Phytotoxic Effects of Treated Wastewater Used for Agricultural Irrigation On Root Hydraulic Conductivity and Plant Growth

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Abstract

Aims

To determine the effects of treated wastewater (TWW) and dialyzed TWW (DTWW) through dialysis tube with a cut-off at 6000-8000 Da, on the water transport characteristics of maize seedlings (Zea mays L).

Methods

Laboratory experiments were conducted to determine the effect of TWW on the hydraulic conductivity of excised roots. Moreover, the effect on transpiration, plant growth, root cell permeability and on the plant fresh and dry weight was determined.

Results

Pressurized water flow through the excised primary roots was reduced by 25%-52%, within 90 min of exposure to TWW or DTWW. In hydroponics, DTWW affected root elongation severely by 58 %, while cell-wall pore sizes of same roots were little reduced (by 6%). Additionally, the exposure to TWW or DTWW caused inhibition of both leaf growth rate by (26%-70%) and transpiration by (14%-64%). While in soil growth, the plant fresh and dry weight was also significantly affected but not with secondary DTWW.

Conclusions

These impacts appeared simultaneously to involve phytotoxic and physical clogging impacts. First, the inhibition in hydraulic conductivity through live roots (phytotoxic and physical effects) after exposure to secondary DTWW was by 22%, while through killed roots accepted after hot alcohol disruption of cell membranes (physical effects only); was only by 14%. Second, although DTWW affected root elongation severely by 58%, cell-wall pore sizes of same roots were little reduced by 6%.

We conclude that large molecules, such as polypeptides, remained after the dialysis process, may have produced hormone-like activity that affected root water permeability.

Introduction

Treated wastewater (TWW) is used commonly for irrigation in semi-arid and arid zones all over the world (Dragonetti et al., 2020, Rahav et al., 2017). In Israel, most fruit tree plantations are irrigated with TWWs of varying quality (Rahav et al., 2017). In contrast, as compared to irrigation with fresh water (FW), irrigation with TWW, depending on the original water source and level of treatment, might result in increased salinity, high levels of organic and inorganic compounds, and levels of living organisms (Chojnacka et al., 2020, Syed et al., 2020), as well as changes in soil structure (Paudel et al., 2017).

The hydraulic conductance of plants is greatly affected by soil characteristics and water quality, especially salt concentration (Aroca et al., 2012). In roots, hydraulic conductance influences water uptake capacity, which depends on the plant's root surface area, root anatomy, and root water permeability (Niu et al., 2020, Meunier et al., 2018, canals et al., 2021). The dominating driving force for water uptake is the water potential gradient, which depends on osmotic gradients (Grimm et al., 2020, Bazihizina et al., 2017, Deluliis et al., 2021).

As opposed to FW, TWW contains a variety of organic and inorganic compounds, in addition to suspended and dissolved solids (Kharitonova et al., 2020). The organic substances include peptides, carbohydrates, lignin, fats, detergents, pharmaceuticals, and synthetic industrial waste materials (Jablonsky et al., 2018). The inorganic components include heavy metals, such as arsenic, cadmium, chromium, copper, lead, mercury, and zinc (Rathna et al., 2019). Phytotoxicity can limit the agricultural use of TWW for crop irrigation (Margenat et al., 2017, Werfelli et al., 2021). It can lead to a breakdown in soil structure, reduce the hydraulic conductivity of soil, increase osmotic potential, decrease aeration, and reduce root growth (Skaalsveen et al., 2020). Reductions in root function and water uptake that follow TWW irrigation may be responsible for deceases in the performance of plantations, as has been found in the case of avocado, grapefruit, almond, peach, and other fruit trees species (Syed et al., 2020, Romero-Trigueros et al., 2017).

Recently, some investigators have described the phytotoxic risk posed by polypeptides that have been extracted from plants in laboratory experiments. Some researchers reported that endogenous plant polypeptides known as rapid alkalinity factors (RALFs) rapidly increase the pH of plant suspension cell-culture medium and inhibit root growth (Pearce et al., 2001, Covey et al., 2010, Bergonci et al., 2014, Haruta et al., 2014). Another polypeptide, defensin, which is a small cysteine-rich antimicrobial protein that is an important component of the innate immunity of plants, can inhibit plant growth (Allen et al., 2008). The authors reported that KP4 (a killer toxin from the smut fungus *Ustilago maydis*) and three plant "defensin" types – MsDef1, MtDef2, and RsAFP2 – all inhibit root growth in germinating *Arabidopsis* seeds at low micro-molar concentrations (Allen et al., 2008). In a pollen-specific tomato (*Solanum lycopersicum*), a new phytotoxic polypeptide called RALF (SlPRALF) has been identified (Covey et al., 2010). The SlPRALF gene encodes a pro-protein that appears to be processed and released from the pollen tube as an active peptide. Furthermore, a synthetic SlPRALF peptide based on this putative active peptide did not affect pollen hydration or viability but inhibited the elongation of normal pollen tubes in an in-vitro growth system. Inhibitory effects by SlPRALF were detectable at concentrations as low as 10 nM, and complete inhibition was observed at one µM of peptide. A greater effect was observed in a low-pH-buffered medium. Thus, exogenous SlPRALF acts as a negative regulator of pollen tube elongation within a specific developmental window (Covey et al., 2010).

