Evaluation of phytotoxicity of effluents from activated sludge and constructed wetland system for wastewater reuse

Ana María Leiva, Adrián Albarrán, Daniela López and Gladys Vidal

ABSTRACT

The aim of this study was to evaluate the phytotoxicity of wastewater treated with horizontal subsurface flow (HSSF) constructed wetlands (CWs) and activated sludge (AS) system using disinfection treatment such chlorination and ultraviolet (UV) system. To assess the impact of the reuse of different effluents (HSSF-Cl, HSSF-UV, AS-Cl and AS-UV), bioassays using seeds of *Raphanus sativus* (*R. sativus*) and *Triticum aestivum* (*T. aestivum*), were performed on both Petri dishes and soil. Different treated wastewater concentrations were varied (6.25%, 12.5%, 25%, 50% and 100%) and the percentage of germination inhibition (PGI), percentage of epicotyl elongation (PEE) and germination index (GI) were determined. Positive effects (PGI and PEE < 0% and GI > 80%) of HSSF-Cl, HSSF-UV, AS-Cl and AS-UV effluents on germination and epicotyl elongation of *R. sativus* and *T. aestivum* were observed in Petri dishes bioassays. However, toxic effects of HSSF-Cl, HSSF-UV and AS-Cl on seeds germination and epicotyl elongation of both plant species were detected in soil samples (PGI and PEE > 0% and GI < 80%). Only *R. sativus* seeds to be irrigated with AS-UV achieved GI values above 86% for all concentrations evaluated. These results indicated that AS-UV effluent had a positive effect on seeds germination and can be recommended for treated wastewater reuse in agricultural irrigation.

Key words | activated sludge, constructed wetlands, disinfection treatments, phytotoxicity, wastewater reuse

INTRODUCTION

It is reported that two thirds of the world’s population currently live in areas that experience water scarcity for at least one month a year (UNWWAP 2017). One possible solution for the sustainable management and conservation of water resources is the reuse of treated wastewater (Andreo-Martínez et al. 2017). In fact, its application is being an attractive alternative for agriculture irrigation in many countries (Norton-Brandão et al. 2013).

The reuse of treated wastewater provides two essential resources: nutrients and water (Greenway 2005). Despite their benefits, its application involves health and environmental risks (Vidal et al. 2007; Chamorro et al. 2010, 2013). For this reason, much attention has been given to the potential of different wastewater treatment technologies for a sustainable reuse of wastewater. The application of activated sludge (AS) effluents for irrigation was well documented (Vera et al. 2013). Zhang & Farahbakhsh (2007) determined that the final effluent from AS wastewater treatment process can be potentially reused with concentration of biological oxygen demand (BOD₅), total suspended solids (TSS), total phosphorus (TP) and ammoniacal nitrogen (NH₄⁺-N) close to 1.0, 3.2, 0.3 and 0.6 mg/L, respectively (Vera et al. 2013). Nowadays, constructed wetlands (CWs) are an attractive solution for improving the quality of wastewater and reusing it for irrigation (Akratos & Tsihrintzis 2007; Vera et al. 2014). Andreo-Martínez et al. (2017) evaluated the performances of horizontal subsurface flow constructed wetlands (HSSFs) to produce effluents suitable for agriculture reuse. In this study, the HSSF effluents achieved concentrations of BOD₅, total nitrogen (TN) and TP of 17, 16 and 1.0 mg/L, respectively, and they are consistent with...
of wastewater (Üstün et al. 2019). Chlorination reduces in 3 Log units the FC concentrations dose used and the wastewater treatment (Sommer et al. 2016). To ensure a sufficient reduction of microbial pathogens, the good performances of disinfection systems, efficiencies depend on several factors such as an adequate contact time and the categories of agriculture reuse. For food crops and processed food and non-food crops, the concentrations have to be lower than no detectable FC/100 mL and 200 most probable number (MPN)/100 mL, respectively (USEPA 2012b). The concentration of FC in wastewater is in the range of $10^7$–$10^8$ MPN/100 mL, which is reduced by about 2 Log units during disinfection treatment (Henze et al. 2002). The most commonly technologies used for pathogens removal in wastewater treatment are the chlorination and ultraviolet (UV) systems (Aquarec Project 2006). Typically, chlorination reduces in 3 Log units the FC concentrations of wastewater (Üstün et al. 2019). On the other hand, for the UV disinfection, some authors have reported removal efficiencies of FC close to 100% (Bayo et al. 2008). Despite the good performances of disinfection systems, efficiencies depend on several factors such as an adequate contact time to ensure a sufficient reduction of microbial pathogens, the dose used and the wastewater treatment (Sommer et al. 1998).

