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Effects of Simulated Oilfield Produced Water on Early Seedling Growth After Treatment in a Pilot-Scale Constructed Wetland System

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Seed germination and early seedling growth bioassays were used to evaluate phytotoxicity of simulated oilfield produced water (OPW) before and after treatment in a subsurface-flow, pilot-scale constructed wetland treatment system (CWTS). Responses to untreated and treated OPW were compared among seven plant species, including three monocotyledons: corn *(Zea mays)*, millet *(Panicum miliaceum)*, and sorghum *(Sorghum bicolor)*; and four dicotyledons: lettuce *(Lactuca sativa)*, okra *(Abelmoschus esculents)*, watermelon *(Citrullus lanatus)*, and soybean *(Glycine max)*. Phytotoxicity was greater in untreated OPW than in treated OPW. Exposures to untreated and treated OPW enhanced growth in some plant species (sorghum, millet, okra, and corn) relative to a negative control and reduced growth in other plant species (lettuce, soybean, and watermelon). Early seedling growth parameters indicated that dicotyledons were more sensitive to test waters compared to monocotyledons, suggesting that morphological differences between plant species affected phytotoxicity. Results indicated the following sensitivity scale for plant species: lettuce > soybean > watermelon > corn> okra≈millet >sorghum. Phytotoxicity of the treated OPW to lettuce and soybean, although concentrations of COCs were less than irrigation guideline concentrations, suggests that chemical characterization and comparison to guideline concentrations alone may not be sufficient to evaluate water for use in growing crops.

Keywords: phytoremediation, phytotoxicity, seed germination

Introduction

Oilfield produced water (OPW) is water brought to the surface as a result of oil production (Veil *et al.* 2004). OPW is a complex mixture often containing metals and organics that must be treated prior to use for purposes such as irrigation. In recent years, production of oil in sub-Saharan Africa has generated thousands of barrels of OPW (Horner, Castle, and Rodgers 2011). If water produced from oilfields in sub-Saharan Africa can be treated, this large volume of water has the potential for use in irrigation, which could augment inadequate existing water resources. Constructed wetland treatment systems (CWTSs) have been used to treat a variety of wastewaters including water originating from farming practices, mines, petroleum industry processes, flue gas desulfurization, and oilfields (Cronk 1996; Hawkins *et al.*1997; Barton and Karathanasis 1998; Knight, Kadlec, and Ohlendorf 1999; Gillespie *et al.* 2000; Huddleston *et al.* 2005; Mooney and Murray-Gulde 2008; Murray-Gulde *et al.* 2003; Rodgers and Castle 2008) and are a potential treatment method for OPWs. An existing pilot-scale CWTS achieving acceptable levels of chemical treatment (treatment of concentrations of constituents of concern to below irrigation water use guidelines) provided an opportunity to examine phytotoxic responses to exposures of both untreated and treated simulated OPW as part of a comprehensive evaluation of OPW for irrigation (Horner *et al.* 2011).

Simulated OPW used in this investigation was based on characterization of water from specific sub-Saharan oil fields by Horner *et al.* (2011). They measured water chemistry parameters (pH, alkalinity, hardness, total dissolved solids, and others) of the OPW and concentrations of major and trace inorganic constituents. Based on comparison with water use guidelines, Horner *et al.* (2011) determined that concentrations of oil and grease (O & G), iron, manganese, nickel, and zinc in the OPW exceeded guideline concentrations for irrigating crops and deemed these to be constituents of concern (COCs). However, measurement of traditional parameters and comparison with guideline concentrations alone are not sufficient for determination of suitability of a water for irrigation. To be used for irrigation, water must be suitable for seed

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Table 1. Analytical methods, detection limits, and characteristics of untreated OPW and treated OPW used in phytotoxicity tests

Parameter	Method	Units	MDL[1]	OPW analyzed by Horner et al. (2011)	Untreated OPW (inflow)[2]	Treated OPW (outflow)[2]
Temperature	YSI Model 52[3]	°C	0.5	na	26.3 (26.1–26.4)	26.9 (26.5–27.2)
pH	Orion Model 420A[3]	S.U.	0.01	7.83 (7.2–8.5)	7.16 (7.10–7.21)	6.59 (6.48–6.65)
Conductivity	YSI 30[3]	μS/cm	0.1	1139 (838–1500)	212 (209–218)	225 (219–233)
Alkalinity	Standard Methods 2320 B (APHA, 2005)	mg/L as $CaCO_3$	2	326 (300–380)	43 (38–46)	35 (32–40)
Hardness	Standard Methods 2340 C (APHA, 2005)	mg/L as $CaCO_3$	2	10.9 (5–20)	32 (27–39)	46 (38–62)
DO[4]	YSI Model 52[3]	mg/L	0.1	na	7.87 (7.83–7.95)	5.91 (5.82–6.01)
Oil & Grease[5]	EPA Method 1664 (USEPA, 1999)	mg/L	1.4	103.8	102.7 (98.3–105.1)	3.6 (2.7–4.9)
Iron[5]	EPA Method 200.7 (USEPA, 1994)	mg/L	0.0062	0.12 (0.05[6] – 0.5)	0.409 (0.375–0.430)	0.027 (0.015–0.050)
Manganese[5]	EPA Method 200.7 (USEPA, 1994)	mg/L	0.0014	3.51 (0.01[6] –8.1)	1.292 (1.253–1.367)	0.023 (0.019–0.028)
Nickel[5]	EPA Method 200.7 (USEPA, 1994)	mg/L	0.0015	4.27 (0.01[6] – 9.5)	1.327 (1.288–1.381)	0.045 (0.042–0.050)
Zinc[5]	EPA Method 200.7 (USEPA, 1994)	mg/L	0.0018	7.09 (0.02[6] – 17.4)	5.69 (5.50–5.83)	0.119 (0.104–0.147)

na = not available.
[1] Method Detection Limit.
[2] Mean (range), n = 3.
[3] Direct instrumentation.
[4] Dissolved Oxygen.
[5] Identified by Horner *et al.* (2011) as a COC; therefore, concentration in the untreated OPW (column 6) was targeted to be within the range of concentrations in the OPW samples analyzed (column 5).
[6] Detection limit of method used by Horner *et al.* (2011).

