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Effects of FeEDDS and EDDS on Peat-based Substrate pH and Cu, Fe, Mn, and Zn Solubility

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Abstract. **Common chelating agents used in horticultural fertilizers like ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and ethylenediaminedi(*o*-hydroxyphenylacetic) acid (EDDHA) are not readily biodegradable and may persist in the environment, maintaining the capacity to solubilize heavy metals. For this reason, biodegradable chelating agents like ethylenediaminedisuccinic acid (EDDS) are being evaluated for use in horticultural crop production. Therefore, the objectives of the study were to determine the effects of FeEDDS and EDDS on substrate pH and copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) solubility in peat-based substrate compared with various Fe and chelate-ligand sources. Extractions were performed using the 1:2 by volume substrate analysis method with an incubation period of 24 hours. The control was distilled deionized water extractions. Iron-source (FS) extractants consisted of 1 mg·L^{-1} Fe solutions derived from FeEDDS, FeEDTA, FeDTPA, FeEDDHA, and $FeSO_4$. Iron-source extractant solution pH ranged from 7.1 (FeEDDS) to 5.4 ($FeSO_4$). The extract pH for all Fe-source treatments was similar at pH 6.7, demonstrating the buffering capacity of the peat-based substrate. Iron recovery rates for FS treatments were determined after subtracting Fe that was freely extracted with distilled-deionized water: $FeSO_4$ (13%), FeEDDHA (68%), FeEDDS (73%), FeEDTA (102%), and FeDTPA (121%). Iron-source treatments were not different for Mn, averaging 0.03 mg·L^{-1}, and Cu (0.04 mg·L^{-1}) and Zn (0.24 mg·L^{-1}) were greatest in the FeEDDS treatment. Chelate-ligand (CL) extractants consisted of 5 mM solutions of EDDS, EDTA, and DTPA. Chelate-ligand extractant solution pH ranged from 9.7 (EDDS) to 2.3 (DTPA), and extract solution pH ranged from 7.2 (EDDS) to 4.7 (DTPA). Extractant solutions of EDDS and DTPA resulted in the lowest and highest levels of Cu (0.06 and 0.14 mg·L^{-1}, respectively) and Fe (4.3 and 13.1 mg·L^{-1}, respectively) in extract solutions. Overall, these results suggest that there are no negative implications for the use of FeEDDS with peat-based substrate in terms of horticultural crop production based on substrate Fe solubility, which was not different from FeEDTA.**

Soluble fertilizers are typically formulated with metal-aminopolycarboxylic acids [APCA (i.e., chelating agents)] of Cu, Fe, Mn, and Zn. These metal–APCA complexes, however, are also applied as single-metal chelate solutions to foliage, soil/substrate, or both for correcting micronutrient deficiency like Fe chlorosis (Mortvedt, 1991). Common chelating agents used in fertilizers include EDTA, DTPA, and EDDHA (Lucena, 2003). These chelating agents differ in stability/formation constants, i.e., the chemical bond-strength of the ion-ligand complex with metals as a function of pH, but they share a common trait; they are synthetically produced and not readily biodegradable (Borowiec et al., 2007; Sillanpää, 1997). In Europe, these chelating agents, especially EDTA, are persistent in the environment and are believed to have the ability to extract and mobilize heavy metals from sediments in surface and groundwaters; transporting extracted metals in the water column (complete review in Albano, 2011). For these reasons, in Europe, EDTA is being replaced where possible with biodegradable chelating agents like [S, S′]-EDDS (EDDS), which is a structural isomer of EDTA that is reported to have similar functionality as a chelate (Metsärinne et al., 2001; Neal and Rose, 1973). However, several other readily biodegradable ‘‘green’’ chelating agents are also being considered as replacements including methylglycinediacetic acid, L-aspartic acid N, N-diacetic acid, sodium diethanolglycine/2-hydroxyliminodiacetic acid, iminodisuccinic acid with salts, glutamic acid diacetic acid, and N-(1,2-dicarboxyethyl)-D,L-aspartic acid (Glauser et al., 2010; Lucena et al., 2008).

Little is known about the use of EDDS in horticultural crop production. Its use as an Fe-chelating agent in the production of marigold was reported by Albano (2011) and Albano and Merhaut (2012) where it was found that plants supplied with FeEDDS were not different in growth or foliar Fe concentration than plants supplied with FeEDTA, FeDTPA, FeEDDHA, or $FeSO_4$. Leachate solution spectral properties and chemistry differed, however, between these Fe chelates. It was found that FeEDDS maximally absorbed at a shorter wavelength (238 nm) than either FeEDTA (258 nm) or FeDTPA (260 nm) and that Fe-catalyzed photodegradation of FeEDDS occurred at a rate close to twice that of FeEDTA when exposed to light (Albano, 2011). It was also found that FeEDDS leached less Fe and Mn than FeEDTA, FeDTPA, or FeEDDHA during the production cycle of marigold (Albano and Merhaut, 2012). Therefore, the broad objectives of this study were to gain knowledge on FeEDDS and EDDS interactions with peat-based substrate. Specific objectives were to 1) compare effects of Fe source (FeEDDS, FeEDTA, FeDTPA, FeEDDHA, and $FeSO_4$); and 2) compare effects of chelate-ligand (EDDS, EDTA, and DTPA) on peat-based substrate pH and solubility of Cu, Fe, Mn, and Zn.