Another phytotoxic polypeptide, called POLARIS (PLS), was found to regulate indole acetic acid (IAA) transport and root growth via effects on ethylene signaling in *Arabidopsis* (Chilley et al., 2006). Hydraulic conductivity in roots is an essential factor controlling root growth and plant development (Asli and Neumann 2009). The minimum concentration of IAA that can dramatically reduce root hydraulic conductivity is 10^{-6} M (Hose et al., 2000), and such reduction inhibits root growth (Asli and Neumann 2010).

Municipal wastewater contains about 0.5 % protein (Rebhun and Manka 1971). Thus, when TWW is used in agriculture, exogenic polypeptides may reach the plant root zone. These polypeptides may have phytotoxic effects on plant growth and development. Therefore, it has been reported that using irrigation water from a river which is polluted with municipal wastewater reduced the growth of Chinese kale and *Dendrobium* orchids under greenhouse conditions, as well as the growth of tomatoes and Chinese kale under sterile conditions (Sarawaneeyaruk et al 2014). Moreover, wastewater reduced the amount of rhizosphere microorganisms in Chinese kale to five times less than that in tap water. Thus, the use of wastewater in irrigation may affect whole plant growth and decrease annual yields (Sarawaneeyaruk et al 2014).

The aim of this study is to investigate the effects of a specific fraction from TWW, such as polypeptides, on plant water balance and growth. The hypothesis is that these polypeptides apparently behave as hormone-like molecules and affect cell metabolism. Consequently, root hydraulic conductivity is affected, and plant growth is inhibited.

Material And Methods

Plant material

Seeds of *Zea mays* L (Cv PR32w86, Merhav Agro, Israel) were germinated and grown in aerated hydroponic media in a light- and temperature-regulated growth chamber for 12-hour photoperiods, as has been previously described (Asli and Neumann 2009).

Wastewater preparation

Primary and secondary treated wastewater was collected from a wastewater treatment plant in Haifa. Israel. Samples were maintained at 4°C during the collection period. After collection, the samples were homogenized and again stored at 4°C until use, following the method of Keith (1988). The primary treated wastewater was collected after the sedimentation process, while the secondary wastewater was collected after the activated sludge process.

Dialyzed treated wastewater preparation

The effluents (from the primary and secondary stages) were processed with dialysis tubes with a cutoff of 6000–8000 Da (Sigma Aldrich) to remove small molecules. The dialysis tube with its wastewater content was flooded overnight with 10 liters of distilled water. To ensure that the salt fraction of the tube content was removed, the process was performed three times until an electrical conductivity of 500 µS. cm^{-1}, similar to that of tap water, was reached. All molecules smaller than 6000–8000 Da were filtered from the solution.

Wastewater characterization

Following the methods of the American Public Health Association described by Asli et al., (2021), the samples were incubated in an orbital shaker incubator (LOM-500-D2) at 25°C for 2 h, homogenized, and assayed for total solids, oil and grease, nitrogen, and chemical oxygen demand (COD) (Table 1).

Root hydraulic conductivity

One-centimeter pieces of the cut ends of 6-cm lengths of excised primary roots ~ 1 mm in diameter were fitted into marked glass capillary tubes. Silicon grease and Parafilm wrapping were used to carefully seal the junctions. The protruding 5 cm of up to 10 roots were immersed vertically in 1-liter lightproof containers (to prevent any photo-oxidative interaction between UV light and the effluent). The containers were filled with continuously stirred solutions of 0.1 mM of $CaCl_2$, with or without the addition of secondary effluent (from the Haifa wastewater treatment plant), with several dilutions of the stock effluent, at pH 6.8 and 27°C. Water flow through the roots was followed by measuring the rise of menisci in the protruding capillaries after the root container was sealed and pressurized to 10 KPa. Hydraulic conductivity was based on flow rates assayed from 30 to 40 min after the commencement of pressurization. Roots showing pressurized flow rates > 0.3 µL min^{-1} in the first 30 min also allowed aberrant transport of a high mol weight of dextran blue (0.4 g L^{-1}) through to the glass capillary; these roots were considered leaky and were discarded.

For killed root assays, the protruding 5 cm of roots fitted to capillaries, as described above, were treated with hot (80 ºC) ethanol for 1 min and gently rinsed in water prior to the flow assay. Killed roots with pressurized flow rates > 0.55 µL min^{-1} were considered leaky and were discarded. Axial hydraulic conductivity of the xylem was found to increase from a point 2 cm behind the tip (not shown). Thus, only the 3-cm-long exposed region of more mature tissue starting 2 cm behind the tip was assumed to contain open xylem and was taken to represent the effective root region for water uptake in the calculation of hydraulic conductivity. Hydraulic conductivity was calculated as m^3 of solution transported per m^2 of effective root area per second per MPa of applied pressure.

Leaf and root growth rates

Leaf elongation was assayed at 5–10 h intervals over 72 h by following length increases in the emerging primary leaves of uniform maize seedlings. Leaf growth rates were based on up to 20 h of linear growth before the end of the logarithmic phase of elongation. Primary root elongation rates were based on the increase in length of marked roots over 3 d.

For soil growth experiments, uniform seedlings were planted in well drained pots containing 500 g of local clay soil with three maize seedling per pot and three pots per treatment. The pots were flood irrigated with 0.1 strength nutrient solution at 48 h intervals for 5 weeks. The treatment was continued with or without addition of TWW or DTWW for a further 7 days. The shoots then had transferred to determine fresh and dry weight.