germination and plant growth. Concentrations of COCs may be less than irrigation guideline concentrations, but potential synergistic effects of multiple constituents in complex mixtures such as OPW may result in toxicity. These synergistic effects can be revealed through bioassays such as phytotoxicity testing (Belin, McCaskey, and Black 2000; Banks and Schultz 2005; Andrade *et al.* 2010). Therefore, this research utilized phytotoxicity tests to determine if the OPW was potentially suitable for irrigation to supplement the comparison of OPW chemical characteristics with irrigation guideline concentrations by Horner *et al.* (2011). Seed germination and early growth bioassays were used to evaluate potential phytotoxicity of the simulated OPW from specific oilfields in sub-Saharan Africa before and after treatment in a pilot-scale CWTS. In this study, pre- and post-treatment waters were considered phytotoxic if plant health was visually affected (wilting of plant, discoloration of plant, necrosis) or measured quantitatively (decreased seed germination percentage, shoot elongation, root elongation, fresh mass, and dry mass). The sensitivities of plant species to COCs in OPW may differ considerably, so several monocotyledons and dicotyledons grown as food crops in the region (FAOSTAT 2011) were evaluated. Specific objectives of this investigation were: 1) to compare untreated and treated simulated OPW in terms of phytotoxicity, and 2) to contrast responses among different plant species to untreated and treated simulated OPW.

Materials and Methods

Pilot-Scale CWTS and Simulated OPW

A subsurface-flow (SSF), pilot-scale CWTS designed and constructed by Pham, Castle, and Rodgers (2011) and used by Horner *et al.* (2012) to assess treatment of the OPW was also used for this investigation. This system was designed based on biogeochemical pathways to reduce aqueous concentrations of targeted COCs (O & G, Fe, Mn, Ni, Zn) in the simulated OPW. The CWTS consisted of four wetland cells aligned in series. Each cell was contained in a 378–L Rubbermaid® utility tank, 123 cm long by 64 cm wide by 61 cm deep. Hydrosoil in cells was composed of 20 cm of pea gravel (5–10 mm in diameter) overlain by 40 cm of medium-sized granitic gravel (20–30 cm in diameter). Cells were planted with *Phragmites australis* (common reed), a macrophyte native to sub-Saharan Africa. The system was loaded with simulated OPW at an inflow rate providing a nominal 4-day hydraulic retention time (HRT) (24-h for each wetland cell). Use of simulated OPW reduces transportation costs associated with using actual OPW and allows precise control of water composition. Simulated OPW was formulated by addition of Shell Rotella T® motor oil and high purity salts (Fisher Scientific Inc., Fair Lawn, NJ) of $FeCl_3$, $MnCl_2 \bullet 4H_2O$, $NiCl_2 \bullet 6H_2O$, and $ZnCl_2$ to a 3,785-L polypropylene carboy retention basin containing municipal water from Clemson, South Carolina.

Table 2. Environmental conditions of bioassays

	Description
Plant Species	Monocots: corn (*Zea mays*) millet (*Panicum miliaceum*) sorghum (*Sorghum bicolor*) Dicots: lettuce (*Lactuca sativa*) okra (*Abelmoschus esculents*) watermelon (*Citrullus lanatus*) soybean (*Glycine max*)
Replicates	30 per seed species
Test waters	Simulated untreated OPW, treated OPW, and municipal water
Experimental chamber	Molded plastic container (10-cm deep)
Growth substrates	Quartz sand and Miracle Grow® potting soil
Exposure	250 mL test water at start of test in each container, additional water to maintain moisture in soil
Duration	21 days per test
Test conditions	Climate-controlled greenhouse at 25 ± 5°C and natural light/dark cycle
Seed germination parameters	Percent germination
Measured early seedling growth parameters	Root elongation, shoot elongation, total fresh mass, total dry mass
Visually observed early seedling growth parameters	Root necrosis, browning of leaves, wilting of leaves, hardening of leaves, growth stunting

Concentrations of COCs in the simulated water were targeted to be within the concentration ranges reported in actual sub-Saharan OPW by Horner *et al.* (2011). Additional salts ($NaHCO_3$, $MgSO_4 \bullet 7H_2O$, KNO_3, and $CaCO_3$) were added to simulate hardness, pH, and ionic composition of OPW. Nominal ion concentrations (mg/L) were 9.56 Cl, 6.14 Ca, 1.3 Mg, 15.5 K, 18.0 Na, 14.9 CO_3^{2-}, 38.4 HCO_3^-, and 3.2 SO_4^{2-}. Simulated OPW flowed from the retention basin to the first cell of the pilot-scale CWTS via Fluid Metering Inc. (FMI®) piston pumps operating at a constant flow rate.

General water chemistry parameters (temperature, pH, conductivity, alkalinity, hardness, and dissolved oxygen concentration), O & G concentration, and concentrations of metals (iron, manganese, nickel, and zinc) were measured in treated and untreated waters to verify exposures (Table 1). Untreated OPW was collected from the retention basin, and treated OPW was collected from outflow of the fourth (final) wetland cell. Temperature, pH, conductivity, and dissolved oxygen concentration were measured using direct instrumentation, and alkalinity and hardness were measured in accordance with standard methods (APHA 2005). EPA method 1664 (USEPA 1999), a gravimetric method involving n-hexane extraction, was used for measuring O & G concentrations using the StepSaver™ apparatus (Environmental Express, Mount Pleasant, South Carolina, USA). Metal concentrations were measured using inductively coupled plasma-atomic emission spectrometry [EPA method 200.7 (USEPA 1994); ICP-AES, SPECTROFLAME-EOP, Spectro Analytical Instruments, Kleve, Germany].