Materials and Methods

Extractions. Substrate analysis was performed using the 1:2 by volume extraction method (Sonneveld and van den Ende, 1971; Sonneveld et al., 1990). In 250-mL LDPE bottles, 100 cm^3 of peat-based substrate (Fafard 4P; Conrad Fafard, Inc., Agawam, MA) composed of Canadian sphagnum peatmoss (45%), processed pine bark, perlite, vermiculite, dolomite, wetting agent, and a preplant fertilizer (i.e., nutrient starter charge) (<http://www.fafard.com/Products/MiddleWeightMixes.aspx> accessed 15 Nov. 2011) (moisture content was 45% ± 1%, n = 6) was combined with 200 mL extractant solution. Bottles were capped and placed on a flatbed shaker set at 160 rpm for a 24-h incubation period. Extract solutions were gravity filtered (Whatman 541; Whatman, Int., Kent, U.K.) with filtrates analyzed for pH using a glass electrode, temperature-compensated pH meter (AR50 pH meter; Fisher Scientific, Suwannee, GA), and subsequently analyzed for Cu, Fe, Mn, and Zn by inductively coupled plasma–optical emission spectroscopy (iCAP 6500; ThermoScientific, West Palm Beach, FL) according to U.S. EPA Method 6010B (1997) with quality assurance as described by Hoskins and Wolf (1998). The extractants for Expt. 1 were 1 mg·L^{-1} Fe solutions derived from FeEDDS, FeEDTA, FeDTPA, FeEDDHA, or $FeSO_4$ (FS). The extractants for Expt. 2 were 5 mM solutions of EDDS, EDTA, or DTPA (CL). Extractants in Expts. 1 and 2 were not buffered, and the control in both experiments was distilled-deionized (DI) water extractant. Chemical reagent sources were: [S, S′]-EDDS trisodium salt (Fluka Analytical, Steinheim, Germany), EDTA disodium salt dihydrate (Sigma-Aldrich, Inc., St. Louis, MO), DTPA (Sigma-Aldrich), FeEDDS (prepared as described in Albano, 2011), FeEDTA sodium salt hydrate (Sigma-Aldrich), FeDTPA disodium

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salt hydrate (Sigma-Aldrich), FeEDDHA (Sequestrene 138; Becker Underwood, Inc., Ames, IA), and Fe(II)SO_4 heptahydrate (Fisher Scientific, Fair Lawn, NJ).

Ultraviolet/Visible absorbance of iron source and chelate-ligand extract solutions. All absorption spectra were determined using a scanning ultraviolet/visible spectrophotometer (Beckman DU 800; Beckman Coulter, Inc., Brea, CA). Spectra of extracts for Expt. 1 (FS) and Expt. 2 (CL) were determined by scanning from 200 to 500 nm and 200 to 400 nm, respectively, at 1-nm intervals at 1200 nm per minute. Extract solutions for analysis by spectrophotometer received an additional filtration (initial filtration was Whatman 541) through a syringe filter disc (Puradisc 25TF, PTFE, 1.0-µm pore size; Whatman) before scans. In Expt. 1, extract solutions were blanked against the DI water control extract, and in experiment 2, extract solutions were blanked against the DI water control extract with the CL extractant solution absorbance subtracted to yield spectra specifically associated with Fe-chelates.

Statistics. Extractions were run in triplicate. Data were analyzed by one-way analysis of variance to determine the main effects of FS or CL on the dependent variables pH, Cu, Fe, Mn, and Zn. Calculations were performed by the general linear model procedure of SAS (Version 9.2; SAS Institute, Cary, NC). Means were separated and planned comparisons were made using Tukey when $P \leq 0.05$.