Transpiration

Transpiration rates were assayed gravimetrically using whole seedlings selected for uniform size. These had open first and second leaves and an emerging third leaf. The seedling roots were loosely sealed in lightproof plastic vials, which were 3/4 filled with 0.1mM of $CaCl_2$, with or without several appropriate effluent type. Plants with their foliage removed and the remaining stump capped with Parafilm were used as controls. Transpiration was assayed by monitoring weight loss for 3 h with an electronic balance, subtracting control values, and dividing by the leaf surface area. A factor of 1 g of leaf fresh weight to 65.1 cm^2 of open leaf area was calculated for 30 plants and used to convert the leaf fresh weight of the assayed plants to leaf area (circa 13 cm^2 per plant). Oxygen levels in the root media at the beginning and end of 3-h transpiration assays were measured using a dissolved-O_2 probe (Cyberscan, DO 300, USA). Similar decreases of approximately 20% were measured via treatment and control experiments after 3 h.

Pore size in root-cell walls

The mean diameters of the pores limiting particulate transport through the cell walls of living maize roots were determined through the observation of cytorrhysis (root collapse) (Carpita et al., 1979). This was induced by sequential exposure to solutions of PEG molecules with hydrodynamic diameters ranging from 0.9 nm to 7.2 nm (Kuga 1981). Concentrations of PEG 200, 1500, 4000, 6000 and 10000 (57.84, 180.71, 204.17, 201.62 and 210.54 g of PEG per 1000 g of water, respectively) were fixed using the calibration described by Money (1989) to give the same-solution water potential of -0.7 MPa for all the PEG treatments. Batches of 10 wastewater-treated roots and controls were gently rinsed and transferred to Petri dishes containing 50 ml of PEG 200 solution and then transferred at 50-min intervals to PEG solutions with higher molecular weights and hydrodynamic diameters. When the external PEG molecules were too large to penetrate the pores in the cell walls, an osmotically induced efflux of cell water resulted in inward collapse and root cytorrhysis. Flattened, ribbon-like roots could then be observed through a binocular microscope and average pore diameters calculated.

Statistics

All experiments were replicated one or more times with similar results. Differences between treatments were estimated by one way ANOVA, using the analysis tool box in GraphPad Prism version 8.00 for Windows, Graph-Pad Software, San Diego California USA.

Results

Characterization of treated wastewater

The values of the parameters (means ± SE) that were established in this study for TWW and DTWW are described in Table 1. During the dialysis process, micro-molecules were filtered out with the use of a dialysis tube. Only macromolecules with molecular weights greater than 6000−8000 Da remained in the dialysis tube to be used in the experiments. It can be noticed from Table 1 that the total amount of solids in the samples were decreased significantly after the dialysis process. Hence, soluble molecules of proteins and other organic materials were partly filtered out based on their molecular weight. Small molecules such as salts, fatty acids, nucleic acids, amino acids and small peptides, and other organic monomers were clearly filtered out.

Effluent effects on root hydraulics

The assays of water flow and hydraulic conductivity were performed on the excised roots using a custom-built system that enabled simultaneous assays on up to 10 roots. The rate of pressurized water transport through the roots remained linear for 140 minutes (Fig. 1A) and then declined slowly (not shown). Therefore, all the flow assays were completed in only 140 minutes.

The results for the sensitivity of water flow through the roots treated with either TWW or DTWW are shown in Fig. 1B and 1C, respectively. The secondary effluents caused significant changes in flow (69–78% of the control values) when either TWW (Fig. 1B) or DTWW (Fig. 1C) was used, while for the primary effluents, the flow was visibly decreased in a progressive manner, reaching 54% of the control value with the use of TWW (Fig. 1B) and 78% with the use of DTWW (Fig. 1C). In summary, the inhibitory effects of TWW and DTWW on flow through the roots appeared to be concentration dependent and progressive.

To investigate the degree to which the inhibition of flow was reversible, roots previously exposed to either TWW or DTWW were transferred back to wastewater-free solutions. This led to invisible increases in flow, compared to the flow rates during TWW or DTWW exposure (Fig. 1B and C). Associated differences in root hydraulic conductivities are quantified in table 2. Exposure to primary TWW caused hydraulic conductivities to decline to 54% of the control value, with no significant recovery after the TWW was removed (it increased to only 57% of the control values). Exposure to secondary TWW caused a reduction to 69% of the control value, with no reversible response when the TWW was removed. Also, primary, and secondary DTWWs showed a reduction to 78% of control values, with no reversible response in either case when the DTWWs were removed. These findings suggest that most particles became irreversibly attached to the root cell walls.

Confirmation of physical interactions between colloidal particles from wastewater and root-cell walls was provided by experiments in which the effects of the TWW and DTWW on flow through hot-alcohol-extracted root "ghosts" were assayed (Table 2). The hot alcohol treatment was expected to disrupt lipid-based cell-membrane barriers, while leaving the polysaccharide polymers of the cell walls relatively intact. The hydraulic conductivity of the alcohol-extracted control roots (17.60 ± 1.30 m. s^{-1} MPa^{-1}. 10^{-7}, n = 11) were reduced to 86% of the control value after the roots were exposed for 40 min to colloidal suspensions of DTWW (Table 2).