Quality assurance and quality control (QA/QC) for ICP-AES metal analyses involved a standard recovery and standard addition every ten samples. Analyses were considered acceptable if recoveries of standards were within ±10% of calibration concentrations for metals and recoveries of standard additions were between 70–130 % (USEPA 1994). For O & G analyses, QA/QC was confirmed by measurement of motor oil standards and matrix spikes every ten samples. Recoveries within 78–114 % for standards and matrix spikes were considered acceptable (USEPA 1999).

Phytotoxicity Testing

Effects of OPW on seed germination and early seedling growth were determined using OECD (2003) methods, with slight modifications to compare phytotoxicity between untreated and treated OPW and to compare responses among plant species (Table 2). Plant species were selected for experimentation based on agricultural importance in sub-Saharan Africa (FAOSTAT 2011), sensitivity, availability, physical differences (seed size), and differences in morphology (monocots and dicots). Species selected included three monocots [corn (*Zea mays*), millet (*Panicum miliaceum*), and sorghum (*Sorghum bicolor*)] and four dicots [lettuce (*Lactuca sativa*), okra (*Abelmoschus esculents*), watermelon (*Citrullus lanatus*), and soybean (*Glycine max*)]. All species selected for evaluation are food crops that grow in this climate (FAOSTAT 2011).

Two test waters (simulated untreated OPW and treated OPW), one negative control water (Clemson, SC municipal water), one test growth substrate (quartz sand), and one negative control test growth substrate (Miracle Grow® potting soil) were used in this experiment. Quartz sand was used as the test growth substrate to comply with accepted methods (USEPA 1996; OECD 2003) and to maximize bioavailability of COCs. Each plant species was exposed to the following treatments: 1) untreated OPW in quartz sand; 2) treated OPW in quartz sand; 3) municipal water in quartz sand; and 4) municipal water in potting soil. The experimental design constrained effects on early seedling growth due to COCs by comparison of results from treatments in quartz sand with untreated OPW, treated OPW, and municipal water; all variables but water were constant in these treatments. The municipal water and potting soil treatment provided sufficient nutrients to confirm seed viability. Observed seed germination percentages in the municipal water and potting soil treatment were

Table 3. Mean values ± one standard deviation (n = 30) measured for early seedling growth parameters and seed germination parameters

		Elongation		Mass			
Plant	*Treatment	Root (cm)	Shoot (cm)	Fresh (g)	Dry (mg)	Root necrosis, %	Seed germination, %
Corn	1	12.30 ± 6.04*a*	21.90 ± 3.79*a*	0.870 ± 0.237*b*	263.1 ± 61.8*b*	0	90
	2	11.34 ± 5.73*a*	22.54 ± 3.74*a*	1.472 ± 0.317*a*	330.9 ± 80.9*a*	0	94
	3	10.09 ± 4.81*a*	18.77 ± 3.12*b*	1.337 ± 0.264*a*	263.7 ± 70.9*b*	0	89
	4	10.29 ± 5.46	43.61 ± 7.48	2.991 ± 1.070	442.3 ± 172.6	0	97
Millet	1	nm 6.0pt1,0.0pt	4.08 ± 0.64*a*	0.048 ± 0.012*a*	14.47 ± 3.22*a*	5	97
	2	nm	4.29 ± 0.60*a*	0.045 ± 0.008*a*	13.21 ± 2.70*a*	2	92
	3	nm	3.12 ± 0.48*b*	0.034 ± 0.005*b*	12.75 ± 2.58*a*	10	91
	4	nm	23.58 ± 2.99	0.453 ± 0.149	60.80 ± 19.25	0	99
Sorghum	1	12.80 ± 4.82*ab*	14.87 ± 2.26*a*	0.219 ± 0.063*ab*	80.3 ± 20.2*a*	0	92
	2	14.25 ± 4.34*a*	15.15 ± 1.72*a*	0.245 ± 0.057*a*	81.1 ± 17.5*a*	0	94
	3	11.02 ± 4.11*b*	13.38 ± 1.74*b*	0.191 ± 0.056*b*	67.4 ± 15.5*b*	0	91
	4	12.98 ± 3.62	40.94 ± 4.04	1.088 ± 0.275	354.9 ± 85.4	0	96
Lettuce	1	nm	0.68 ± 0.25*b*	0.030 ± 0.011*b*	5.0 ± 2.3*b*	0	86
	2	nm	0.87 ± 0.24*a*	0.040 ± 0.010*a*	5.9 ± 2.0*b*	0	88
	3	nm	0.95 ± 0.29*a*	0.046 ± 0.009*a*	7.7 ± 1.7*a*	0	84
	4	nm	12.29 ± 1.42	4.521 ± 1.469	269.5 ± 85.4	0	92
Okra	1	10.75 ± 1.53*a*	6.78 ± 0.67*a*	1.100 ± 0.209*a*	200.1 ± 150.1*a*	5	45
	2	7.55 ± 1.38*b*	4.52 ± 0.57*b*	0.679 ± 0.215*b*	166.9 ± 112.7*a*	0	38
	3	4.54 ± 1.48*c*	3.35 ± 0.72*c*	0.510 ± 0.154*b*	124.2 ± 89.4*a*	10	33
	4	8.08 ± 2.05	11.60 ± 3.16	2.595 ± 2.044	390.1 ± 296.0	0	63
Soybean	1	4.46 ± 2.85*c*	5.72 ± 2.50*b*	0.743 ± 0.505*a*	141.6 ± 61.7*b*	20	83
	2	7.41 ± 3.76*b*	6.81 ± 3.22*b*	0.947 ± 0.636*a*	159.8 ± 66.1*b*	15	77
	3	10.09 ± 3.02*a*	10.16 ± 2.42*a*	1.030 ± 0.506*a*	253.4 ± 78.7*a*	0	91
	4	6.54 ± 2.37	25.84 ± 1.75	2.589 ± 0.512	673.6 ± 137.6	0	95
Watermelon	1	nm	5.84 ± 1.67*a*	0.611 ± 0.124*a*	67.8 ± 17.8*b*	0	48
	2	nm	5.89 ± 2.10*a*	0.662 ± 0.111*a*	70.4 ± 20.6*ab*	0	50
	3	nm	6.09 ± 2.24*a*	0.688 ± 0.114*a*	83.7 ± 17.8*a*	0	55
	4	nm	18.37 ± 3.15	4.378 ± 1.096	397.9 ± 120.5	0	84