Results

Expt. 1: Iron-source interaction. Effects of FS were significant for pH, Cu, Fe, and Zn at $P \leq 0.001$ and not significant for Mn. Extractant solutions were unbuffered and ranged in pH from 7.1 (FeEDDS) to 5.4 ($FeSO_4$) (Fig. 1). The pH of extract solutions as a mean of all treatments including the DI water control after 24-h incubation was similar at 6.7 ± 0.1 demonstrating the buffering capacity of the substrate (Fig. 1). This mean pH (6.7) is at the upper limits of what is broadly considered suitable for the production of bedding plants in peat-based soilless substrate (pH 5.4–6.8; Nelson, 1999) and is consistent with the initial substrate pH for the same brand substrate used in the study as reported by others (Dekkers et al., 2000). Bedding plants sensitive to high substrate pH, however, like petunia (*Petunia ×hybrida*) and calibrachoa (*Calibrachoa ×hybrida*) would likely be expressing Fe deficiency symptoms at this substrate pH (Fisher et al., 2003; Šrámek and Dubský, 2009, 2011).

Excluding the DI water control, FS Cu ranged from 0.01 mg·L^{-1} ($FeSO_4$) to 0.04 mg·L^{-1} (FeEDDS), Fe ranged from 0.85 mg·L^{-1} ($FeSO_4$) to 1.93 mg·L^{-1} (FeDTPA), and Zn ranged from 0.14 mg·L^{-1} ($FeSO_4$) to 0.24 mg·L^{-1} (FeEDDS) (Fig. 2). Iron-source was not significant for Mn and averaged 0.03 mg·L^{-1}. Levels of Cu (0.04 mg·L^{-1}) and Zn (0.24 mg·L^{-1}) were significantly greater in the FeEDDS treatment by 75% and 38%, respectively, than for the other FS treatments or the DI water control combined, which

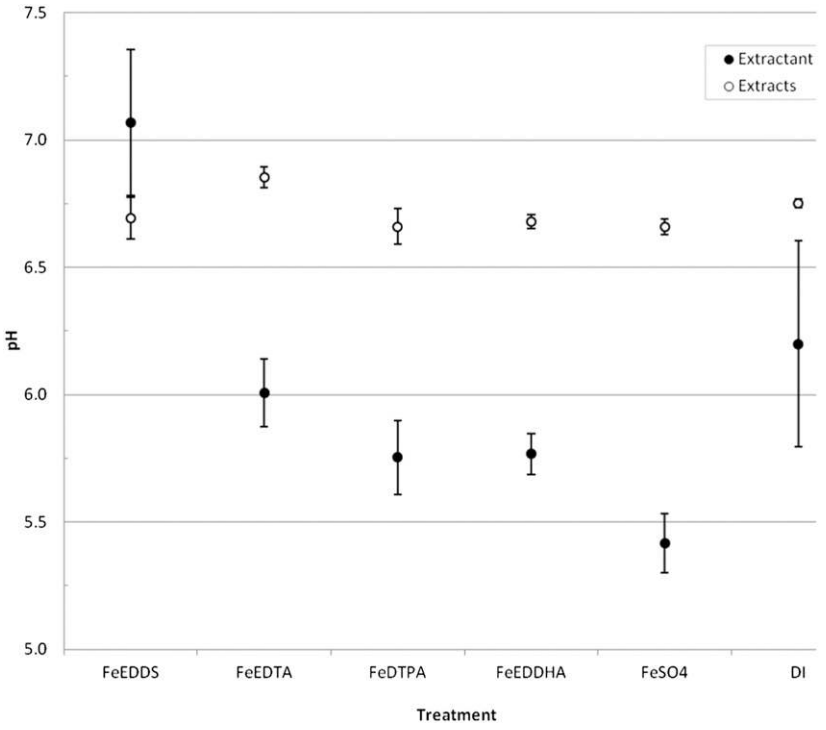

Fig. 1. Extraction (•) and extract (◦) solution pH for Expt. 1 [iron-source (FS)]. Vertical bars represent SEM. When not present, bars fall within the symbol. n = 3.

