeated measures. Absolute growth curves were created using total dry mass data. The data was fitted to logistic growth curves using the NLINMIX macro in the SAS computer package (SAS, Cary, NC).

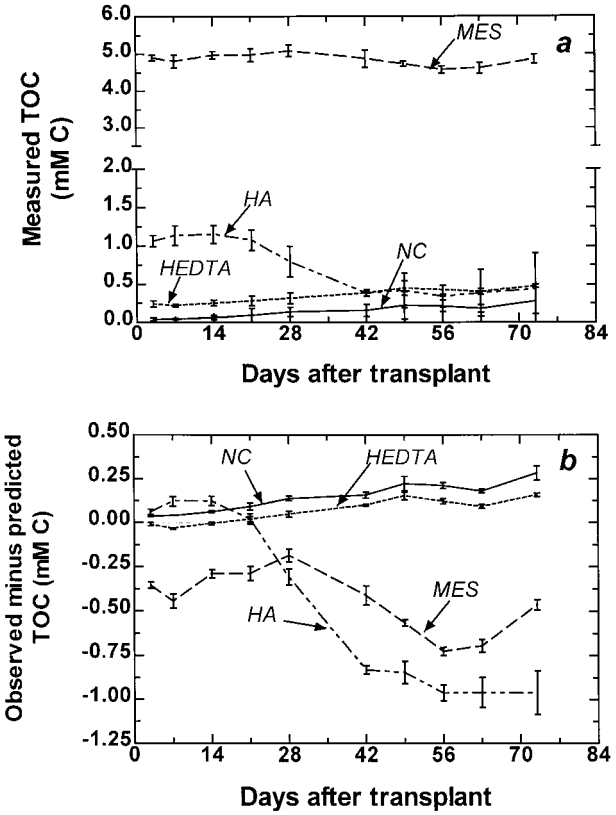

Fig. 1. Nutrient solution total organic C (TOC) levels over time. (a) TOC measured (observed) in solution, and (b) difference in TOC concentration (observed − predicted), where predicted values represent C from no chelate (NC), HEDTA, MES-buffer, and humic acid (HA) treatments if no uptake or degradation of these substances occurred. Vertical bars equal 95% confidence intervals.

RESULTS AND DISCUSSION

Nutrient Solution Total Organic Carbon

The TOC analysis was used to indirectly monitor MES, HEDTA, and HA levels in solution at 7-d intervals (Fig. 1a). The MES concentration remained relatively stable throughout the study (Fig. 1a), which concurs with the findings of Bugbee and Salisbury (1985). Figure 1b represents the difference between C measured in solution and C predicted to be in solution (observed minus predicted). Values greater than zero represent unaccounted C gain in solution and values less than zero represent unaccounted C loss from the system. Carbon gain may have been from root exudation and C loss may have been attributed to plant uptake, mineralization and subsequent loss as $CO_{2(g)}$, or precipitation and subsequent settling as C solids.

Solution TOC levels in the MES treatment were about 0.4 m*M* lower than what was predicted (Fig. 1b) but this was still within 10% of the set point (Fig. 1a). Total C from the MES treatment varied <10% over time (Fig. 1). The HA solution TOC values remained

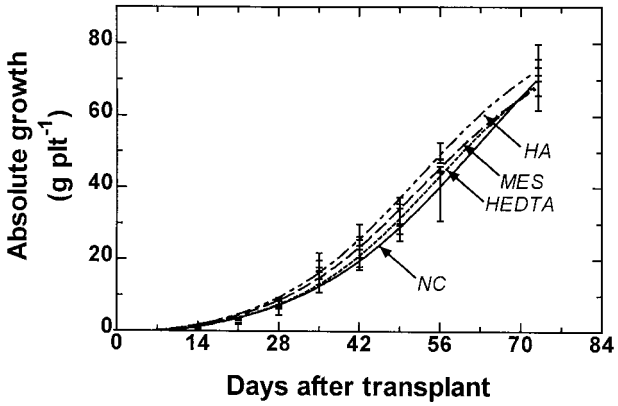

Fig. 2. Plant growth over time for no chelate (NC), HEDTA, MES-buffer, and humic acid (HA) treatments. Absolute growth (g plant^{-1}) was fitted to logistic growth curves. The coefficient of determination, r^2, for the fitted model was 0.98, indicating a very good fit of the model to the data. The *P* value for the *F*-test comparing the four treatment groups was 0.0335. Post hoc pairwise comparisons of the treatment groups showed NC and HA were the most dissimilar ($P = 0.0916$).