*Treatment 1 = untreated OPW, quartz sand; treatment 2 = treated OPW, quartz sand; treatment 3 = municipal water, quartz sand; treatment 4 = municipal water, potting soil.
nm = not measured.
Letters indicate Tukey's grouping; treatment 4 was not included in Tukey's analysis because a different growth substrate was used (potting soil); same letters indicate no statistical significance (alpha = 0.05).

compared to the following minimum standard germination percentages: 75% for corn, 75% for millet, 75% for sorghum, 80% for lettuce, 50% for okra, 70% for watermelon, and 75% for soybean (SCCOR 2011). All variables but growth substrate were kept constant in the municipal water treatments.

Experiments, including treatment in the CWTS and phytotoxicity testing, were performed in a climate-controlled greenhouse located in Clemson, South Carolina, from May 5 to July 12, 2013. Temperatures through the duration of the experiment were 25 ± 5°C. Soils were dispensed into molded plastic containers 10−cm deep and 11−cm diameter. Depending on their size, 5–15 seeds were placed 1–2 cm below the soil surface in each container. Seed cultures were exposed to natural light/dark cycles in the greenhouse; no artificial lighting was used. Seedlings were watered daily to keep the soil moist. Indicators of phytotoxicity (chlorosis, stunted growth, and browning of leaves) were observed and recorded daily throughout the experiment.

Twenty-one days following planting, seedlings were harvested, and the following parameters were measured: 1) seed germination percentage, 2) root elongation, 3) shoot elongation, 4) fresh mass of seedling, and 5) dry mass of seedling (dried for 24 hours at 80°C). Seedlings from each container were removed and the roots were gently rinsed using water to remove soil particles. Seed germination percentage was calculated for all plant species (Equation 1).

$$Seed\ germination\,\% = \frac{\#\ of\ seeds\ germinated}{\#\ of\ seeds\ planted} \times 100 \qquad (1)$$

Root and shoot elongation were measured using a ruler. An analytical balance capable of weighing 0.0001 gram was used to measure mass. Root elongation was not measured for millet, lettuce, and watermelon due to the fibrous root structure of these species.

Table 4. Observations of early seedling growth

	Seed characteristics		Early growth observations		
		Seed mass[1] (mg)	*Treatment 1	*Treatment 2	*Treatment 3
Monocots	Corn	200.1 ± 25.4	development of support roots, second leaves present, no observed abnormalities	development of support roots, second leaves present, no observed abnormalities	development of support roots, second leaves present, yellowing of leaf tips
	Millet	7.6 ± 1.3	browning of leaves, dark brown/black coloration (necrosis) of roots, stunted growth	browning of leaves, root necrosis, stunted growth	browning of leaves, root necrosis, stunted growth
	Sorghum	39.8 ± 5.5	development of support roots, second leaves present, no observed abnormalities	development of support roots, second leaves present, yellowing of leaf tips	development of support roots, second leaves present, yellowing of leaf tips, thickened tap root
Dicots	Lettuce	1.4 ± 0.3	stunted growth, browning of leaves	stunted growth, browning of leaves	stunted growth, browning of leaves
	Okra	53.2 ± 7.8	wilting of leaves, root necrosis	yellowing of leaf tips, death after germination	yellowing of leaf tips, root necrosis, death after germination
	Soybean	194.4 ± 22.2	browning of leaves, hardening of leaves, root necrosis, blunt roots, death after germination	browning of leaves, hardening of leaves, root necrosis, blunt roots, death after germination	browning of leaves, blunt roots
	Watermelon	51.8 ± 6.3	browning of leaf tips	browning of leaf tips	browning of leaf tips

[1]Mean mass (± one standard deviation) of 20 individual seeds (n = 20) from the same seed stock used in experiments.
*Treatment 1 = untreated OPW, quartz sand; treatment 2 = treated OPW, quartz sand; treatment 3 = municipal water, quartz sand.
Note: No abnormalities were observed for treatment 4 (municipal water, potting soil).

Data Analysis

Phytotoxicity of untreated and treated OPW was compared using analysis of variance (ANOVA) and Tukey's tests (Statistical Analysis System - SAS Institute 2002) to determine differences in mean values of early seedling growth parameters (root elongation, shoot elongation, fresh mass, and dry mass) among the quartz sand treatments. Early seedling growth parameters were checked for equal variances with an F test (Microsoft® Excel) and normality using a normal probability plot (SAS Institute 2002). In addition to quantitative tests, visually observed early seedling growth parameters were compared qualitatively to assess differences in phytotoxicity between untreated and treated OPW. To estimate phytotoxicity of quartz sand, T-tests using Microsoft® Excel were performed to determine statistical differences in means of the municipal water treatments. The alpha level for all statistical tests was 0.05.

A sensitivity scale was formulated from comparison of responses among plant species to untreated and treated OPW. Based on calculated statistical differences (using ANOVA and Tukey's tests) among values of early seedling growth parameters including root elongation, shoot elongation, fresh mass and dry mass, a sensitivity scale was developed by ranking plant species on a relative scale of sensitivity to test waters. A mean value of a specific early growth parameter (i.e., fresh mass) determined to be statistically greater by Tukey's tests in test waters compared to negative control water indicated low

