Influence of FeEDDS, FeEDTA, FeDTPA, FeEDDHA, and FeSO₄ on Marigold Growth and Nutrition, and Substrate and Runoff Chemistry

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Abstract. The objectives of the study were to determine effects of iron (Fe) source on plant growth, plant nutrition, substrate chemistry, and runoff chemistry. Iron source (FS) treatments consisted of Fe-aminopolycarboxylic acid (APCA) complexones iron ethylenediaminetetraacetic acid (FeEDTA), iron [S, S']-ethylenediaminedisuccinic acid (FeEDDS), iron diethylenetriaminepentaacetic acid (FeDTPA), and iron ethylenediaminedi(*o*-hydroxyphenylacetic) acid (FeEDDHA) and non-chelated iron sulfate (FeSO₄) added to a base nutrient solution at the rate of 1 mg·L⁻¹ Fe final concentration. Marigold (*Tagetes erecta*) 'First Lady' was grown in peat-based media fertilized with FS treatments over a period of 22 d. Iron source treatments were nonsignificant for foliar Fe, manganese (Mn), or zinc (Zn) averaging 162 µg·g⁻¹ Fe, 228 µg·g⁻¹ Mn, and 35 µg·g⁻¹ Zn but were significant for foliar copper (Cu). Main effect of FS on pour-through (PT) leachate pH was statistically different but not practically significant, averaging 6.42. The FeDTPA treatment resulted in higher levels of Cu, Fe, and Zn in PT extracts. Leachate-runoff (LR) was collected and analyzed over the course of the study. Results of LR were similar to PT with levels of Cu, Fe, and Zn for the FeDTPA treatment resulting in higher concentrations of these metals. In both PT and LR, the highest concentration of Mn was associated with the FeEDTA treatment. Spectrophotometer analyses of PT and LR leachates determined the presence of all Fe chelates tested in those solutions.

Most soluble fertilizers used in floral crop production are formulated with micronutrient metals Cu, Fe, Mn, and/or Zn complexed with synthetic APCA complexones, known commonly as chelating agents like EDTA, DTPA, and EDDHA. These compounds are used to maintain solubility of these multivalent metals in chemical environments where they would otherwise be insoluble (e.g., concentrated fertilizer stock solutions or as a result of substrate pH) and, as such, unavailable for plant uptake. Of metal chelates typically used in fertilizers, chelated Fe is the most common among formulations. Iron chelates are used to prevent or correct Fe deficiency and have been reported to be more effective than other non-APCA sources

of Fe, including Fe citrate, Fe glucoheptonate, and Fe sulfate (Broschat, 2003). Iron chelates, however, have also been found to contribute, possibly in association with substrate pH, to Fe toxicity in certain bedding plants, including marigolds and geraniums (*Pelargonium × hortorum*) (Albano et al., 1996; Broschat and Moore, 2004; Wik et al., 2006).

Chelating agents are involved not only in the direct supply of micronutrient metals in fertilizers, but also through their interaction with solid-phase substrate components. Standard soil testing methods use chelating agents, particularly DTPA, to estimate extractable metals from peats and mineral soils, and peat sources have been found to vary considerably in extractable Fe (Berhage et al., 1987; Broschat and Donselman, 1985; Handrek, 1989; Wik et al., 2006). Work by Albano et al. (1996) and Bachman and Halbrooks (1994) documented that repeated application of soluble fertilizers containing Fe chelate or chelate-ligand resulted in significantly higher levels of soluble Fe in leachate vs. the non-chelated Fe source. The significance of these results is twofold: 1) chelating agents may continue to supply metals in the substrate–rhizosphere environment in excess of that applied through APCA-mediated metal extraction from solid-phase components; and 2) Fe che-

lates, chelate-ligands, or both may be leached in runoff from horticultural production areas into receiving water resources.

Synthetic APCAs like EDTA, DTPA, and EDDHA are characterized by low susceptibility to biodegradation. As a result, these chelating agents can persist in the environment, maintaining the capacity to extract and solubilize heavy metals from sediments. Therefore, a search for a replacement for these synthetic APCAs, especially EDTA, is ongoing. A promising candidate compound is [S, S']-EDDS, a biodegradable chelating agent with a structure and chemistry similar to that of EDTA (Albano, 2011).