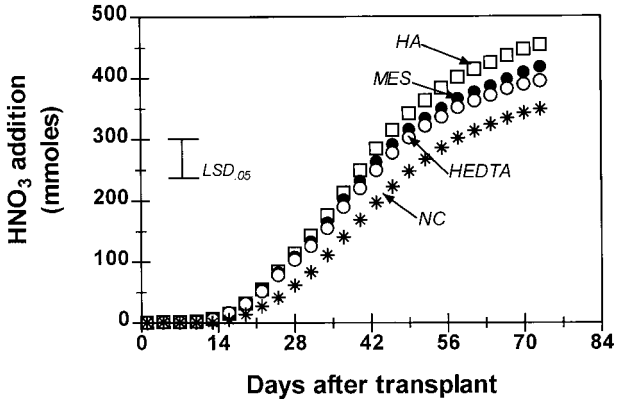

Fig. 3. Nutrient solution acid (HNO_3) requirement for pH control over time, an indication of NO_3^- uptake rates for no chelate (NC), HEDTA, MES-buffer and humic acid (HA) treatments. Least significant difference ($LSD_{0.05}$) was used to compare cumulative acid additions at a fixed date.

steady (~1 m*M* C) until 28 DAT, after which the TOC dropped to levels similar to those in the HEDTA treatment (Fig. 1a). This resulted in a 30% drop from the predicted values (Fig. 1b). A dark brown precipitate formed at the bottom of the HA-treated tubs. After the study was terminated some precipitate was collected, solubilized with 0.1 *M* NaOH and analyzed by ICP. Analysis showed high levels of K, Ca, Mg, Fe, and P associated with the precipitate. The GEOCHEM-PC model predicted 56% of the HA in the starter solution would form a complex with Ca and 23% would form a complex with Mg. The calculations also predicted formation of a $FePO_4$ solid but no interaction between K^+ and HA was noted. We surmise that an accumulation of solution Ca, Mg, and HA in solution over time resulted in flocculation and subsequent precipitation of HA from the nutrient solution. We hypothesize that the HA-Ca flocculent acted as an exchange complex for K^+ sorption.

The TOC in HEDTA and NC treatments increased over time (Fig. 1a). This may have been partly because of root turnover and exudation that were otherwise masked in the high C treatments. The HEDTA treatment also gained C from the HEDTA replenishments which was accounted for. The TOC observed–TOC predicted lines for HEDTA and NC treatments should have been comparable if there was no HEDTA degradation. Linear regressions were performed on these lines ($r^2 = 0.80$) including slope and intercept comparisons. The lines had equivalent slopes but significantly different intercepts. This suggests that C accumulation from root turnover and exudation was similar (equal slope) between the two treatments and HEDTA degradation was trivial.

Biomass

There were no total dry mass or yield differences among treatments at 73 DAT. Although a final harvest measurement provides an integrated measure of the effect of stress on productivity, growth curves can reflect treatment differences over time. Absolute growth was compared among treatments and fitted to logistic growth curves, with $r^2 > 0.98$ for all treatments (Fig. 2). An ANOVA test found significant treatment differences ($P = 0.034$), where the NC treatment was most dissimilar to the HA treatment ($P = 0.092$). It is suspected that the NC plants produced less biomass during early growth because of Fe deficiency stress (demand greater than supply) but they later recovered, as shown by the high biomass values towards the end of the study (Fig. 2). Increased root biomass may improve phytosiderophore (PS) production and subsequent Fe availability near the roots. Biomass recovery may have been because of later tiller development rather than additional growth in the main tiller. The planting density in this study was low (~30 plants m^{-2}), so there was ample space for tiller development. If denser plantings were used to minimize tillering, biomass values from the NC treatment may not have recovered. Further testing of plant density interactions with tillering and Fe bioavailability would be useful.