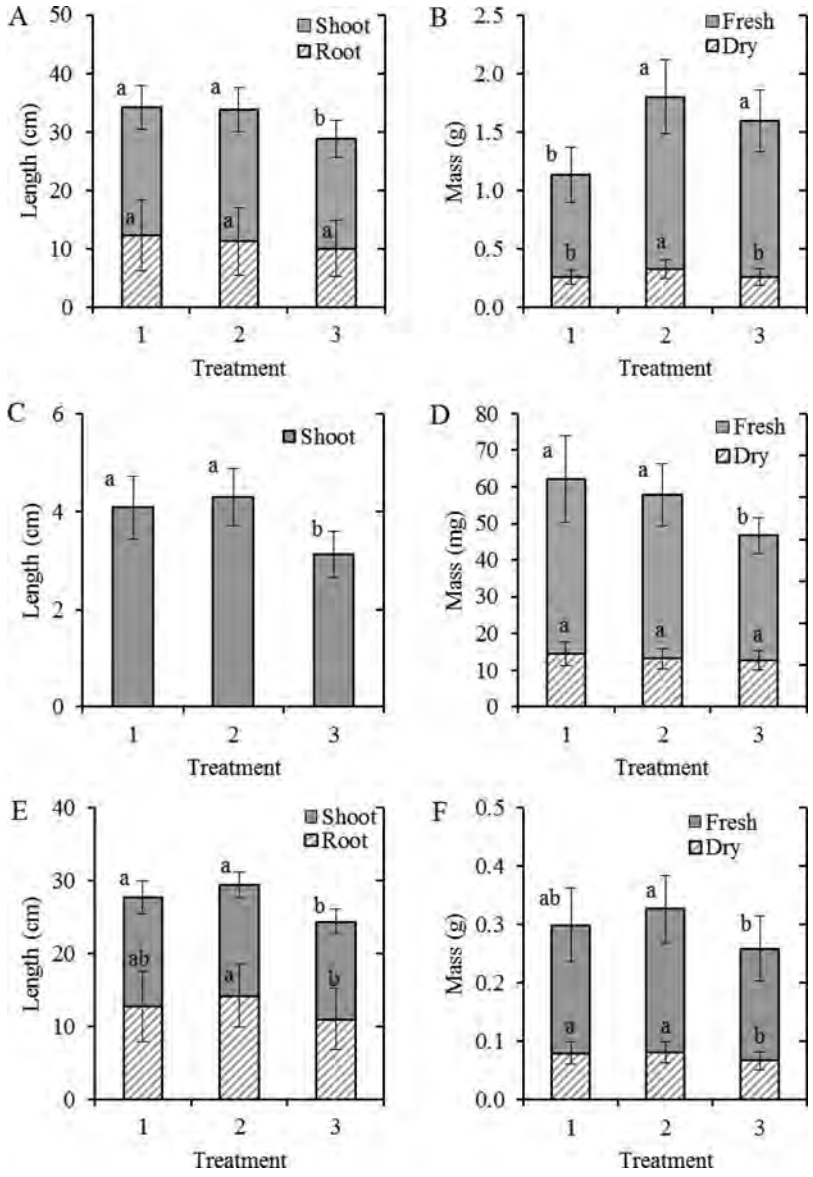

Fig. 1. Means and standard deviations for early seedling growth parameters measured for monocots in quartz sand. A) shoot elongation and root elongation for corn; B) fresh mass and dry mass for corn; C) shoot elongation for millet; D) fresh mass and dry mass for millet; E) shoot elongation and root elongation for sorghum; and F) fresh mass and dry mass for sorghum. Treatment 1 = untreated OPW; treatment 2 = treated OPW; treatment 3 = municipal water (negative control water). Letters represent different Tukey's groupings; same letters indicate no statistical difference. Error bars represent ± one standard deviation from the mean (n = 30).

sensitivity; a mean value of a specific early growth parameter determined to be lesser by Tukey's test in test waters compared to negative control water indicated high sensitivity. The degree of sensitivity for a specific plant was based on the number of early growth parameters that were statistically different between test waters and negative control water. For example, if two early growth parameters were less in test waters compared to negative control water for corn and all four early growth parameters were less in test waters compared to negative control water for okra, okra would be considered more sensitive. Seed germination percentages were not used for developing the sensitivity scale because differences in seed germination percentages among plant species do not necessarily indicate differences in sensitivity (Meyer *et al.*1973).

Results

Viability of Seeds and Nutrient Phytotoxicity

In the municipal water and potting soil treatment, germination percentage of corn, millet, sorghum, lettuce, and soybean was ≥90% (Table 3). Watermelon germination was