In a previous study (Albano, 2011), FeEDDS was deemed to be a suitable Fe chelate source in comparison with FeEDTA and FeDTPA with plants not being different in leaf dry mass or foliar Fe or Mn among Fe treatments. Spectral properties and vulnerability to photodegradation of FeEDDS was also determined and it was discovered that FeEDDS photodegrades more rapidly than FeEDTA. In the current study, FeEDDS was compared with Fe sources with Cu, Mn, and Zn supplied as sulfate salts opposed to the previous study (Albano, 2011) where they were supplied as EDTA chelates. Also in the current study, the effects of Fe source on substrate and leachate runoff chemistry during the production of marigold were determined. Therefore, objectives of the study were to assess the performance of FeEDDS in comparison with Fe chelates (FeEDTA, FeDTPA, FeEDDHA) and with a non-chelated (FeSO₄) Fe source on 1) marigold growth (including leaf greenness, plant height, true-leaf pair count, and leaf dry mass) and foliar Cu, Fe, Mn, and Zn; and 2) substrate and leachate runoff chemistry (including Cu, Fe, Mn, and Zn; and pH and electrical conductivity) during the production of marigold.

Materials and Methods

Growing conditions. Marigold 'First Lady' seeds were sown in six-cell grow-packs (40 cm³ per cell) containing a moistened soilless peat-based medium (Fafard 4P; Conrad Fafard, Inc., Agawam, MA). Seeds germinated 2 to 3 d after sowing (DAS). Iron source treatments were initiated 24 DAS when the second true leaf pair (TLP) was fully expanded and subsequently applied on 26, 28, 31, 32, 33, 35, 36, 37, 39, 40, 41, 43, 44, and 45 DAS. Morning and afternoon treatment applications were made on 43, 44, and 45 DAS. Clear-water leaching with distilled, deionized (DI) water occurred on 30, 34, 38, 42, and 46 DAS. Plants were harvested 47 DAS. The experiment was a completely randomized design with five FS treatments (FeSO₄, FeEDTA, FeEDDS, FeDTPA, and FeEDDHA) and 10 replications per treatment with a replication consisting of six plants. The study was conducted in a double-walled polycarbonate-glazed greenhouse located in Fort Pierce, FL (lat. 27.41° N, long. 80.35° W), 28 Feb. (sowing) to 15 Apr. (harvest), 2008. Growing temperatures over the course of the experiment

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averaged 27/22 °C day/night. Treatment applications (300 mL) were evenly distributed among cells of a grow-pack, and leaching fractions over the course of the study averaged 40% ± 5%.

Nutrient solution treatments. A base nutrient solution (BNS) was laboratory-prepared and contained the following concentrations (mg·L⁻¹) of essential plant nutrients: 200 nitrogen (N), 44 phosphorus (P), 164 potassium (K), 113 calcium (Ca), 49 magnesium (Mg), 1 Fe, 0.5 boron (B), 0.5 Mn, 0.05 Zn, 0.05 molybdenum (Mo), and 0.02 Cu. Source reagents for preparing BNS were: KNO₃, KH₂PO₄, MgSO₄, Ca(NO₃)₂, NH₄NO₃, (NH₄)₂H₂PO₄, CaHPO₄, H₃BO₃, MnSO₄, CuSO₄, ZnSO₄, and MoO₃. Treatments consisted of FeSO₄ [control (Sigma-Aldrich, Inc., St. Louis, MO)], FeEDTA (Sigma-Aldrich), FeEDDS [prepared as described in Albano, 2011 (FS, Sigma-Aldrich; EDDS source, Fluka Analytical, Steinheim, Germany)], FeDTPA (Sigma-Aldrich), and FeEDDHA (Sequestrene 138; Becker Underwood, Inc., Ames, IA) added to BNS at 1 mg·L⁻¹ Fe final concentration. As a result of differences in reagents, treatments varied slightly in sulfur (S) and sodium (Na) as determined by inductively coupled-plasma optical emission spectroscopy [ICP-OES (IRIS 1000 HR DUO; ThermoElemental, Franklin, MA)] according to U.S. EPA Method 6010B (1997). Sulfur concentration in the Fe chelate treatments was 64.13 mg·L⁻¹ S and 64.70 mg·L⁻¹ S in the FeSO₄ treatment. Sodium concentration in FS was 0.02 mg·L⁻¹, FeSO₄; 0.40 mg·L⁻¹, FeEDTA; 1.68 mg·L⁻¹, FeEDDS; 1.36 mg·L⁻¹, FeDTPA; and 0.90 mg·L⁻¹, FeEDDHA. Iron chelate reagents were formulated as monosodium FeEDTA and FeEDDHA, disodium FeDTPA, and trisodium FeEDDS compounds. Treatment solutions were prepared with DI water and were adjusted to pH 5.8 with HCl or KOH (0.1 N solutions) before application.