Water and pH Control Requirements

There were no differences in cumulative water uptake or water use efficiency (WUE), where WUE averaged 3.6 g dry mass per kg H_2O. Acid-base efficiency averaged 0.67 g dry mass per mmol H^+. Acid-base efficiency is a function of the acid or base added to solution to counteract the amount of either acid or base exuded by roots to maintain charge balance within the plant. Plant acid-base efficiency was not affected by MES in this study. Although MES slows pH changes in solution, the same total amount of acid or base will be required for pH control for long duration plant studies. The cumulative acid use requirement was lower for the NC treatment than the HA treatment after 66 DAT (Fig. 3). Unlike Strategy I plants (mostly dicots) that release pH lowering organic acids to reduce Fe^{3+} to Fe^{2+}, Strategy II plants (mostly grasses) release PS which have little effect on pH (Römheld, 1987). We observed that under

the period of highest Fe stress (early growth), the pH decreased in the NC treatment, suggesting that the plants either released organic acids or the cation/anion uptake ratio had increased. Although we measured TOC increases in the NC solution, it could not be determined how much, if any was because of organic acids. Additionally, electrical conductivity measurements were taken to estimate nutrient solution status, but changes in cation/anion ratios within the solution were unknown. To better understand this period of pH decline, more frequent and extensive tissue and nutrient solution sampling may be required during early plant growth.

Nutrient Uptake

A review by Guerinot and Yi (1994) suggested that plants require at least 10^{-9} *M* soluble Fe for optimal growth. Solution Fe activity in all treatments was controlled by solution equilibrium with solid phase $FePO_4$, which was about 10^{-14} *M* for all treatments. An additional $10^{-4.7}$ *M* Fe was complexed with HEDTA and 10^{-6} *M* Fe was complexed with HA in these treatments, but Fe^{3+} solution activity in the NC treatment was only 10^{-14} *M* Fe. The NC treatment had particularly low leaf Fe (55 mg kg^{-1}) at 28 DAT, which is approaching deficiency (50 mg kg^{-1}) (Marschner, 1995), and additional sampling earlier in the study might have shown significantly lower Fe values in the NC treatment. However, there was no significant difference in leaf Fe concentrations among treatments after 28 DAT, which averaged 92 mg kg^{-1}.

Graminaceous species release Fe complexing PS under nutrient stress conditions (Römheld, 1991). Even under Fe sufficient conditions (0.1 m*M* Fe-EDTA), Röhmheld and Marschner (1990) found that the application of PS to solution increased Fe uptake several fold. Young plants from the NC treatment had interveinal leaf chlorosis, suggesting Fe deficiency as a result of insufficient synthesis and release of PS. As PS release increased, Fe uptake probably increased, allowing recovery to a maximal growth rate (Fig. 2). The stability constant for mugeneic acid, a PS, is similar to HEDTA (Nomoto et al., 1987), so it is feasible that PS production in the NC treatment may have resulted in plant recovery.

Geochemical modeling indicated that the Zn, Cu, and Mn solution activities were not regulated by the solubility of solid phases. The Mn^{2+} activities and free concentrations were similar among treatments, with free concentrations averaging 10^{-5} *M*. Thus, there was no significant difference in leaf Mn levels among treatments (mean = 300 mg kg^{-1}). In the case of Cu and Zn, leaf concentrations tended to decrease with age as the nutrients were remobilized during grain fill (Fig. 4) (Marschner, 1995). In addition, leaf Cu and Zn concentrations were different among treatments, where the NC treatment resulted in the highest leaf concentrations over time (Fig. 4). Perhaps PS production in the NC treatment enhanced Cu and Zn bioavailability. For example, Treeby et al., (1989) found that barley (*Hordeum vulgare* L.) plants grown with added PS resulted in PS