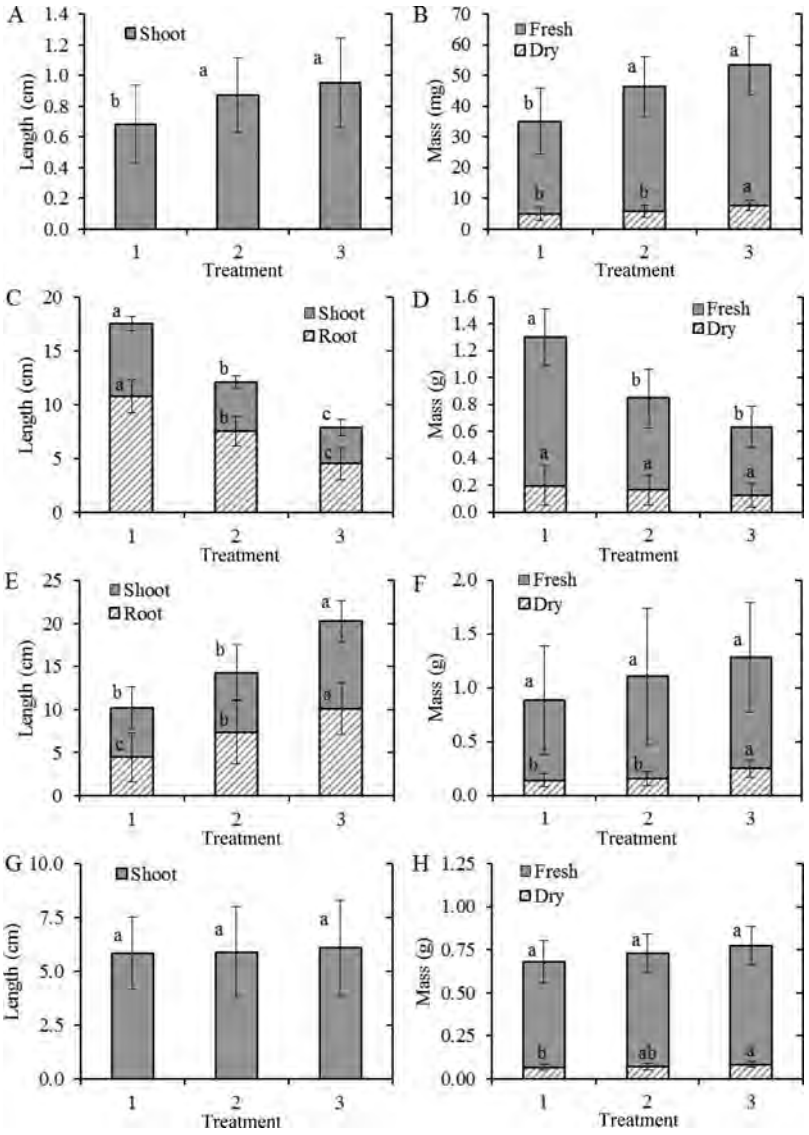

Fig. 2. Means and standard deviations for early seedling growth parameters measured for dicots in quartz sand. A) shoot elongation for lettuce; B) fresh mass and dry mass for lettuce; C) shoot elongation and root elongation for okra; D) fresh mass and dry mass for okra; E) shoot elongation and root elongation for soybean; F) fresh mass and dry mass for soybean; G) shoot elongation for watermelon; H) fresh mass and dry mass for watermelon. Treatment 1 = untreated OPW; treatment 2 = treated OPW; treatment 3 = municipal water (negative control water). Letters represent different Tukey's groupings; same letters indicate no statistical difference. Error bars represent ± one standard deviation from the mean (n = 30).

84%, and okra germination was 63%. Germination percentages for all plant species were greater than minimum germination percentages (SCCOR 2011), which confirmed seed viability.

With exposure to municipal water, the following were statistically greater for the potting soil substrate than for the quartz sand substrate, indicating possible nutrient deficiencies in the quartz sand: shoot elongation, fresh mass, and dry mass for all plant species; and root elongation for okra. Soybean root elongation was greater in the municipal water and quartz sand treatment compared to the municipal water and potting soil treatment.

Comparison of Phytotoxicity Between Untreated and Treated OPW

Chemical analyses indicated that concentrations of metals (iron, manganese, nickel, and zinc) and O & G were higher in the untreated OPW than in the treated OPW (Table 1). Among treatments in quartz sand with untreated OPW, treated OPW, and municipal water, percent germination of seeds ranged from 84–88% for lettuce, 77–91% for soybean, 48–55% for watermelon, and 33–45% for okra (Table 3). Percent germination of corn, millet, and sorghum seeds ranged from 89 to 97%. Percent seed germination was greatest for millet and

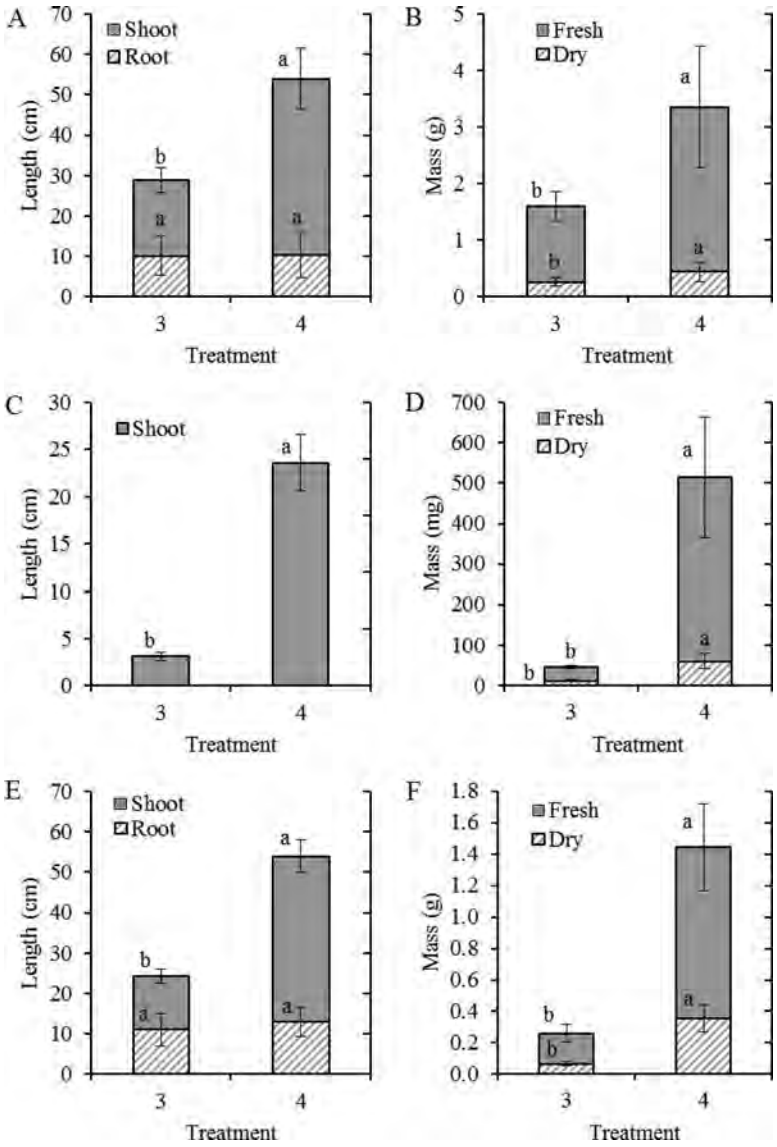

Fig. 3. Means and standard deviations for early seedling growth parameters measured for monocots in municipal water showing differences in growth due to substrate (quartz sand in treatment 3; potting soil in treatment 4). A) shoot elongation and root elongation for corn; B) fresh mass and dry mass for corn; C) shoot elongation for millet; D) fresh mass and dry mass for millet; E) shoot elongation and root elongation for sorghum; and F) fresh mass and dry mass for sorghum. Letters represent different t test groupings; same letters indicate no statistical difference. Error bars represent ± one standard deviation from the mean value (n = 30). Values for all early seedling growth parameters, except for root elongation of corn, are statistically higher in the treatment with potting soil compared to the treatment with quartz sand.

okra in the untreated water; corn, sorghum, and lettuce in the treated water; and soybean and watermelon in the municipal water (negative control). Germination percentages were greater for monocots (corn, millet, and sorghum) than for dicots (lettuce, okra, soybean, and watermelon) in untreated and treated OPW.