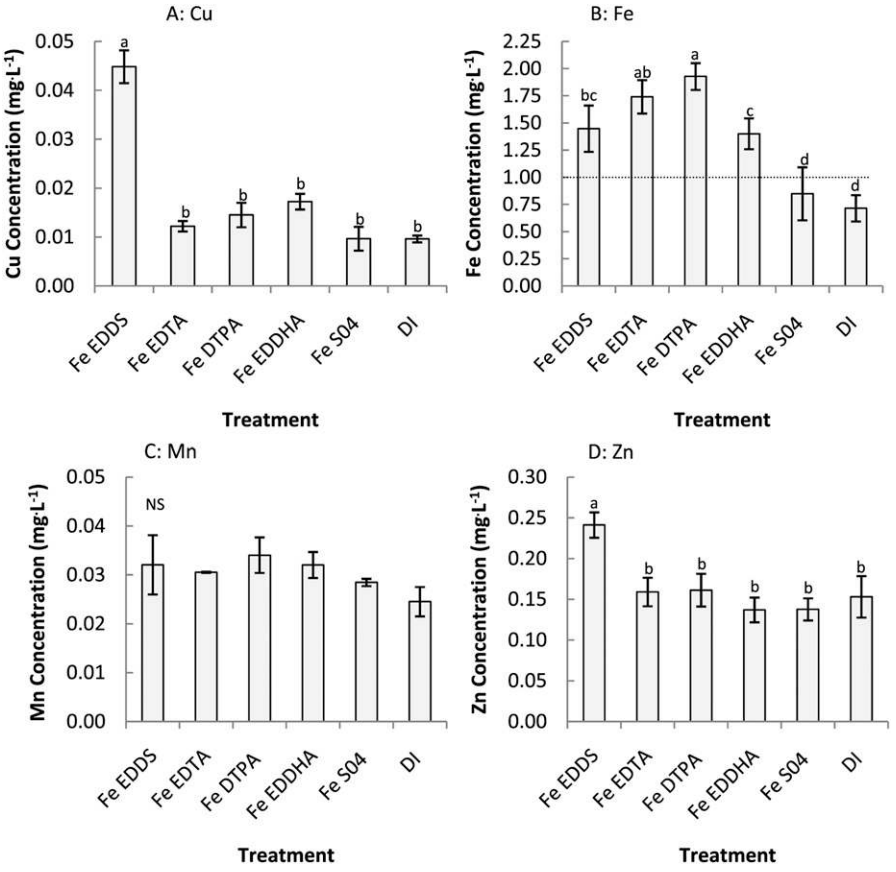

Fig. 2. Extract levels of copper (Cu) (**A**), iron (Fe) (**B**), manganese (Mn) (**C**), and zinc (Zn) (**D**) for 1 mg·L^{-1} Fe extractant solutions derived from Fe-ethylenediaminedisuccinic acid (EDDS), Fe-ethylenediaminetetraacetic acid (EDTA), Fe-diethylenetriaminepentaacetic acid (DTPA), Fe-ethylenediaminedi(*o*-hydroxyphenylacetic) acid (EDDHA), and $FeSO_4$. The control was distilled-deionized water (DI). Extraction incubation period was 24 h and the 1:2 by volume substrate analysis method was used. The dotted line in **B** represents the 1 mg·L^{-1} Fe rate applied. Data are means ± SE (n = 3). Within any metal, bars with different letters are significantly different at $P \leq 0.05$ (Tukey).

were not different (Fig. 2). Iron-EDDS with an Fe extract concentration of 1.45 mg·L^{-1} was not significantly different from FeEDTA or FeEDDHA but was significantly lower than the FeDTPA treatment by 25%. Iron recovery rates for FS treatments were determined after subtracting Fe that was freely extracted with DI water: $FeSO_4$ (13%), FeEDDHA (68%), FeEDDS (73%), FeEDTA (102%), and FeDTPA (121%).

Spectral scans of the DI water control extract reveals the extraction of a large amount of ultraviolet-absorbing compounds (Fig. 3A). To ascertain spectrums associated with Fe treatments, extracts were blanked against the DI water control extract solution. Iron sulfate absorbance was very low at ≈10-fold less across the scanned spectrum (Fig. 3B) compared with the Fe-chelate treatments (Fig. 3C–F). The spectra for FeEDDS and FeEDTA were similar and like FeDTPA, had extract solution absorbance over the scanned spectrum higher than for the corresponding extractant solution (Fig. 3C–E). In contrast, FeEDDHA extract spectrum was in general lower in absorbance than for the extractant solution (Fig. 3F). Comparisons at $^{\lambda}$max between the extractant and extract solutions were made and shifts in $^{\lambda}$max absorbance units (AU) were: +53%, +37%, +37%, and –24% for FeEDDS, FeEDTA, FeDTPA, and FeEDDHA, respectively (Fig. 3C–F).

Expt. 2: Chelate-ligand interaction. Effects of CL were significant for pH, Cu, Fe, and Zn at $P \leq 0.001$ and Mn at $P \leq 0.05$. Extractant solutions prepared with DI water were unbuffered and ranged in pH from 9.7 (EDDS) to 2.3 (DTPA) (Fig. 4). Extract solution pH after 24-h incubation had significantly decreased for EDDS (7.2) and increased for DTPA (4.7) (Fig. 4). The control, DI water, significantly increased from 6.2 to 6.8 for the extractant and extract solutions, respectively, consistent with Expt. 1 (Figs. 1 and 4). For CL, excluding the DI water control, Cu ranged from 0.06 to 0.14 mg·L^{-1}, and Fe ranged from 4.33 to 13.08 mg·L^{-1} for EDDS and DTPA, respectively (Fig. 5A–B). Manganese ranged from 1.01 to 2.67 mg·L^{-1}, and Zn ranged from 0.33 to 0.40 mg·L^{-1} for EDDS and the mean of EDTA and DTPA (which were not significantly different), respectively (Fig. 5C–D). Levels of Cu and Fe in extract solutions followed the profile DTPA > EDTA > EDDS corresponding to these chelating agent's stability constants for the metals (Fig. 5A–B). Levels of Mn and Zn followed similar profiles in extract solutions, DTPA = EDTA > EDDS (Fig. 5C–D). The DI water control relative to the mean of chelating agents extracted very low levels of Cu (91% less), Fe (92% less), Mn (98% less), and Zn (62% less) demonstrating the chelating agents' capacity to extract metals from peat-based substrate. Finally, when complexed with Fe, EDDS, EDTA, and DTPA are chromaphores that maximally absorb at 238, 258, and 260 nm, respectively, and these peaks were present in extract solution spectra, confirming the formation of these Fe-chelates (Fig. 6).