Harvest and leaf tissue analysis. Plants were harvested 47 DAS at which time TLP number, leaf greenness by SPAD reading of TLP 6 using a SPAD-502 chlorophyll meter (Minolta Camera Co., Japan), and plant height from cotyledonary node to shoot apex were recorded for each of six plants of a grow-pack replication with a mean generated for the replication for use in statistics. Subsequently, leaf tissue was collected and processed as described in Albano (2011). Briefly, washed leaf tissue was dried, weighed, and milled to pass a 20-mesh screen. Milled plant tissue was digested using a closed-vessel microwave-assisted procedure according to U.S. EPA Method 3052 (1997). Digestates were analyzed for Cu, Fe, Mn, and Zn, by ICP-OES. Minimum detection limits for Fe, Mn, Cu and Zn on the ICP-OES used in the study were 6, 3, 3, and 3 µg·L⁻¹, respectively.

Pour-through method extract collection and analysis. One h after DI leach on 30, 34, 38, 42, and 46 DAS, PT method extractions (Sonneveld and van den Ende, 1971; Sonneveld et al., 1990) were performed by placing grow-packs on 14-cm² containers (590-mL capacity) with holes drilled into the lid and applying

a sufficient volume of DI to collect between 50 and 80 mL leachate (Lang, 1996). Extract pH and electrical conductivity (EC) were determined followed by gravity filtration (Whatman 541; Whatman, Int., Kent, U.K.) and subsequent analysis for Cu, Fe, Mn, and Zn by ICP-OES (only pH and EC determined for 38 DAS).

Leachate runoff collection and analysis. Leachate-runoff at each irrigation was collected by placing grow-packs on collection containers as described previously. Grow-packs were allowed to drain for at least 10 min after application of FS treatment or DI. Leachates were processed and analyzed as described for PT.

Ultraviolet/visible absorbance of pour-through and leachate runoff solutions. Samples for analysis by spectrophotometer were filtered through a syringe filter disc (Puradisc 25TF, polytetrafluoroethylene, 1.0-µm pore size; Whatman) before scans. Absorption spectra were determined using a scanning ultraviolet/visible spectrophotometer (Beckman DU 800; Beckman Coulter, Inc., Brea, CA). Spectra (200–800 nm) of 2 mg·L⁻¹ Fe solutions from FeSO₄, FeEDTA, FeEDDS, FeDTPA, and FeEDDHA were determined, serving as standard spectral profiles for comparison with LR and PT solutions. Composite samples were prepared for LR collected on 28, 33, 37, 41, and 45 DAS [from morning treatment application for 45 DAS (FS application events)] and for PT [30, 34, 42, and 46 DAS (DI application events)] by combining 2 mL from each replicate of a treatment across collection dates. Composite samples from LR and PT were scanned 200–800 nm as described previously by Albano (2011).

Statistical analyses. Data were analyzed by analysis of variance to determine the main effect of FS. Data were also analyzed using binary independent variables coded chelated (0) or non-chelated (1). Calculations were performed by the general linear model procedure of SAS (Version 9.2; SAS Institute, Cary, NC). Means separation and planned comparisons were made using Tukey when $P \leq 0.05$.

Results and Discussion

Plant growth. Parameters measured at harvest included plant height, leaf greenness, true-leaf pair count, and total leaf dry mass. Growth parameters did not differ significantly among FS treatments (data not shown). Plant height measured from the cotyledonary node to primary shoot apex was 17.1 ± 1.6 cm. Plants had 8 ± 1 true-leaf pairs and total leaf dry mass per six plant replicates of 8.81 ± 1.11 g. Leaf greenness, estimated by SPAD index readings (an indirect measurement of chlorophyll) of TLP 6, representing recently matured, fully expanded leaves, was 54 ± 2 units. SPAD index values fell within the range of what has been observed in normally growing floricultural crops, ≈40 to 70 SPAD index units for plants receiving 200 mg·L⁻¹ N (Bi et al., 2010; Jeong et al., 2010).