To further investigate the phenomenon of root clogging by colloidal suspensions, the mean pore diameters of the root-cell walls were assayed before and after 4 h of flow-inhibiting exposure to the wastewater (Table 3). We reasoned that the accumulation of colloidal particles filtered out at the cell-wall surface might reduce the mean diameter of pores in the cell-wall matrix. Table 3 shows clearly that prior exposure to TWW led to a reduction in the mean diameters of the root pores by 14%-27%, whereas the exposure to DTWW by 6%-9% only. In summary, nanoparticles of both TWW and DTWW suspended in the water flowing into roots appeared to attach irreversibly to the root cell-wall surfaces, reducing effective cell-wall pore diameters and root hydraulic conductivities (Table 3).

Whole plant effects

Since suspensions of TWW and DTWW had clear inhibitory effects on water transport through the excised roots, it was of interest to determine whether they could cause water-stress responses by inhibiting water transport in whole plants. Both leaf growth (Fig. 2A and C) and transpiration (Fig. 3) in maize seedlings are sensitive to reductions in water supply; therefore, we tested for possible changes in these parameters after adding TWW (Fig. 2A and B) or DTWW (Fig. 2C and D) to the root media of intact hydroponic seedlings.

Transpiration rates were significantly reduced (by 47–62%) after 3 h of exposure to either primary TWW or primary DTWW (Fig. 3). After these 3-h exposures, the transpiration rates were reduced to 75% and 87%, respectively.

Leaf growth rates were also significantly reduced during 3-d treatments, by 30% of the control value for primary TWW (Fig. 2A) and by 44% of the control value for primary DTWW (Fig. 2C), while the effect of secondary TWW on leaf growth was 54%, and the effect of secondary DTWW 74% (Fig. 2A and C). Furthermore, the effect of both primary TWW and DTWW on root growth rates was 26% of the control value, while the effect of secondary TWW was 45%, and that of secondary DTWW 42% (Fig. 2B and D).

More important, besides the inhibition of leaf growth, elongation of the roots was affected by the 3-d treatments. Despite continuous contact with suspensions of TWW or DTWW, a portion of the roots retained a healthy appearance (Fig. 2B and D). Thus, the suspensions assayed here appear to produce their stressful effects on the shoots via toxic effects on the roots.

To determine whether similar levels of TWW and/or DTWW in soil media might also reduce water availability to the shoots, potted maize plants were flood-irrigated with nutrient solution with or without TWW and/or DTWW at 48 h intervals for 5 weeks. When primary TWW was used for irrigation, this treatment resulted in significant reduction in shoot fresh weight by 26% and dry weight by 27%, while using primary DTWW resulted in significant reduction in shoot fresh weight by 9% and shoot dry weight by 21%, as compared to control plants irrigated with nutrient solution only (Table 4). Using secondary TWW resulted in significant reduction in shoot fresh weight (by 12%) and shoot dry weight (by 17%) as compared to control plants irrigated with nutrient solution only. Using secondary DTWW in irrigation resulted in significant reduction in shoot fresh weight (by 5%) as compared to control plants irrigated with nutrient solution only but no significant reduction was accepted in shoot dry weight.

Discussion

Physical flow inhibition

For primary maize roots, the mean diameter of the cell-wall pores was determined to be approximately 6.6 nm (Table 3). This diameter falls within the range of values reported by others using isolated cells or cell-wall powders derived from different tissues and plants (Asli and Neumann 2009, Carpita et al., 1979, Baron-Epel et al., 1988, Chesson et al., 1997).

TWW contains several types of particles and colloids (polypeptides, sugars, lipids, microplastic, TiO_2, etc.) of different dimensions. Thus, during agricultural irrigation by TWW, these particles, especially the larger ones, would not be expected to effectively penetrate the outer part of the cell-wall pores. Discussion of the detailed mechanisms of the root flow inhibition induced by colloidal particles in TWW is beyond the scope of this report. However, it seems likely that particles rejected at the surface of the cell can physically limit root water transport by forming surface "cake" layers or a boundary layer and thereby decrease the hydraulic conductivity of the root-cell wall (Table 2). An inhibition of flow resulting from a similar formation of cake layers is well known to occur whenever colloidal solutions are filtered through excised maize roots (Asli and Neumann 2009, 2010) or through synthetic nano-filtration membranes (Deshmukh et al., 2018).

The findings of some previous investigations of biophysical interactions between large polymer molecules or colloid particles and plant-cell walls are consistent with our findings. Thus, Asli and Neumann (2009) were able to follow the diffusion of bentonite clay sheets and TiO_2 nanoparticles through the root-cell walls of *Zea mays* L. Bentonite clay sheets showed hindered or zero passage, consequently, the hydraulic conductivity of the excised roots was inhibited. Similarly, Asli and Neumann (2010) reported that solutions of polyethylene glycol 6000 (with a mean hydrodynamic diameter of 5.3 nm) caused a non-osmotic inhibition of pressurized water flow through the excised roots; the authors concluded that the inhibition of hydraulic conductivity was due to the ability of PEG to clog the cell-wall pores of maize roots.