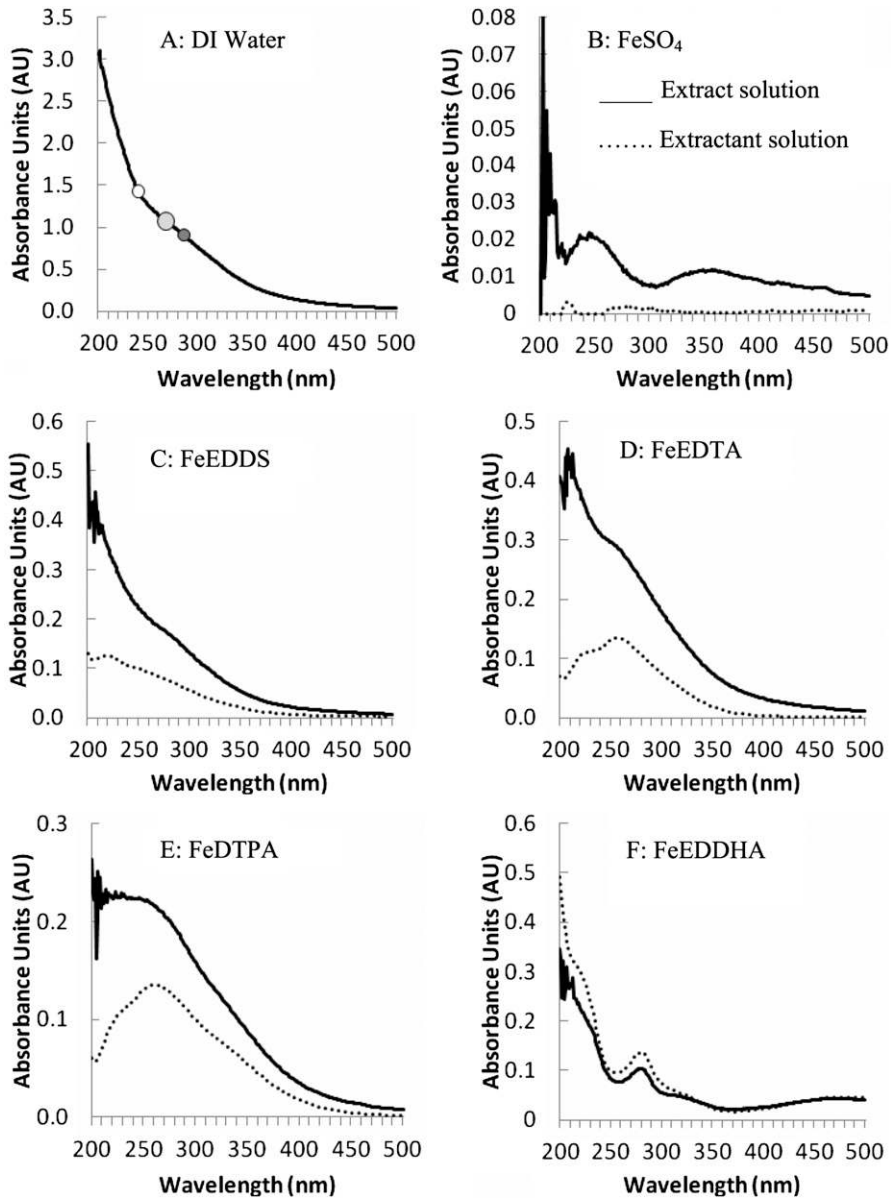

Fig. 3. (**A**) The spectra of the distilled-deionized water control extract with circles representing peak absorbance, from left to right, for FeEDDS (238 nm), Fe-ethylenediaminetetraacetic acid (EDTA) (258 nm), and Fe-diethylenetriaminepentaacetic acid (DTPA) (260 nm) (combined) and Fe-ethylenediaminedi(*o*-hydroxyphenylacetic) acid (EDDHA) (280 nm). Spectra of 1 ppm of iron-source (dotted line) and extract (solid line) are presented for $FeSO_4$ (**B**), Fe-ethylenediaminedisuccinic acid (EDDS) (**C**), FeEDTA (**D**), FeDTPA (**E**) and FeEDDHA (**F**). n = 3.