The application of treated wastewater for irrigation may alter the physicochemical characteristics of soil such as acidity, salinity and organic matter and nutrients concentrations, and they may contribute for the accumulation of potentially toxic chemical and biological compounds (Chamorro et al. 2013; Becerra-Castro et al. 2015). In general, the effects of treated wastewater on soil and plants are actually not known. Ravindran et al. (2016) studied the phytotoxicity effects of untreated and treated wastewater on four commercial seeds of crops: tomato (Lycopersicon esculentum), radish (Raphanus sativus), carrot (Daucus carota) and onion (Allium cepa). The results of this study indicated that both effluents evaluated showed no phytotoxicity effects with a germination index (GI) above 80%. For this reason, establishing the phytotoxicity is fundamental for the design and the sustainability of different wastewater treatments (Venegas et al. 2018).

This study is the first approach that considered a comparison of phytotoxicity between effluents from different wastewater treatments for evaluating the reuse of treated wastewater. In this case, the effluents came from a conventional, AS, and a non-conventional treatment, HSSF, that were compared using different disinfection systems which are commonly used in wastewater treatment worldwide: chlorination and UV.

Taking the above into account, the aim of this study was to evaluate the phytotoxicity of wastewater treated with HSSFs and AS systems using chlorination and UV disinfection treatment on seeds of Raphanus sativus (R. sativus) and Triticum aestivum (T. aestivum) and to determine the potential reuse of these effluents for agriculture irrigation.

**MATERIAL AND METHODS**

**Description of different wastewater treatments**

**AS system**

This system is an extended aeration AS of the wastewater treatment plant located in Hualqui (56°59’26.93” south latitude and 72°56’47.23” west longitude), Biobío Region, Chile and it is designed for a rural community of 20,000 inhabitants. This biological reactor consists of an aeration tank with fine bubble diffusers distributed at the bottom of the system. Table 1 shows the operational characteristics and removal efficiencies of the AS system. The organic loading rates (OLRs) and the hydraulic retention times (HRTs) fluctuated between 0.1 and 0.3 g·DBO$_5$/m$^3$·d and 12 and 36 d, respectively. Regarding removal efficiencies of BOD$_5$, TN and TP, these values varied from 93–98%, 15–25% and 10–20%, respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Wastewater treatment system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HSSF system</td>
</tr>
<tr>
<td>Medium support</td>
<td>–</td>
<td>Gravel</td>
</tr>
<tr>
<td>OLR g·BOD$_5$/m$^3$·d</td>
<td>3.3–4.2</td>
<td>0.1–0.3$^*$</td>
</tr>
<tr>
<td>HRT d</td>
<td>4–8$^*$</td>
<td>12–36$^d$</td>
</tr>
<tr>
<td>COD removal efficiency %</td>
<td>57–60$^{h,b}$</td>
<td>90–99$^d$</td>
</tr>
<tr>
<td>BOD$_5$ removal efficiency %</td>
<td>70–80$^a$</td>
<td>93–98$^d$</td>
</tr>
<tr>
<td>TN removal efficiency %</td>
<td>15–28$^c$</td>
<td>15–25$^d$</td>
</tr>
<tr>
<td>TP removal efficiency %</td>
<td>9–13$^c$</td>
<td>10–20$^d$</td>
</tr>
</tbody>
</table>

OLR, organic loading rate; HRT, hydraulic retention time; COD, chemical oxygen demand; BOD$_5$, biological oxygen demand; TN, total nitrogen; TP, total phosphorus.