More recently, Ranathunge et al. (2004) observed that in rice roots, a water flow reduction of 25–30% occurred when China ink particles (with diameters of about 50 nm) were pressurized through the outer part of the root. Finally, Proseus and Boyer (2005) tested the ability of pressurized aqueous suspensions of gold nanoparticles of varying diameters to permeate or to pass through algal cell walls. After several experiments using confocal laser microscopy, they reported that gold nanoparticles with a diameter of about 10 nm could not penetrate algal walls, even when 0.5 MPa of pressure was applied.

Toxic and physiological effects

Some particles in TWW may have toxic effects that do not depend on their size or diameter. The effect of low concentrations of particles from TWW on plant roots and shoot growth was investigated. Particles larger than 6000–8000 Da, such as polypeptides (Table 1), inhibit plant growth (Fig. 2B and D). The ability to inhibit hydraulic conductivity (Fig. 1B and C) and plant growth despite their low concentration is an evidence to phytotoxicity effect. Apparently, polypeptides may behave as hormone-like molecules that play an important role in the regulation of cellular metabolism in animals and plants, and consequently, may suppress plant growth. Since secondary DTWW inhibited the root hydraulic conductivity differently through live (by 22%) and killed roots (by 14% only) (Table 2), the mechanism in which the process is governed might be simultaneously by physical and phytotoxicity effects. Furthermore, although secondary DTWW affected the root elongation severely (by 58%) (Fig. 2B and D), the cell-wall pore sizes of the same plant root were little reduced (by 6%) (Table 3).

This appears to be the first report to establish that polypeptides from TWW in root media can reduce the hydraulic conductivity of maize primary roots in both physical and phytotoxic ways. The physical effect was reflected in the inhibition of the plant water balance, inducing symptoms of water stress (reduced transpiration and leaf growth) in the shoots, while the phytotoxicity effect more likely proceeded from an alteration of biochemical pathways in the cell, which inhibited the capacity of the root to absorb water. Thus, both the physical and phytotoxic effects of TWW on plants should be considered when evaluating the risk potential of agricultural and environmental applications. For example, as freshwater availability decreases, TWW, which can contain high concentrations of suspended particles and dissolved biopolymers, will be increasingly used for crop irrigation in drought-prone regions of the world (Grant and Verburg 2020). Further research will be needed to determine whether the use of such recycled waters can limit root water uptake under field conditions.

Plant -soil interaction

TWW and DTWW decreased shoot and root elongation rate either in hydroponics experiments (Fig. 2A-D) or in soil growth (Table 4). It was noticed that the inhibition effect of TWW and/or DTWW was larger in hydroponics growth in comparison with soil irrigation. Apparently, due to the high probability of exposure between the root surface area and the wastewater contents in hydroponics. Conceivably, soils can filter out some of the nanoparticles suspended in the water supply and thereby limit the rate of polypeptide accumulation on the root surfaces of transpiring plants. Also, microorganisms in the rhizosphere during natural biodegradation may consume these polypeptides; and spontaneously, natural neutralization process might be existed. Another possibility is that, over time, plants can invest more carbon resources in root surface-area accretion and the maintenance of higher root-to-shoot ratios. A continuous and rapid production of new, roots and root hairs could then compensate for the decreased hydraulic conductivity of existing roots already inhibited by polypeptides. Thus, the maintenance of "excess" root surface area (Vysotskaya et al., 2004), and hence root water-supply capacity, could represent an adaptation to potentially stressful root-inhibition caused by polypeptide in soil waters.

In conclusion, our findings establish that nanoparticles of inorganic and organic nanomaterials of domestic origin in root water supplies can accumulate irreversibly on root cell-wall surfaces with toxic consequences, and can, moreover, lead to both a physical and phytotoxic inhibition of root water-transport capacity (Fig. 1B and C), leaf growth (Fig. 2A and C), root growth (Fig. 2B and D), and transpiration (Fig. 3).

Declarations

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Sare Asli], [Nedal Massalha] and [Mohamad Hugerat]. The first draft of the manuscript was written by [Sare Asli] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1: Characteristics of the treated wastewater used in this study. Whereas EC: Electrical conductivity; COD(T): chemical oxygen demands-total; COD(S): chemical oxygen demands-soluble; TS: total solids; TDS: total dissolved solids; TSS: total suspended solids and TKN: total kjeldahl nitrogen.

Parameter	Value (means ± SE, n = 3)			
	TWW		DTWW	
	Primary	Secondary	Primary	Secondary
pH	6.13±0.15	6.66±0.09	6.37±0.09	6.45±0.17
EC ($mS \cdot m^{-1}$)	2.18±0.04	2.56±0.04	0.57±0.03	0.48±0.03
COD(T) (mgL^{-1})	714±6.35	285±6.49	329±6.23	176±8.50
COD(S) (mgL^{-1})	581±5.86	215±5.86	497±10.14	144±4.93
TS (mgL^{-1})	1359±15.76	1070±28.04	778±33.80	194±8.97
TDS (mgL^{-1})	823±8.57	547±8.65	328±5.49	124±8.82
TSS (mgL^{-1})	453±9.54	386±9.91	328±9.06	90±6.69
TKN (mgL^{-1})	224±10.14	144±10.68	106±6.93	84±4.00
Proteins (mgL^{-1})	1048±8.97	772±15.06	1013±12.71	608±13.69

Table 2. Contributions of salts and colloids in primary and secondary effluents to the reduction of root hydraulic conductivity.