Discussion

In Expts. 1 and 2, the inherent pH and buffering capacity of the commercial peat-based substrate represented the extent of controlling the incubation pH for testing effects of FS and CL. This experimental approach is similar to Boxma (1981) where pH to study the behavior of Fe-chelates in peats was varied by adjusting the pH of substrate with lime and not by altering the pH of Fe-chelate solutions with buffers. When having to supply an Fe-chelate solution for correcting Fe deficiency, the results of Expt. 1 support Boxma (1981) and work by others (Wik et al., 2006) on the importance of selecting the appropriate chelating agent based on the specific substrate pH. For example, FeEDDHA is the only currently available APCA that effectively maintains Fe solubility in high pH substrate (i.e., pH greater than 7.0).

For the common Fe-chelates (i.e., FeEDTA, FeDTPA, and FeEDDHA), their interaction with peat as it relates to chelate-associated Fe solubility is 1) a function of substrate pH; 2) chelating agent stability constants with other cations like Ca, Cu, Mn, and Zn; and 3) relative abundance of these cations (Boxma, 1981; de Kreij, 1998; Lindsay and Norvell, 1969; Norvell and Lindsay, 1969). These interactions would also apply to FeEDDS; however, with this chelating agent, there is another consideration: the potential loss of the FeEDDS (EDDS) compound through biodegradation. Therefore, Fe recovery rates of

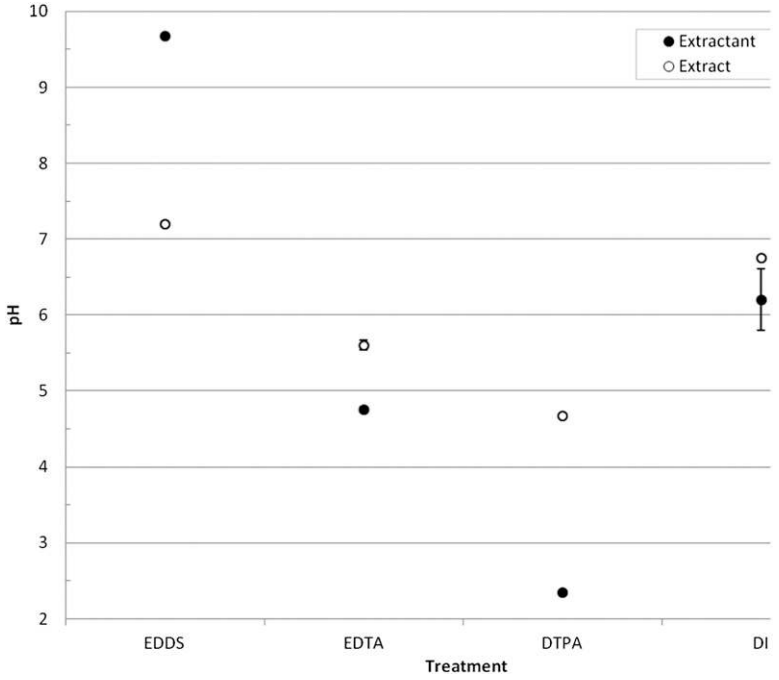

Fig. 4. Extraction (•) and extract (◦) solution pH for Expt. 2 (chelate-ligand). Vertical bars represent SEM. When not present, bars fall within the symbol. n = 3.