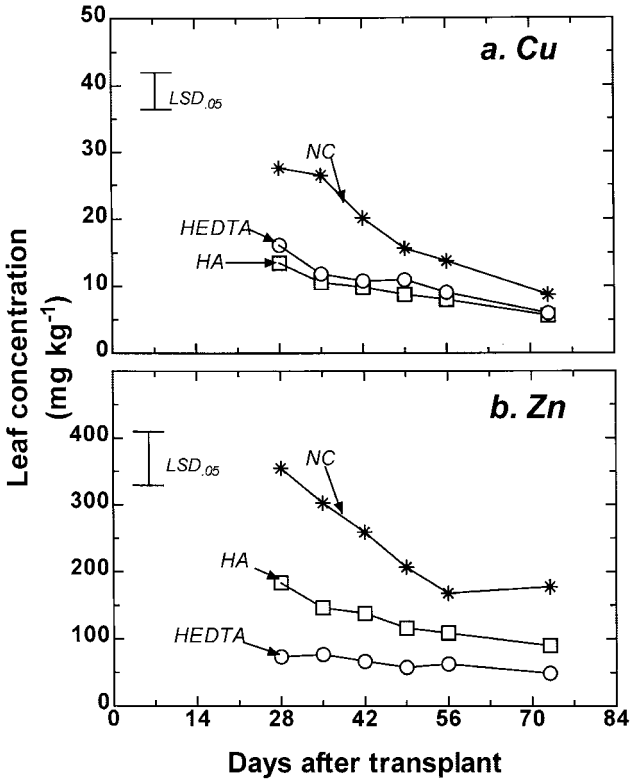

Fig. 4. Leaf tissue (a) Cu and (b) Zn concentrations over time for no chelate (NC), HEDTA, and humic acid (HA) treatments. Least significant difference ($LSD_{0.05}$) was used to compare leaf Cu and Zn concentrations at a fixed date. Leaf tissue from the MES-buffer treatment was not analyzed.

forming complexes with Cu and Zn, which led to higher leaf concentrations. In contrast, HEDTA forms strong Cu and Zn complexes, which would render them less bioavailable. Although the NC treatment had the highest leaf Cu levels, there were no differences in leaf Cu levels between the HA and HEDTA treatments (Fig. 4a). The calculated free Cu^{2+} concentration was $10^{-6.04}$ *M* for the NC treatment, $10^{-9.05}$ *M* for the HA treatment, and $10^{-11.4}$ *M* for the HEDTA treatment (Table 4). Based on leaf Cu levels, the calculated Cu^{2+} free concentration should have been the same for HA and HEDTA treatments. This suggests that the Cu^{2+} equilibrium constant ($-\log K^0$) may have been too low for HA (Table 1). The calculated Zn^{2+} free concentration was similar between the NC and HA treatments ($10^{-5.43}$ *M*) (Table 4) and lower for the HEDTA treatment ($10^{-7.71}$ *M*). However, the leaf Zn concentration was greater for the NC treatment than the HA treatment (Fig. 4b), again suggesting that the selected Zn^{2+} equilibrium constant ($-\log K^0$) for HA may have been low (Table 1). The discrepancies associated with the Cu and Zn results for the HA treatment were not surprising since the HA equilibrium constants were a compilation of data from various sources (Table 1). In contrast, micronutrient leaf tissue data for the NC and HEDTA treatments corresponded well to the free metal concentrations predicted by the GEOCHEM-PC model. These data indicate that equilibrium models can predict nutrient uptake if accurate equilibrium constants are implemented.

Table 4. Metal interactions among treatments as computed by the GEOCHEM equilibrium model.

Treatment†	Metal	Free concentration −log value	Complex or solid formed
		M	%
No chelate, NC	Fe^{3+}	13.74	99.99 solid with PO_4^-
	Mn^{2+}	5.54	4.2 complexed with SO_4^{2-}
	Cu^{2+}	6.04	5.1 complexed with SO_4^{2-}
	Zn^{2+}	5.43	4.1 complexed with SO_4^{2-}
Humic acid, HA	Fe^{3+}	13.93	99.98 solid with PO_4^-
	Mn^{2+}	5.54	<1 complexed with HA
	Cu^{2+}	9.05	99.9 complexed with HA
	Zn^{2+}	5.43	<1 complexed with HA
HEDTA	Fe^{3+}	13.95	44 solid with PO_4^-
	Mn^{2+}	5.59	11 complexed with HEDTA
	Cu^{2+}	11.11	100 complexed with HEDTA
	Zn^{2+}	7.71	99.0 complexed with HEDTA

† The 4-morpholine ethane sulfonic acid (MES) treatment results were comparable with the HEDTA treatment.