Plant nutrition. Foliar Fe, Mn, or Zn, averaging 162 µg·g⁻¹ Fe, 228 µg·g⁻¹ Mn, and 35 µg·g⁻¹ Zn, did not differ significantly among FS treatments (Table 1). These results are consistent with the previous study (Albano, 2011) for Fe and Mn for the common FS treatments, FeEDDS, FeEDTA, and FeDTPA. Foliar Cu did differ significantly among FS treatments ($P = 0.0041$) ranging from 4.58 µg·g⁻¹ Cu for FeSO₄ to 7.11 µg·g⁻¹ Cu for FeEDDHA (Table 1). Iron-EDDS foliar Cu levels were not different from that of FeEDTA, FeDTPA, or FeEDDHA but were significantly higher than the FeSO₄ treatment. Regardless of FS treatment, foliar levels of Fe, Mn, Zn, and Cu were interpreted to be within the range of what is considered sufficient for normal growth for bedding plants (Vetanovetz, 1996; Table 1). Data were also analyzed to compare a chelated vs. a non-chelated Fe source. Using this method of analysis, leaf tissue had significantly greater and lower levels of Cu and Mn, respectively, in the Fe chelate than in FeSO₄ treatments (Table 2).

Foliar Fe in the previous (Albano, 2011) and present studies for the common Fe source treatments (FeEDDS, FeEDTA, and FeDTPA) were the same averaging 141 ± 1 µg·g⁻¹ Fe. Work by Broschat (2003) compared various Fe sources for correcting Fe chlorosis in the nursery crop ixora (*Ixora* spp.) and found that plants grown in a peat-based substrate supplied with FeDTPA or FeEDDHA at 6 months' growth had foliar Fe levels that were not statistically different, averaging 126 µg·g⁻¹ Fe, in which in the this study, the mean value for these same Fe chelates was similar at 170 µg·g⁻¹. Wik et al. (2006) compared FeSO₄, FeEDTA, and FeEDDHA in geranium and calibrachoa (*Calibrachoa* × hybrid) grown at low and high substrate pH, respectively. At the 1 mg·L⁻¹ Fe, 200 mg·L⁻¹ N treatment in their study (same levels of N and Fe supplied in the present study), foliar Mn for geranium and foliar Fe for calibrachoa were not significant between Fe sources.

Foliar Mn in the previous (Albano, 2011) and present studies differed considerably, averaging 88 and 223 µg·g⁻¹ Mn, respectively, for the common Fe treatments FeEDDS, FeEDTA, and FeDTPA. Factors that may have contributed to this difference include the form of Mn supplied [EDTA (previous) vs. sulfate (present)] and age or development of plants at the time of harvest. Marigolds in the previous (Albano, 2011) and present studies were harvested with five (35 DAS) and eight (47 DAS) true-leaf pairs, respectively. Other differences include the season that experiments were conducted: summer in the previous study (Albano, 2011) and winter/spring in the current study, resulting in warmer and cooler growing temperatures for the previous and current studies, respectively. Foliar Mn levels, however, for both the previous (Albano, 2011) and present studies were within the range of what has been observed in African-type marigolds [80 to 300 µg·g⁻¹ Mn (Albano and Miller, 1998; Albano et al., 1996)].

Substrate chemistry. Substrate analysis was conducted by PT on 30, 34, 38 (pH and

EC only), 42, and 46 DAS. Extract solution data and statistics are presented in Table 3. In general, Fe and pH increased; Cu remained steady; and Mn, Zn, and EC decreased in PT extracts (leachates) over the course of the study (data not shown). Compared with FeSO₄, Fe chelate treatments had significantly greater values for pH, Cu, Fe, and Zn (Table 2). Extract Fe and other micronutrients, however, have been found to correlate poorly with plant tissue concentrations of these nutrients (de Krijt et al., 1996). These observations hold true for the current study because there were no significant differences observed between FS treatments for foliar Fe, Mn, or Zn.