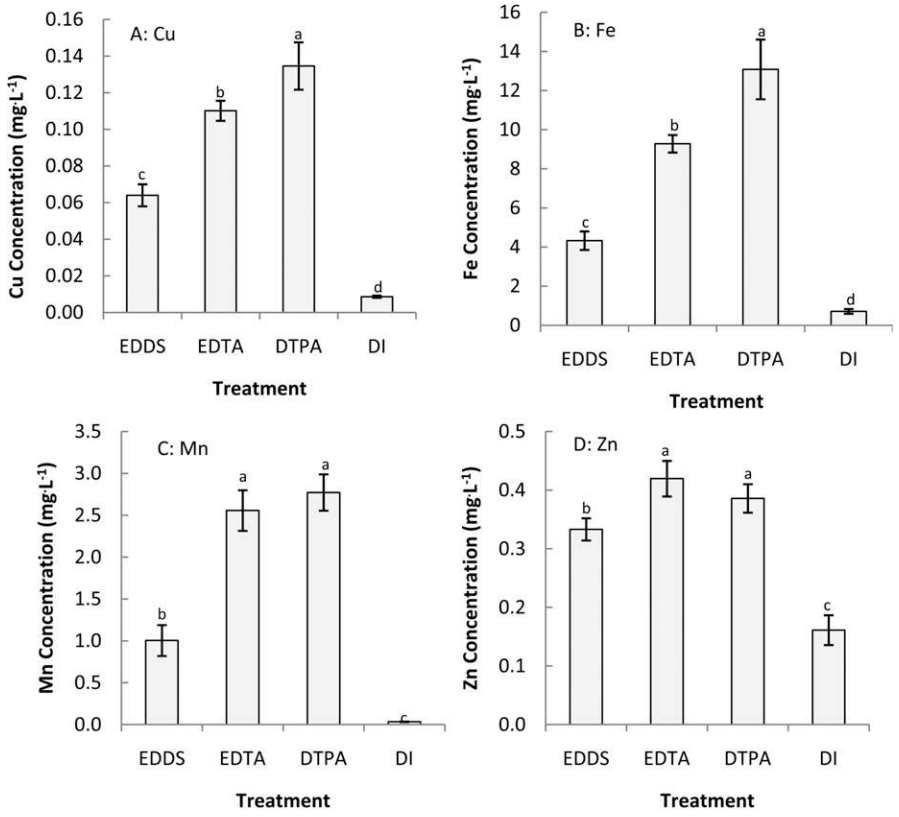

Fig. 5. Extract levels of copper (Cu) (**A**), iron (Fe) (**B**), manganese (Mn) (**C**), and zinc (Zn) (**D**) for 5 mM extractant solutions of ethylenediaminedisuccinic acid (EDDS), ethylenediaminetetraacetic acid (EDTA), and diethylenetriaminepentaacetic acid (DTPA). The control was distilled-deionized water (DI). Extraction incubation period was 24 h and the 1:2 by volume substrate analysis method was used. Data are means ± SE (n = 3). Within any metal, bars with different letters are significantly different at $P \leq 0.05$ (Tukey).

less than 100% for FeEDDS and FeEDDHA in Expt. 1 could be the results of cation substitution for Fe, chelating-agent fixation to peat, degradation of the chelating agent (for FeEDDS), or a combination of these possibilities. Based on the extract spectral profile for FeEDDHA (Fig. 3), it appears that this Fe-chelate became bound to physical substrate components because its extract solution spectral profile is lower (based on AU) than the profile for the 1 ppm Fe, FeEDDHA extractant solution (Fig. 3F). Boxma (1981) describes FeEDDHA adsorption to peat at pH of 4.35. The present study shows that FeEDDHA adsorption to peat-based substrate may occur at higher pH as well. The Fe-EDDS spectrum is similar to FeEDTA but with a slightly lower absorbance at FeEDDS' peak absorbance wavelength at 238 nm (FeEDDS) compared with FeEDTA at 258 nm. This difference in absorbance (discussed later) at the same concentration Fe for these Fe-chelates is consistent with Albano and Merhaut (2012). This suggest that FeEDDS' less than 100% Fe recovery rate is the result of Fe substitution with other cations, and based on Figure 2, Fe was substituted on the EDDS complexone with Cu and Zn in that order. This supports work by Orama et al. (2002) in which stability constant profiles for [S, S′]-EDDS (the isomer used in the present study) as a function of pH shows that at pH ≈6.7 (the substrate pH observed in the present study), EDDS has affinity for Cu, Zn, Fe, and Mn in that order. Further evidence that the EDDS chelate did not biodegrade is work by Tandy et al. (2006) where it was reported that EDDS associated with metals in soil took up to 2 weeks to degrade. Iron recovery rates of 121% for FeDTPA is likely the result of a combination of factors including the extract pH for this APCA, which falls within the pH range (4.0–7.0) that DTPA has the highest stability/affinity for Fe, the extractable Fe associated with the substrate's preplant fertilizer, and/or the extractable Fe associated with the substrate's physical components/amendments (Boxma, 1981; Reed, 1996; Wik et al., 2006).

In Expt. 2, the extraction incubation pH was the result of the interaction of CL with peat-based substrate. In this experiment, the buffering capacity of the peat-based substrate was not sufficient to moderate CL extracts to a common pH as was observed for FS treatments in Expt. 1. Therefore, it is not possible to distinguish chelate effects from pH effects in this study. Although the objective of Expt. 2 was not the development or modification of peat-based substrate testing methods for determining plant-available micronutrients, information from the study can be used for evaluating current methods including the acidity of DTPA solutions prepared with DI water, consistent with Berghage et al. (1987), and the apparent adequacy of 5 mM DTPA concentration for extracting micronutrients, consistent with Berghage et al. (1987) and Warncke (1998). Regardless, when using chelating agents like DTPA in soil/substrate tests to estimate plant-available micronutrients, careful consideration needs to be given to the extraction method [e.g. 1:2 vs. saturated media extract (SME)], pH of the incubation solution, metal-loading of insoluble (i.e., what would be plant-unavailable) metals, and concentration of non-essential metals (i.e., metals other than Cu, Fe, Mn, or Zn; like cadmium and lead) for interpreting and comparing