References: aLópez et al. (2015); bSepúlveda-Mardones et al. (2017); cLópez et al. (2016); dVera et al. (2016).
HSSFs system

The CWs system consists in a HSSF pilot plant which was also located in the treatment plant of Hualqui. Six parallel HSSF units with different plant species were implemented. Two HSSF systems were planted with *Phragmites australis* (*P. australis*), the other two with *Schoenoplectus californicus* (*S. californicus*) and, finally, the other two were planted with ornamental plants. Specifically, one of them was planted with *Cyperus papyrus* (*C. papyrus*) and the other with a mixture of *C. papyrus* and *Zantedeschia aethiopica* (*Z. aethiopica*). The support medium used in all HSSF units was gravel with a size and porosity of 19–25 mm and 0.6%, respectively (Sepúlveda-Mardones et al. 2013; Leiva et al. 2013; López et al. 2019).

During the operation, the HRTs and the OLRs varied in the range of 4–8 d and 3.3–4.2 g·BOD₅/m²·d, respectively. Moreover, the removal efficiencies of BOD₅, TN and TP were between 70% and 80%, 15% and 28% and 9% and 13%, respectively (Table 1).

Disinfection systems

**Chlorination**

For chlorination, a sodium hypochlorite solution (NaOCl) of 50 g/L was used in a stirred batch reactor (500 mL) with the samples of AS system and HSSF CWs separately. With this concentration of NaOCl, the dose of Cl applied was equivalent to 200 mg·min/L. Moreover, the experiments were performed under pH-neutral conditions (7.3 ± 0.7) at room temperature (17.5 °C ± 3.5).

**UV disinfection**

Four UVC lamps (λ = 254 nm) of 8 W were used as an UV source and they were placed into a closed system to avoid the deviation of UV irradiation and to ensure its incidence directly and perpendicularly on the samples. The UV source was focused onto a Petri dish filled with 200 mL of effluent which was magnetically stirred. At the center of the solution surface, the incident intensity was approximately 2.5 mW/cm² and it was measured by a UVX shortwave radiometer (Meter UVP J-225). For all experiments, the UV dose was 2.5 mJ/cm² with pH neutral conditions (7.3 ± 0.7) at room temperature (17.5 °C ± 3.5) (Jarpa et al. 2015).

**Physicochemical and microbiological characterization of AS and HSSF effluents**

Figure 1 shows the schematic diagram of the different effluent treatments used in this study. Water samples were collected after the disinfection treatments (UV disinfection or chlorination) of different wastewater treatments (AS and HSSF). To evaluate the quality of HSSF-UV, HSSF-Cl, AS-UV and AS-Cl effluents, the samples were filtered using a 0.45 μm pore size membrane. The physicochemical...
parameters measured were chemical oxygen demand (COD) (colorimetric method, 5210-B), BOD₅ (modified Winkler azide method, 5210-B), TSS, volatile suspended solids (VSS) (gravimetric method, 2540-D and 2540-E, respectively), NH₄⁺-N (distillation methods by total Kjeldahl nitrogen (TKN) equipment), nitrate (NO₃⁻-N), TN, TP (Spectroquant-Nova 60, Merck kits), phosphate (PO₄³⁻-P) (colorimetric method), free chlorine (FC) and total chlorine (TCI) (DPD colorimetric method, 4500-Cl G). For microbiological analysis, FC were determined using the multiple-tube fermentation technique (9221-E). In addition, pH, electrical conductivity (EC) and temperature were measured using portable equipment OAKTON (PC650–480485) and the dissolved oxygen (DO) concentration was analyzed by a portable oxygen sensor (HANNA OXI 330i/set HI 9146-04). All techniques used in this study were based on the protocols described in Standard Methods (APHA 1998).