Effects of time (DAS) ($P < 0.001$) and FS ($P = 0.004$) were significant for pH; however, there was no significant interaction between main effects ($P = 0.605$) (Table 3). Mean substrate pH increased from 6.26 to 6.56 from the first to the final PT event (Table 3). Iron-DTPA had the highest pH for the final PT event at 6.63 (data not shown). However, PT FeSO₄ and FeEDDHA had the lowest and highest mean substrate pH at 6.36 and 6.47, respectively (Table 3). Substrate pH for FeEDDS was not different from the other Fe chelates but was significantly higher than the FeSO₄ treatment by 0.07 pH units (Table 3). Peat-based media are amended with liming agents to maintain a substrate pH suitable for the production of horticultural crops. A substrate pH of 5.4 to 6.8 is considered suitable for greenhouse bedding crops grown in peat-based media (Nelson, 1999), and over the course of the study, substrate pH for FS treatments fell within this range with a mean pH of 6.42 (Table 3).

Effects of time ($P < 0.001$) and FS ($P = 0.014$) were significant for EC, but there was no significant interaction between main effects ($P = 0.536$) (Table 3). Mean substrate EC decreased from 1.13 mS·cm⁻¹ to 0.53 mS·cm⁻¹ from the first to final PT (Table 3). Iron-EDTA and FeEDDHA had the highest (0.83 mS·cm⁻¹) and FeDTPA the lowest (0.74 mS·cm⁻¹) substrate EC levels (Tables 3). Iron-EDDS had significantly higher EC than FeDTPA treatment by only 0.07 mS·cm⁻¹ and was not different from the other Fe sources (Table 3). According to Lang (1996), EC between 3.0 to 5.0 mS·cm⁻¹ is generally associated with normal plant growth when interpreting results from the PT method. However, in this study, EC levels were much lower than these values (Table 3), but no signs of nutrient disorders were observed visually or determined by SPAD index readings; and foliar nutrient levels for Cu, Fe, Mn, and Zn were interpreted as sufficient for normal plant growth (Table 1). The observed lower EC levels are probably the result that PTs were conducted in conjunction with DI application and not nutrient (i.e., FS) application.

Effects of time (DAS) ($P < 0.005$), FS ($P < 0.001$), and interaction between main effects ($P < 0.001$) were significant for Cu (Table 3). Except for FeDTPA, substrate Cu levels were generally consistent over time with the FeSO₄ treatment resulting in substrate Cu levels that matched or were below the ICP-OES mini-

mum detection limit for the metal at 3 µg·L⁻¹ (data not shown). Iron-DTPA substrate Cu level of 34 µg·L⁻¹ in extracts was five times greater than the mean of the other FS treatments combined at 6 µg·L⁻¹. Iron-EDDS substrate Cu was only significantly different from the FeDTPA treatment (Table 3).

Effects of time (DAS), FS, and their interaction were significant at $P < 0.0001$ each

for PT Fe (Table 3). Iron sulfate and FeEDDS were not different and had relatively low levels of substrate Fe (less than 40 µg·L⁻¹) (Table 3). In contrast, FeEDTA, FeEDDHA, and FeDTPA treatments resulted in substrate Fe levels greater than 200 µg·L⁻¹ (Table 3) For FeEDTA, FeEDDHA, and FeDTPA treatments, Fe concentration in PT extract increased an average of 42% from the first to

Table 1. Marigold foliar plant tissue nutrient concentration for plants grown in five differing iron sources (FS in article) was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES); n = 10.

Fe source	Metals (µg·g ⁻¹)			
	Fe	Manganese	Zinc	Copper
FeEDDS	130.72	232.53	36.48	6.39 ab ^z
FeEDTA	161.32	227.75	34.75	5.66 bc
FeDTPA	133.41	210.20	33.79	7.04 ab
FeEDDHA	207.14	210.12	33.91	7.11 a
FeSO ₄	176.64	259.11	35.99	4.58 c
Mean	161.85	227.94	34.98	6.16
Sufficiency range ^y	90–250 ^y	75–300	25–100	5–28
Significance (<i>P</i>)	0.3924	0.3035	0.7861	0.0041

^zMeans followed by the same letter in the same column are not significantly different (Tukey; $P \leq 0.05$). Where no letters appear in a column, effects of FS were not significant.

^ySufficiency range interpreted according to Vetanovetz (1996).