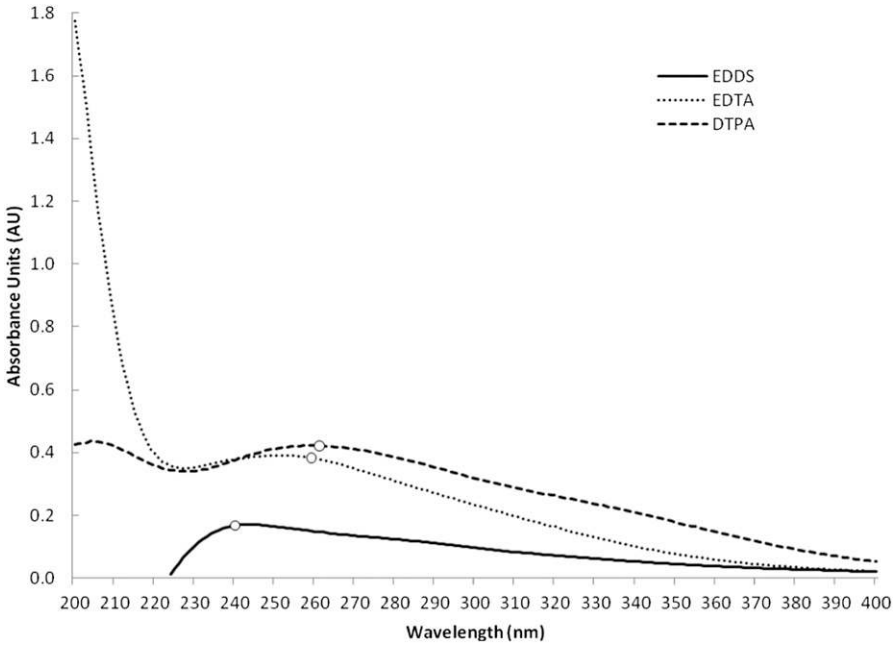

Fig. 6. Spectra for Expt. 2 chelate-ligand extracts with circles representing the peak absorbance for each specific chelate-ligand complexed with iron: 238, 258, and 260 nm for ethylenediaminedisuccinic acid (EDDS), ethylenediaminetetraacetic acid (EDTA), and diethylenetriaminepentaacetic acid (DTPA), respectively. Data presented are values of a 20-fold dilution (n = 3).

results (O'Connor, 1988). For reference, a commonly cited procedure for estimating micronutrient availability in greenhouse substrates is described by Warncke (1998) where an unbuffered 5 mM DTPA solution is the extractant and SME the extraction method with 30-min incubation.

Expt. 2 spectral scans support Expt. 1 and work by Orama et al. (2002) that EDDS' greatest stability with Fe occurs at a pH lower than 6.7 based on the formation of Fe-chelates from CL treatments as determined by spectrophotometry. In previous work (Albano, 2011) it was discovered that at the same concentration of Fe, FeEDDS absorbance at 238 nm ($^{\lambda}$max) was ≈8% to 10% lower than the peak wavelength absorbance for FeEDTA (258 nm) or FeDTPA (260 nm). In the current study, FeEDDS formation absorbance was ≈50% lower than FeEDTA or FeDTPA formation based on peak wavelength absorbance for these chelates when complexed with Fe (Fig. 6). The extract pH (Fig. 4) favored Fe complexation with EDTA (pH 4.0–6.3) and DTPA (pH 4.0–7.0). Therefore, it is likely that a lower incubation pH for EDDS (pH ≈4.0–6.0) would have equated to higher absorbance at 238 nm for this Fe-chelate complex.

In summary, FeEDDS was associated with the highest solubility of Cu and Zn from peat-based substrate and with an Fe recovery rate of less than 100% under the conditions of the current study. Regardless, Fe solubility in peat-based substrate for the FeEDDS treatment was not statistically different from FeEDTA or FeEDDHA, and all Fe-chelates had Fe solubility levels that were significantly greater than either the non-chelated, $FeSO_4$ or DI water control, which were not statistically different. This research supports previous work (Albano, 2011; Albano and Merhaut, 2012) on the suitability of FeEDDS as an Fe source for horticultural crop production in peat-based substrate. Also, the addition of CL to peat-based substrate resulted in significantly greater solubility of Cu, Fe, Mn, and Zn, supporting the premise that APCA compounds can contribute to heavy metal mobility in water resources receiving contaminated runoff, thus affecting water quality. Future research will need to investigate the stability of EDDS-chelated metals in dry and soluble-stock fertilizers to assess if the chelating agent's biodegradation characteristics will limit the commercial use of this APCA compound in horticultural trade.

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