Phytotoxicity analyses

Petri dish bioassays

These bioassays were conducted for the effluents of HSSF-UV, HSSF-Cl, AS-UV and AS-Cl using seeds of dicotyledonous plants, *R. sativus*, and monocotyledonous plants, *T. aestivum*. These seeds were selected as recommended by the Ecological Effects Test Guidelines (USEPA 2012a). In this study, 5 mL of each effluent was placed onto filter-paper disks (Whatman) covering the bottom of Petri dishes (MiniPlast Ein-Shemer, 90 × 55 mm). Likewise, 10 seeds of each plant species were then incubated in each dish at room temperature (22 °C) with laboratory light during 144 h (6 d). For testing the influence of different concentrations of effluents, several concentrations (6.25%, 12.5%, 25%, 50% and 100%) were prepared. In all experiments, controls with distilled water were always included and each sample and conditions were tested in triplicate. This methodology was adapted from Zucconi *et al.* (1981), Villamar *et al.* (2014) and Venegas *et al.* 2018.

Soil bioassays

For this analysis, artificial soil was used with the following composition: 20% kaolin clay, 10% peat and 70% sand according to OCDE guidelines 208 which indicates that substrates should be composed of inert materials for minimizing the interaction with the test substance (OECD 2006). Ten seeds of *R. sativus* and *T. aestivum* were planted in 300 g of soil contained in plastic pots and they were irrigated with 150 mL of different effluents (HSSF-UV, HSSF-Cl, AS-UV, AS-Cl) during 336 h (14 d). As with the Petri dish bioassays, different concentrations were tested (6.25%, 12.5%, 25%, 50% and 100%) in triplicate with control samples (distilled water) (Villamar *et al.* 2014; Venegas *et al.* 2018).

Phytotoxicity indicators

To evaluate the phytotoxicity of different effluents (HSSF-UV, HSSF-Cl, AS-UV, AS-Cl), the number of seed germinated of *R. sativus* and *T. aestivum* and their epicotyl and root lengths were determined in each sample. With these values, the percentage of germination inhibition (PGI), the percentage of epicotyl elongation (PEE) and the germination index (GI), were calculated for each plant species as follows:

\[
PGI = \frac{(SGC - SGS)}{SGC} \times 100
\]

where SGC is the number of seeds germinated in the control and SGS is the number of seeds germinated in the different samples (HSSF-UV, HSSF-Cl, AS-UV, AS-Cl). With negative PGI (<0%), the germination is stimulated and with positive PGI (>0%), the number of seeds decreases regarding the control and the germination is inhibited (Villamar *et al.* 2014).

\[
PEE = \frac{(EEC - EES)}{EEC} \times 100
\]

where EEC is the epicotyl elongation in the control and EES is the epicotyl elongation in the different samples. With negative PEE (<0%), the growth of epicotyl is stimulated and with positive PEE (>0%), the growth of epicotyl decreases regarding the control and the elongation is inhibited (Villamar *et al.* 2014; Venegas *et al.* 2018).

\[
GI = \frac{(PGS \times PRL)}{100}
\]

where PGS is the percentage of germinated seeds in the different samples divided by the number of germinated seeds in the control, and PRL is the percentage of root length of seeds in the different samples divided by the root length of seeds in the control. According to Trautmann & Krasny (1997) and Pinho *et al.* (2017), the
GI can be classified as: GI > 80%: no inhibition; 60% < GI < 80%: mild inhibition; 40% < GI < 60%: strong inhibition and GI < 40%: severe inhibition.