Table 2. Effects of iron (Fe) source on leaf tissue, pour-through (PT in article), and leachate runoff (LR in article) nutrient concentrations.^z

Fe source	pH	Electrical conductivity (mS·cm ⁻¹)	Metal concn (mg·L ⁻¹)			
			Copper	Fe	Manganese	Zinc
<i>Leaf tissue</i>						
FeSO ₄	— ^y	—	4.58	176.64	259.11	35.99
Fe chelates	—	—	6.55	162.41	220.15	34.73
Significance (<i>P</i>)	—	—	0.001	0.696	0.040	0.539
<i>PT</i>						
FeSO ₄	6.33	0.83	0.003	0.018	0.101	0.029
Fe chelates	6.42	0.82	0.014	0.301	0.121	0.058
Significance (<i>P</i> value)	0.003	0.758	<0.001	<0.001	0.193	0.006
<i>LR</i>						
FeSO ₄	6.06	1.46	0.029	0.068	0.214	0.073
Fe chelates	6.13	1.45	0.036	0.438	0.218	0.085
Significance (<i>P</i>)	0.001	0.828	0.249	<0.001	0.669	0.158

^zFor chelated sources, n = 40 (FeEDDS, FeEDTA, FeDTPA, and FeEDDHA) and for the non-chelated Fe source (FeSO₄), n = 10.

^yData not collected.

Table 3. Summary statistics for pour-through (PT in article) extracts by time [time; days after sowing (DAS)] and by iron (Fe) treatment [Fe source (FS in article)], n = 10.

Electrical conductivity			Metal concn (µg·L ⁻¹)			
	pH	(mS·cm ⁻¹)	Copper	Fe	Manganese	Zinc
DAS			<i>Time</i>			
30	6.26 e ^z	1.13 a	10 b	194 b	218 a	120 a
34	6.35 d	1.08 a	11 b	189 b	131 b	59 b
38	6.50 b	0.73 b	— ^y	—	—	—
42	6.43 c	0.56 c	10 b	284 a	71 c	15 c
46	6.56 a	0.53 c	15 a	309 a	47 c	15 c
Fe source			<i>Fe treatment</i>			
FeEDDS	6.43 ab	0.81 a	6 bc	33 d	80 c	28 c
FeEDTA	6.41 bc	0.83 a	7 bc	234 c	156 a	70 b
FeDTPA	6.42 ab	0.74 b	34 a	545 a	132 ab	111 a
FeEDDHA	6.47 a	0.83 a	10 b	381 b	115 abc	23 c
FeSO ₄	6.36 c	0.81 a	3 c	17 d	101 bc	29 c
Source			<i>Significance (P)</i>			
Time (DAS)	<0.001	<0.001	<0.005	<0.001	<0.001	<0.001
Fe source (FS)	0.004	0.014	<0.001	<0.001	<0.005	<0.001
DAS × FS	0.605	0.536	<0.001	<0.001	0.463	<0.001

^zMeans followed by the same letter in the same column are not significantly different (Tukey; $P \leq 0.05$).

^yData not collected.

final PT event (data not shown). By the final PT event, FeDTPA had substrate Fe levels of $700 \mu\text{g}\cdot\text{L}^{-1}$, $\approx 70\%$ of that supplied (data not shown). In contrast, for the FeEDDS treatment, substrate Fe remained relatively consistent over the course of PT sampling events at less than $100 \mu\text{g}\cdot\text{L}^{-1}$ Fe (data not shown). Spectral scans of PT extracts were performed to qualitatively assess Fe complexed with chelates as determined by ultraviolet absorbance (250–300 nm). In Figure 1A, spectral scans for Fe source treatment standards prepared at $2 \text{ mg}\cdot\text{L}^{-1}$ Fe were determined. There is a unique profile for FeEDDHA characterized by a “hump” at $\approx 280 \text{ nm}$. Iron-EDDHA’s profile is clearly visible in the PT extract scan (Fig. 1B). Based on this and comparing Figures 1A and 1B, and considering PT Fe concentrations, PT extracts contained those Fe chelates. Therefore, under the substrate conditions in the current study, both absorbance and Fe concentration in PT extracts followed the profile $\text{FeDTPA} > \text{FeEDDHA} > \text{FeEDTA} > \text{FeEDDS} > \text{FeSO}_4$.