Early seedling growth results, including statistical analysis, indicated that phytotoxicity of untreated OPW was greater than that of treated OPW. Using quartz sand as the substrate, statistical differences between untreated OPW, treated OPW, and municipal water were found in at least one early seedling growth parameter for each plant species (Table 3, Figures 1, 2). Values of some early seedling growth parameters (i.e., fresh and dry mass for corn, shoot elongation and fresh mass for lettuce, and root elongation for soybean) were greater for treated OPW than for untreated OPW. Shoot elongation, root elongation, and fresh mass for only one plant species (okra) were greater in untreated OPW compared to treated OPW. No statistical difference in early seedling growth parameters was observed between test waters for millet, sorghum, and watermelon. No value of early growth parameters for monocot seedlings was greater for untreated OPW than for treated OPW. There were no apparent differences in visually observed phytotoxicity indicators (root necrosis, browning or wilting of leaves, stunted growth) between untreated and treated OPW for any of the plant species (Table 4).

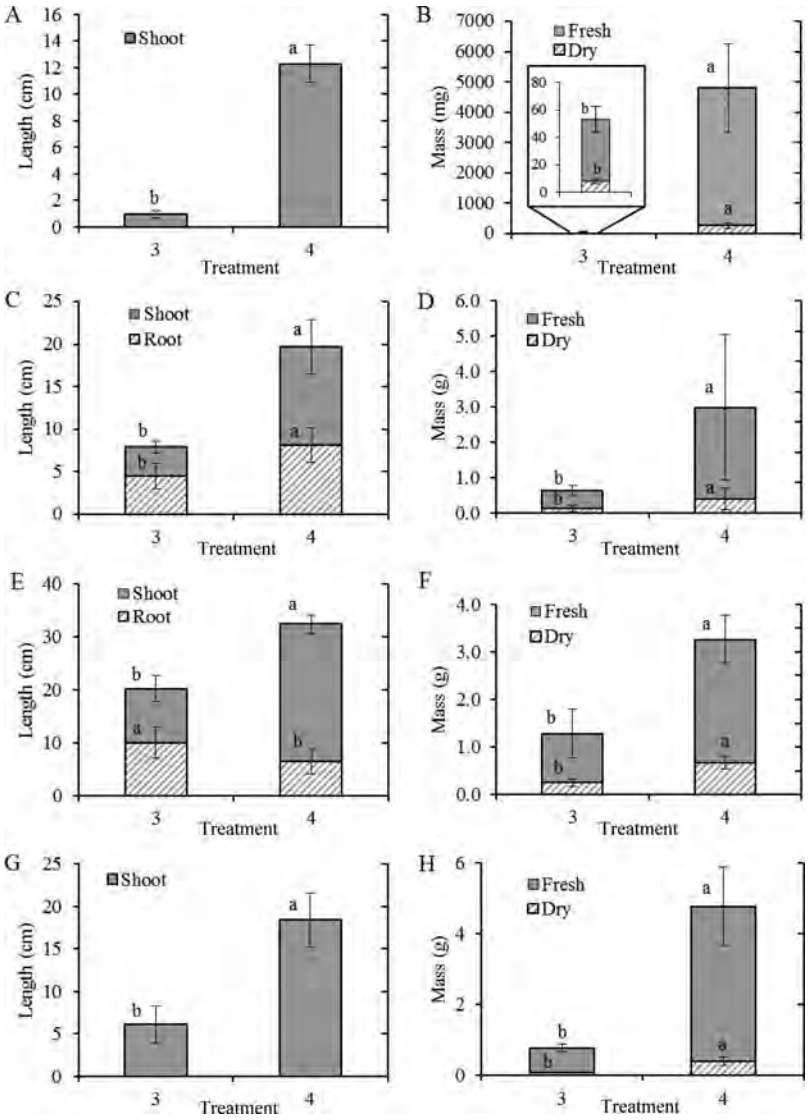

Fig. 4. Means and standard deviations for early seedling growth parameters measured for dicots in municipal water showing differences in growth due to substrate (quartz sand in treatment 3; potting soil in treatment 4). A) shoot elongation for lettuce; B) fresh mass and dry mass for lettuce; C) shoot elongation and root elongation for okra; D) fresh mass and dry mass for okra; E) shoot elongation and root elongation for soybean; F) fresh mass and dry mass for soybean; G) shoot elongation for watermelon; H) fresh mass and dry mass for watermelon. Letters represent different t test groupings; same letters indicate no statistical difference. Error bars represent ± one standard deviation from the mean value (n = 30).

Comparison of Phytotoxicity Among Plant Species

For monocots, the following were greater in test waters (untreated and treated OPW) compared to negative control water: shoot elongation for corn, millet, and sorghum; fresh mass for millet; and dry mass for sorghum. For dicots, shoot and root elongation of okra were greater in test waters compared to negative control water (Table 3). Sorghum was the least sensitive of the plant species examined based on values of all early seedling growth parameters (shoot elongation, root elongation, fresh mass, and dry mass) being greater for sorghum exposed to treated OPW compared to negative control water (Table 3). Values for shoot elongation, fresh mass, and dry mass for lettuce were less in untreated OPW compared to negative control water; therefore lettuce was found to be the most sensitive of the plant species investigated because all early seedling growth parameters measured for this species were statistically less in a test water compared to negative control water. The following were greater in treated and/or untreated OPW compared to negative control water: shoot elongation and fresh mass in both test waters for millet; shoot elongation and root elongation in both test waters for okra, fresh mass in untreated OPW for okra; shoot elongation in both test waters for corn, and dry mass in treated OPW for corn. The following were less in treated and/or untreated OPW compared to negative control water: shoot elongation, root elongation, and dry mass for soybean in treated and untreated OPW; and dry mass for watermelon in untreated OPW.