The primary and secondary effluents contained 1.3 and 1 gL^{-1} total solids, respectively. Colloid-enriched effluent remaining after dialysis to remove smaller molecules from the primary or secondary effluents (with a cut-off of 6000-8000 Da, Ec = 500 $\mu S. cm^{-2}$). To prepare the control solution, 0.1 M of $CaCl_2$ was added to one liter of distilled water; for the treated solution 0.1 M of $CaCl_2$ was added to one liter of each effluent. The treatment solutions were drained and replaced with 0.1 mM of $CaCl_2$ solution, as indicated by the arrows. The points and bars represent means ± SE (n = 7-11 roots). Similar responses were observed in three replications of the experiment. The letters in the rows indicate significant differences based on one-way ANOVA with a 95% confidence level.

Treatment			Hydraulic conductivity ($m. s^{-1}. MPa^{-1} .10^{-7}$)		
TWW	Dialysis	Root	Control	Treatment	Reduction (%)
Primary	No	Live	8.31±0.58 a	4.53±0.22 b	46
	Yes	Live	12.05±1.08 a	9.53±0.74 b	22
Secondary	No	Live	11.22±0.49 a	7.73±0.30 b	31
	Yes	Live	9.22±0.38 a	7.22±0.36 b	22
	Yes	Killed	17.60±1.30 a	15.20±0.80 b	14

Due to technical limitations, table 3 is only available as a download in the Supplemental Files section.

Table 4. Inhibition of shoot growth of potted maize plants irrigated with humic acid.

Plants were grown in a local clay soil in 1 L pots with drainage holes at base and sides and the soil flooded by sub irrigation with 0.1 strength modified Hoagland solution with or without TWW and/or DTWW, at 48 h intervals. Treatment and controls each consisted of three replicate pots with six plants per pot. After 5 weeks the shoots were excised at soil level and weighed. Data are means ± SE for individual shoots, n=18. The reduction in shoot fresh and dry weight was calculated in comparison with the control (zero reduction). Different letters in columns indicate significant weight differences at P<0.05 using One way ANOVA test.

	Shoot fresh weight (g)	Reduction in shoot fresh weight (%)	Shoot dry weight (g)	Reduction in shoot dry weight (%)
Control	14.95±0.09 a	0	2.8±0.15 A	0
Primary TWW	11.08±0.15 b	26	2.04±0.06 B	27
Primary DTWW	13.55±0.21 c	9	2.21±0.05 B	21
Secondary TWW	13.20±0.20 c	12	2.31±0.06 B	17
Secondary DTWW	14.20±0.13 c	5	2.55±0.10 A	9