The commercial HA used in this study has somewhat different complexing properties than those of the humic materials that accumulate in NASA's ALS bioregenerative systems. Recent characterization of the hydrophobic portion isolated from an ALS bioreactor (Grossl and Mackowiak, 1999) indicated that it was most similar to aquatic fulvic acid, FA. For this study, we used about 12 g of dry commercial HA. To process this much ALS bioreactor effluent humic material or purchase this quantity of well-characterized (International Humic Substances Society) FA would have been labor and cost prohibitive. Generally, aquatic FA has greater carboxyl content than HA (Thurman, 1986), suggesting that FA may complex more metal ions. Future work will address humic substances that more closely reflect the products from bioprocessing. This information may then be applicable to other bioprocessing systems, such as biowaste systems that create products intended for land application.

pH Buffering

The pH buffering capacity of a solution is determined by the amount (moles) of strong acid or base needed to produce a unit change in pH. A pH buffer should be chosen that has a pKa value close to the desired pH, which in this case was 5.3. The useful range for a pH buffer is considered to be the pKa ± 1 pH unit (Harris, 1982). Buffering capacity at pH 5.3 for HA is provided mostly by carboxylic acid functional groups, with an average pKa of 4.2 (Thurman, 1986). The MES has one acid dissociation constant with pKa = 6.1 (Kandegedara and Rorabacher, 1999), and HEDTA has one acid dissociation constant with pKa = 5.6 (Norvell, 1991). Thus, HA, MES, and HEDTA would be expected to provide buffering at pH 5.3. We determined the buffer capacity of the complete nutrient solutions sampled at 32 DAT by titrating the solutions with 0.1 *M* HCl from pH 7.0 to pH 4.0 (Table 5). The MES treatment required nearly three times more acid to lower solution pH than the other treatments. The MES acidic functional group has little interaction with metal ions (Yu et al., 1997), unlike HEDTA and HA, which complex metals that could compete with H^+ for binding sites. To compare HEDTA, HA, and MES pH buffering capacities without interference from metals or other buffering constituents, i.e., phosphates, solutions (buffer + distilled water) were compared at 1 m*M* C and acid titrated from pH 7 to pH 4, using 0.01 *M* HCl. The buffering capacities were not significantly different except between the HA and MES treatments (Table 5), where the HA buffering capacity was significantly less than the MES solution. Perhaps not all of the HA C is directly associated with carboxyl groups, thus comparisons based on equimolar C concentrations may not be appropriate. Based on this data, it can be theorized that HEDTA would buffer solution pH as well as MES if it was supplied at roughly the same C level and HA at somewhat higher C levels. Putting this into practice might be difficult since high concentrations of HEDTA would greatly reduce micronutrient bioavailability and higher HA concentrations would likely settle out as HA-Ca and HA-Mg flocculates.

Table 5. The pH buffering capacities of treatment solutions and the individual organic components in distilled water with acid titration from pH 7.0 to 4.0.

Treatment	Nutrient solutions at 32 d after transplant	Organic components in water at 1 m*M* C
	(mol HCl m^{-3})	(mol MCl m^{-3})
No chelate, NC	0.367c†	–‡
HEDTA	0.387bc	0.270ab
Humic acid, HA	0.407b	0.254b
MES§	1.063a	0.284a

† Values followed by the same letter within a column are not significantly different at the 5% level according to the least significant difference (LSD).
‡ There was no organic component to test from the no chelate (NC) treatment.
§ Treatment with 4-morpholineethanesulfonic acid.

SUMMARY AND CONCLUSIONS

Free Fe from Fe phosphate dissolution was inadequate to support normal Fe uptake and rapid plant growth. However, when all aqueous Fe species were considered, enough Fe was available to prevent Fe deficiencies. Under our environmental conditions, complexing agents were only necessary during early plant growth (<35 DAT). Plant release of Fe complexing PS probably allowed the NC treatment to maintain adequate Fe nutrition after an initial stress period. Humic acid improved Fe bioavailability by complexing ~10^{-6} *M* Fe, which prevented early Fe deficiency. In addition, HA improved Zn bioavailability more than HEDTA while maintaining adequate levels of other micronutrients. Therefore, HA was a successful substitute for HEDTA in our system. However, PS and HA equilibrium constants for Fe, Zn, and Cu need to be better established to accurately model nutrient availability in other hydroponic and soil solutions.

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