Effects of time ($P < 0.001$) and FS ($P < 0.005$) were significant for Mn. However, the interaction between main effects was not significant ($P = 0.4627$). Manganese decreased from $218 \mu\text{g}\cdot\text{L}^{-1}$ for the first PT event to $47 \mu\text{g}\cdot\text{L}^{-1}$ for the final PT event, a 78% reduction (Table 3). Iron-EDDS had the lowest Mn level

at $80 \mu\text{g}\cdot\text{L}^{-1}$, whereas FeEDTA had the highest Mn level at $156 \mu\text{g}\cdot\text{L}^{-1}$ (Table 3).

The effects of time (DAS), FS, and their interaction were significant at $P < 0.001$ each for Zn (Table 3). Zinc decreased from $120 \mu\text{g}\cdot\text{L}^{-1}$ to $15 \mu\text{g}\cdot\text{L}^{-1}$ from the first to final PT event, respectively, an 88% reduction (Tables 3). Iron-DTPA had the highest Zn level at $111 \mu\text{g}\cdot\text{L}^{-1}$, whereas FeEDDS along with FeEDDHA and FeSO_4 had the lowest Zn levels with a mean value of $27 \mu\text{g}\cdot\text{L}^{-1}$ Zn (Table 3).

Runoff. Leachate runoff at each treatment application (i.e., FS) and DI leach were collected, averaging $34 \pm 6 \text{ L}$ per treatment over the course of the study (data not shown). Effects of FS on pH, Cu, Fe, Mn, and Zn were significant ($P \leq 0.005$) (Table 4). Runoff pH was lowest and highest in the FeSO_4 (6.06) and FeEDDHA (6.17) treatments, respectively (Table 4). These differences may be statistically significant, but probably not biologically significant. Iron-EDDS was not significantly different from other chelated Fe sources but was significantly higher than the FeSO_4 treatment. Effects of FS were not significant for EC, ranging from 1.42 to $1.46 \text{ mS}\cdot\text{cm}^{-1}$ (Table 4). Runoff Cu, Fe, and Zn was greatest in the FeDTPA treatment, whereas Mn was greatest in the FeEDTA treatment (Table 4). The greatest range in micronutrient concentration for any metal was observed for Fe where FeDTPA and FeSO_4 were $705 \mu\text{g}\cdot\text{L}^{-1}$ and $68 \mu\text{g}\cdot\text{L}^{-1}$ Fe, respectively (Table 4), demonstrating the chelating agent’s ability to extract Fe from peat-based physical components, differences in solubility in the substrate, or both. Data were analyzed comparing Fe chelate vs. FeSO_4 and it was found that chelated sources had significantly higher pH and Fe than the non-chelate source (Table 2), consistent with work by Albano et al. (1996). As was done with PT, LR composite samples were analyzed by spectrophotometer to qualitatively determine the presence of Fe chelates. Comparing the Fe chelate spectra in Figure 1A with that in Figure 1C, Fe chelates were leached in runoff. Under the substrate conditions in the current study, both absorbance and Fe concentration in LR extracts followed the profile $\text{FeDTPA} > \text{FeEDDHA} > \text{FeEDTA} > \text{FeEDDS} > \text{FeSO}_4$ (Table 4; Figs. 1A and 1C). This profile is consistent with that observed for PT.

Stability of a metal–chelate complex is pH dependent. For practical purposes, EDDS, EDTA, DTPA, and EDDHA form stable chelate complexes with Fe from 3.0 to 9.0, 4.0 to 6.3, 4.0 to 7.0, and 4.0 to 9.0, respectively (Bucheli-Witschel and Elgi, 2001; Jones and Williams, 2001; Norvell, 1971; Orama et al., 2002; Reed, 1996). To note, Bucheli-Witschel and Elgi (2001) and Orama et al. (2002) do not provide information on the percent Fe chelated by EDDS within the pH range 3.0–9.0; therefore, to determine EDDS’ practical chelation pH range with Fe for use in horticultural crop production would require further research. Within these specific pH ranges, these chelates preferentially bind with Fe, Cu, Zn, and Mn in that order. The general pH (6.42) observed in the present study favors DTPA in percent of metals chelated. Indeed, the FeDTPA treatment, in general, was associated with higher levels of Cu, Fe, Mn, and Zn in both PT extracts and LR leachates (Tables 3 and 4). In LR extracts, FeEDDS had very low levels of Cu, Fe, Mn, and Zn (Table 3). Very little is known about the interaction of EDDS with peat-based media physical components. To determine this would require studies without plants and as a single applied component (i.e., Fe chelate or chelate ligand). Regardless, the low level of PT or LR metals for FeEDDS may indicate that this chelate is sorbed to peat like has been described for FeEDDHA at low pH (Boxma, 1981).