Figures

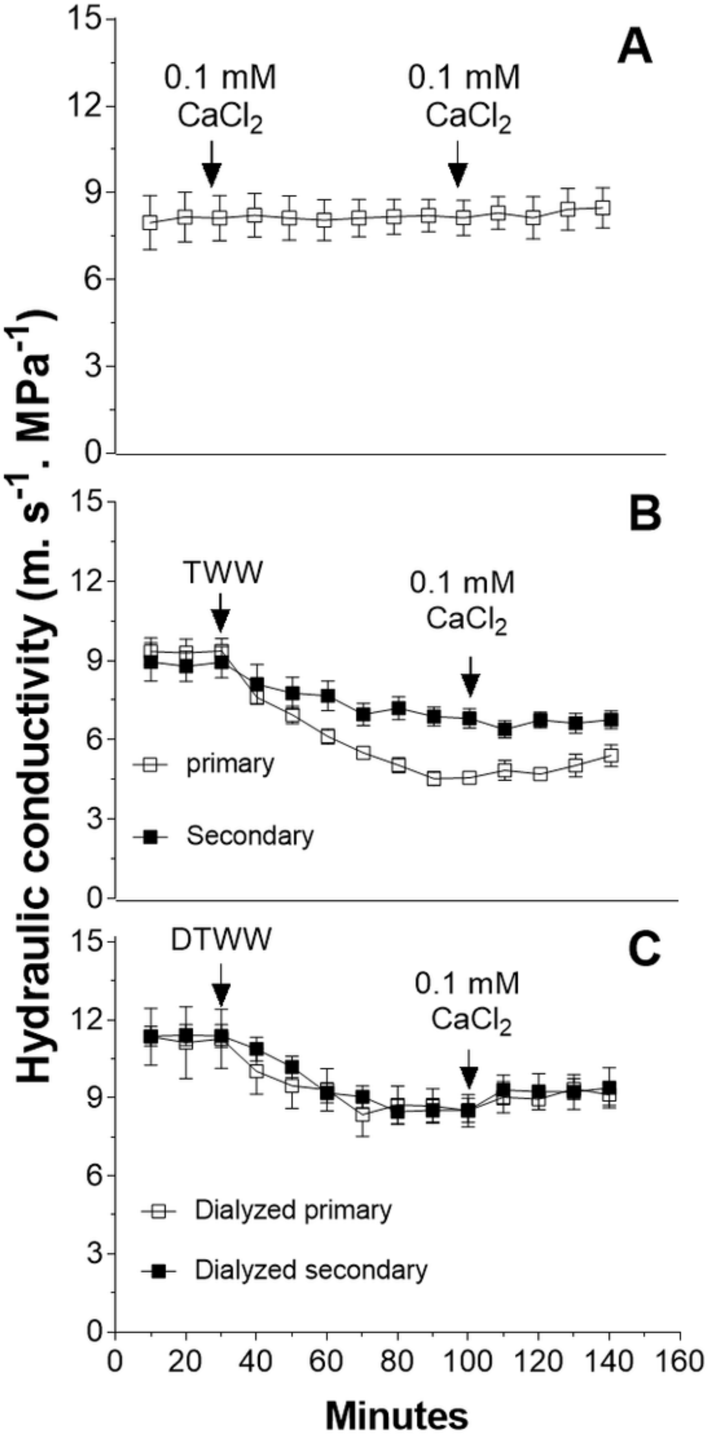

Figure 1

Effluents inhibit hydraulic conductivity through the excised roots of maize (Zea mays L). The roots were experimentally treated as described in Material and Methods. After 90 min of water flow through the excised roots, the control solutions were drained and replaced with 0.1 mM CaCl2 solution, as indicated by the arrows. (A) Hydraulic conductivity through the excised roots under constant exposure for 140 minutes. (B) TWW inhibition of hydraulic conductivity through the excised roots. (C) DTWW inhibition of hydraulic conductivity through the excised roots. To prepare the control solution, 0.1 M CaCl2 was added to one liter of distilled water; for the treated solution, 0.1 M CaCl2 was added to one liter of each effluent. Points and bars represent means ± SE (n = 7-10 roots). Similar responses were observed in three experimental replications. Colloid-enriched effluent remaining after dialysis (with a cut-off of 6000-8000 Da, Ec = 500 µS. cm-1) to remove smaller molecules from the primary and secondary effluents.