Based on the number of early seedling growth parameters for each plant species that were statistically greater (indication of low sensitivity) or statistically less (indication of high sensitivity) in test waters compared to negative control water (Table 3), the following sensitivity scale was formulated: lettuce>soybean>watermelon>corn>okra≈millet>sorghum. This scale indicates greater sensitivity of the dicots (lettuce, soybean, watermelon) than the monocots (corn, millet, sorghum). Okra was the only dicot for which observed early seedling growth (root elongation, shoot elongation, and fresh mass) was greater in test waters compared to negative control water.

Discussion

Phytotoxicity can result from various factors including excess nutrients, nutrient deficiencies, or the deficiency of one nutrient induced by another (Reddy 2006). Some elements (carbon and hydrogen) in organic compounds and most heavy (density >5 g/cm^3) metals are considered micronutrients, and their presence in specific concentrations is required for plants to grow (Reddy 2006). The use of quartz sand as a growth substrate may result in nutrient deficiency, as suggested by comparison of the municipal water and quartz sand treatment to the municipal water and potting soil treatment (Figures 3, 4). Some COCs in this study (iron, manganese, nickel, and zinc) are needed for plant growth (Salisbury and Ross 1992), but excess amounts can lead to phytotoxicity (Reddy 2006). Indications of phytotoxicity due to nutrient stress (excess or lack of nutrients) are yellowing of leaves, chlorosis, stunted growth, and necrosis (Reddy 2006). In each of the quartz sand treatments using untreated OPW, treated OPW, and municipal water, at least five plant species showed one or more phytotoxicity indicators.

In a study by Chaîneau, Morel, and Oudot (2000), petroleum (i.e. O & G) had no effects on corn biomass at an exposure of 850 mg/L with a 40-day duration time; with adverse effects observed at 3,300 mg/kg with a 120-day duration time. Baek *et al.* (2004) found no obvious phytotoxic effects in root elongation for corn and red bean (*Phaseolus nipponesis*) in soils contaminated with 0–1,000 mg/kg of aliphatic hydrocarbons with a 14-day duration time. Salanitro *et al.* (1997) found that heavy and medium crude oils in soils at concentrations from 23,600 to 41,400 mg/kg O & G with 21-day duration time did not negatively affect plant dry weight. In this study, O & G concentration in test waters was below 850 mg/L; therefore, based on previous studies, it is unlikely that O & G is responsible for the observed phytotoxicity.

Lettuce is used widely for phytotoxicity tests because of its agricultural importance and high sensitivity to toxic chemicals (Banks and Schultz 2005). Phytotoxicity values for zinc reported for root elongation of lettuce range from an EC50 (effective concentration of a substance estimated to produce a specific adverse effect in 50% of test subjects) of 1.0 mg/L (Fjällborg *et al.* 2006) to 26.19 mg/L (Ronco *et al.* 2000). Zinc concentration in the untreated OPW (5.69 mg/L) falls within this range, and therefore is interpreted to be a possible source of phytotoxicity for lettuce. In previous studies (Fjällborg *et al.* 2006; Salvatore, Carafa, and Carratù 2008), toxic effects were elicited in lettuce at 1.4 mg/L iron (EC50), 28.0 mg/L manganese (EC50), and 13 mg/L nickel (minimum inhibitory concentration). Although these metal concentrations from the previous studies are greater than those in untreated OPW in the current investigation, the duration of exposure in the previous studies was 72 to 96 hours, compared to three weeks (504 hours) in the current study. Kopittke *et al.* (2010) suggested that metal toxicity increases with duration of exposure and that the phytotoxicity endpoint (i.e., no observed effect concentration, EC50, or lowest observed effect concentration) decreases with increase in exposure duration. Therefore, iron, manganese, and nickel may be sources of phytotoxicity in the current study. Seedling exposure was on the order of weeks in this study; therefore, focus was on short term responses, when plants have minimal biomass and maximum exposure to COCs. Experiments on plant uptake/bioconcentration of COCs with longer exposure durations would likely provide additional data pertinent to use of the OPW for crop irrigation.

Soybean showed root necrosis and seedling death after germination in the treated OPW, but not in the control water suggesting phytotoxicity of the treated OPW. However, concentrations of COCs (O & G, Fe, Mn, Ni, Zn) in the treated water were less than irrigation guideline concentrations reported by Horner *et al.* (2011). Also, growth of lettuce and soybean was less in the treated OPW than in the control water, suggesting phytotoxicity. Toxicity tests (seed germination and early seedling growth) are necessary, but not sufficient, for determining suitability of a specific water for crop irrigation. Other considerations include: water quantity available, water use efficiency (crop coefficient), crop growth rate and productivity at latitude of the location, uptake of COCs to edible portions (phytoconcentration), disease susceptibility, and acceptance by the local culture.

Conclusion

Seed germination and early seedling growth bioassays revealed that phytotoxicity was greater in untreated OPW compared to treated OPW. Probable sources of phytotoxicity were metals in test water and nutrient deficiencies in growth substrate. Differences in seed germination and early seedling growth parameters indicated both untreated and treated OPW enhanced plant growth for some plant species and reduced growth in others. Dicots (lettuce, soybean, and watermelon) were more sensitive to both untreated and treated OPW compared to monocots (corn, millet, and sorghum), indicating that morphological differences between plant species affected phytotoxicity in the experiments. Sensitivities of okra were similar to those observed for monocots. Phytotoxicity of the treated OPW to lettuce and soybean, although concentrations of COCs were less than irrigation guideline concentrations, suggests that chemical characterization and comparison to guideline concentrations alone may not be sufficient to evaluate water for use in growing crops. Therefore, phytotoxicity testing is critical in determining usefulness of OPW for purposes such as irrigation.

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