Conclusions

Results of the present study support those of Albano (2011). Iron-EDDS is a suitable source of Fe for the production of marigold and most likely other horticultural crops. Iron source (FeEDDS, FeEDTA, FeDTPA, FeEDDHA, and FeSO_4) had no influence on marigold leaf count, plant height, leaf dry mass, or leaf greenness nor were there any signs of Bronze Speckle or Micronutrient Toxicity Syndrome (MTS) [Fe, Mn, or a combination of both toxicity (Albano et al., 1996; Hulme and Ferry, 1999)]. There were no statistically significant differences in foliar Fe, Mn, or Zn and no practical difference in Cu between FS treatments. Even the non-chelate treatment, FeSO_4 , where all micronutrients (Cu, Fe, Mn, and Zn) were supplied

Fig. 1. Spectra for iron (Fe) source [FeSO_4 , FeEDTA, FeEDDS, FeDTPA, and FeEDDHA (FS in article)] standards ($2 \text{ mg}\cdot\text{L}^{-1}$ Fe; A), pour-through (PT; B), and leachate runoff (LR; C). For B and C, n = composite samples of 40 and 50, respectively.

Table 4. Leachate runoff (LR in article) was collected over the course of the study [22 collection dates ($n = 22$)]².

Fe source	pH	Electrical conductivity ($\text{mS}\cdot\text{cm}^{-1}$)	Metal concn ($\mu\text{g}\cdot\text{L}^{-1}$)			
			Copper	Fe	Manganese	Zinc
FeEDDS	6.13 ab ²	1.46	36 ab	159 d	181 c	76 b
FeEDTA	6.11 b	1.46	28 b	417 c	250 a	85 ab
FeDTPA	6.12 b	1.42	46 a	705 a	223 b	103 a
FeEDDHA	6.17 a	1.45	31 ab	469 b	218 b	75 b
FeSO_4	6.06 c	1.46	29 b	68 e	214 b	73 b

Significance (P) 0.004

0.730

<0.001

<0.001

<0.001

<0.001

²Copper, Fe, manganese, and zinc were determined by inductively coupled plasma–optical emission spectroscopy.

³Means followed by the same letter in the same column are not significantly different (Tukey; $P \leq 0.05$). Where no letters appear in a column, effects of Fe source were not significant.

as sulfate salts, had foliar Cu, Fe, Mn, and Zn levels that were considered sufficient for normal growth of bedding plants. However, producing concentrated fertilizer stock solutions formulated with non-chelated micronutrients is practically impossible as a result of the low solubility of Cu, Fe, Mn, and Zn salts. For growers to have the convenience of a single fertilizer product that can be maintained as a concentrated stock solution, which contains both macro- and micronutrients, APCA complexones (chelates) are required to sustain solubility of these metals.

Although there were no practical differences between Fe source treatments on plant physical or nutritional characteristics, differences between FS treatments were observed for PT and LR studies. In general, pH and EC were not different for FS treatments for any PT sampling event, but pH and EC significantly increased and decreased, respectively, over time. For any FS treatment, FeDTPA, in general, was associated with or near the highest concentration of Cu, Fe, Mn, or Zn for any PT sampling event. Substrate pH over the course of the study averaged 6.42, which based on DTPA's pH stability constant for these metals, especially Fe, 4.0 to 7.0 pH, supports these findings. Iron-EDDS compared with other Fe chelate treatments, in general, resulted in the lowest levels of Cu, Fe, Mn, and Zn in PT extracts. The significance of this is that FeEDDS/EDDS metal chelates may be a better fertilizer agent to use in formulating fertilizers for bedding plants susceptible to Fe/Mn toxicity problems (Bronze Speckle or MTS), but research on the toxicity profile of FeEDDS would need to be conducted to test this hypothesis. As for runoff (LR), leachate pH for chelated sources of Fe was significantly higher than for the sulfate source, although the difference may be of little practical significance. Iron chelates were found in both PT and LR samples. The significance of this is that it is possible for APCA complexones to be leached intact in runoff, thus leading to the contamination of receiving water bodies that in turn degrade water quality with these chemical compounds.

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