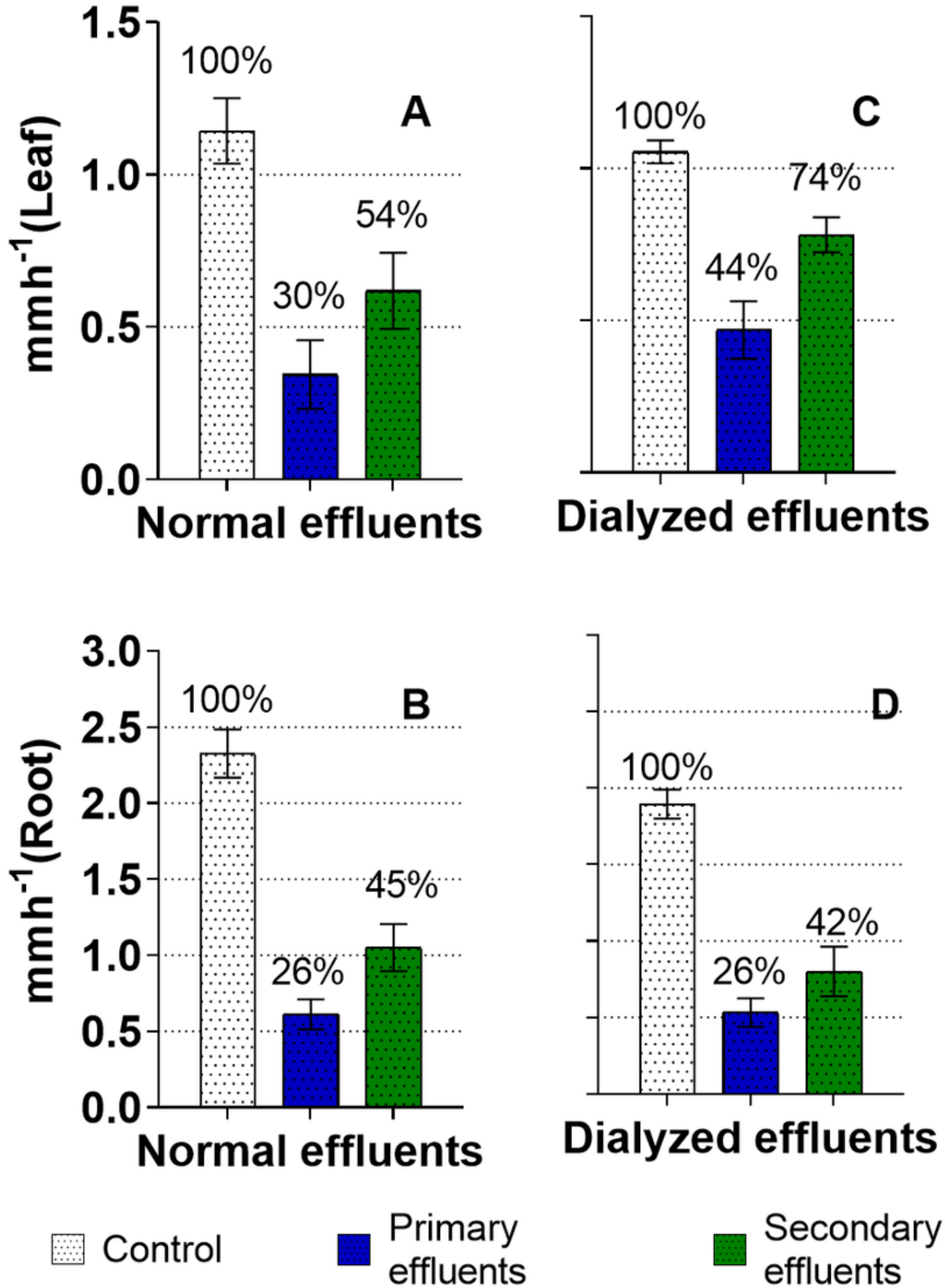

Figure 2

Comparison of reductions in leaf and root lengths in hydroponically grown maize (Zea mays L), caused by effluents. (A, B) Reductions in primary and secondary normal effluents containing 1.3 and 1.0 gL-1 total solids, respectively. (C, D) Reductions in dialyzed primary and secondary effluents obtained by dialysis through dialysis tubes (cutoff: 6000-8000 Da, Ec = 500 µs. cm-1). A 0.1 Hoagland solution was used to prepare the control and the treatment solutions, in which the seedlings were grown for three days. Leaf elongation means were calculated after 20 h of linear growth before the end of the logarithmic phase of elongation. Points and bars represent means ± SE (n = 14-16). Similar responses were observed in three replications of the experiment.

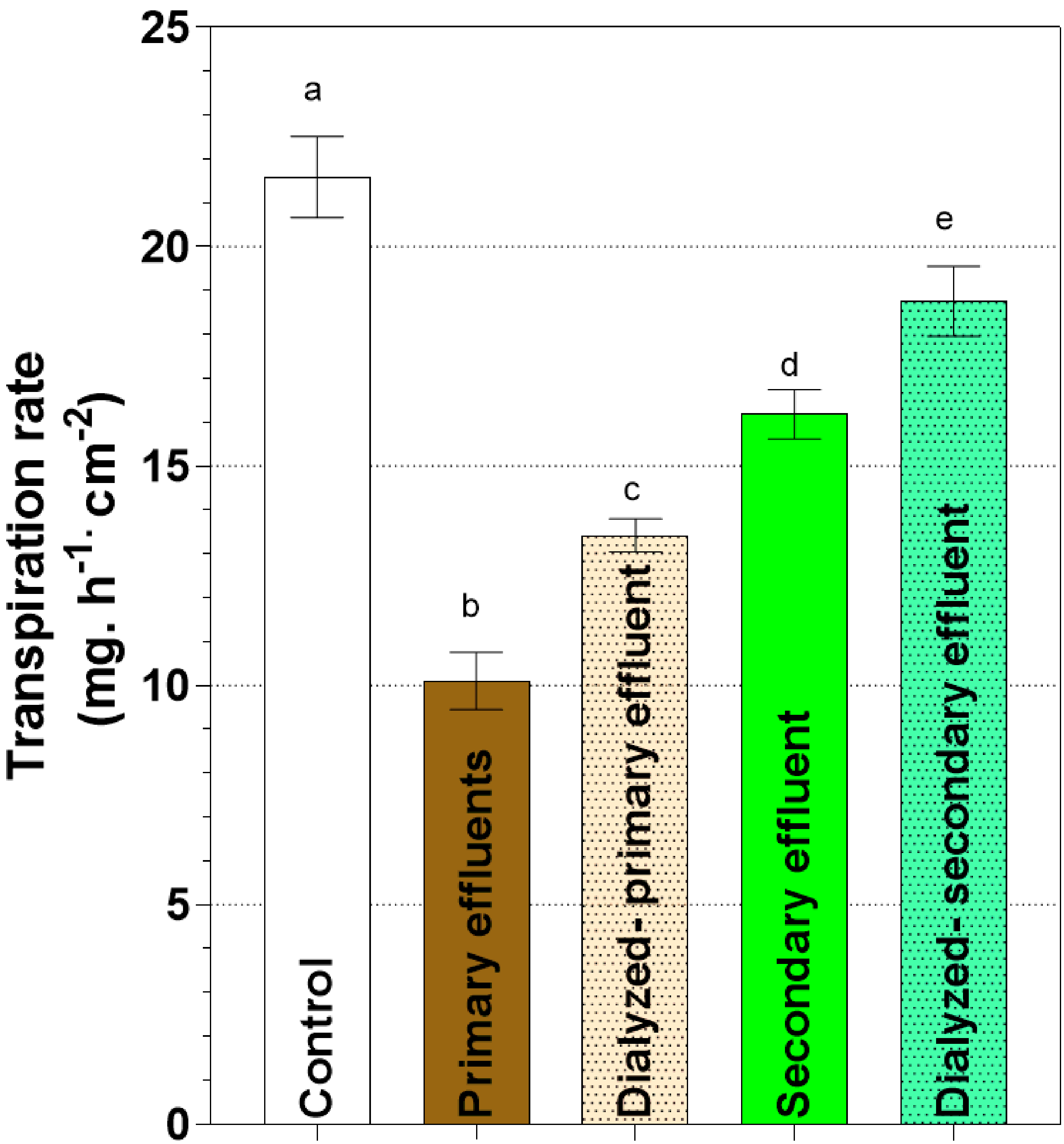

Figure 3

Comparison of the inhibition of transpiration in maize seedlings, caused by effluents in the root media. The experiment was designed as described in Materials and Methods. The values consist of the pooled means ± SE of three similar experiments involving five replicate plants. The letters in the columns indicate significant differences from the control values, as assessed by one-way ANOVA at a 95% confidence level.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table3.jpg