**Statistical analyses**

Analyses were performed to determine the phytotoxicity of different effluents (HSSF-UV, HSSF-Cl, AS-UV, AS-Cl) on *R. sativus* and *T. aestivum* seeds. After checking for normality by the Shapiro–Wilk test, averages of phytotoxicity indicators (PGI, PEE and IG) were compared in different wastewater and disinfection treatments for Petri dishes and soil through ANOVA test (for data with normal distribution) and Kruskal-Wallis test (for data without normal distribution). To compare different wastewater and disinfection treatments in seeds of *R. sativus* and *T. aestivum*, data with a normal distribution were analyzed with a paired t-test and data without normal distribution were subjected to Wilcoxon test. The same tests have been applying to compare physicochemical and microbiological parameters of different effluents. Statistical analyses were done using INFOSTAT software (version 8.0) with a significance level of *p* = 0.05 (Di Rienzo et al. 2011).

**RESULTS AND DISCUSSION**

**Physicochemical and microbiological characterization of HSSF and AS effluents**

Table 2 shows the physicochemical and microbiological characterization of the effluents of HSSF CWs and AS with different disinfection treatments (UV and chlorination). For the effluents of HSSF-UV, HSSF-Cl, AS-UV and AS-Cl, the values of pH and temperature ranged between 7.4 and 8.3 and between 9.6 and 13.8 °C, respectively, without significant difference between disinfection and wastewater treatments (*p* > 0.05). On the contrary, the EC values of HSSF-UV and HSSF-Cl were 50% higher than AS-Cl and AS-UV. These differences are explained by the salinity

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>HSSF</th>
<th>AS</th>
<th>UV</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>8.3 ± 0.64</td>
<td>7.5 ± 0.2</td>
<td>8.0 ± 0.38</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>EC</td>
<td>μS/cm</td>
<td>1,278.5 ± 38.9</td>
<td>671.2 ± 45.5</td>
<td>1,275.5 ± 68.6</td>
<td>645.4 ± 59.9</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>9.6 ± 1.0</td>
<td>13.3 ± 8.6</td>
<td>9.61 ± 1.0</td>
<td>13.8 ± 9.1</td>
</tr>
<tr>
<td>DO</td>
<td>mg/L</td>
<td>0.4 ± 0.3</td>
<td>7.4 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>COD</td>
<td>mg/L</td>
<td>142.6 ± 11.5</td>
<td>79.4 ± 2.9</td>
<td>148.4 ± 20</td>
<td>81.0 ± 5.1</td>
</tr>
<tr>
<td>BOD₅</td>
<td>mg/L</td>
<td>24.0 ± 4.2</td>
<td>24.7 ± 25.9</td>
<td>27.0 ± 4.2</td>
<td>23.7 ± 24.2</td>
</tr>
<tr>
<td>TSS</td>
<td>mg/L</td>
<td>10.6 ± 12.9</td>
<td>6.2 ± 3.0</td>
<td>11.6 ± 6.4</td>
<td>6.3 ± 4.5</td>
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<tr>
<td>VSS</td>
<td>mg/L</td>
<td>10.6 ± 12.9</td>
<td>6.2 ± 3.0</td>
<td>11.6 ± 6.4</td>
<td>6.3 ± 4.5</td>
</tr>
<tr>
<td>NH₄⁺–N</td>
<td>mg/L</td>
<td>47.5 ± 37.6</td>
<td>4.2 ± 7.2</td>
<td>75.8 ± 13.3</td>
<td>3.5 ± 6.0</td>
</tr>
<tr>
<td>NO₃⁻–N</td>
<td>mg/L</td>
<td>ND</td>
<td>1.2 ± 1.1</td>
<td>ND</td>
<td>1.3 ± 0.7</td>
</tr>
<tr>
<td>TN</td>
<td>mg/L</td>
<td>77.0 ± 3.5</td>
<td>7.9 ± 6.5</td>
<td>83.3 ± 13.3</td>
<td>8.2 ± 6.4</td>
</tr>
<tr>
<td>TP</td>
<td>mg/L</td>
<td>12.4 ± 0.8</td>
<td>6.3 ± 1.1</td>
<td>13.0 ± 1.4</td>
<td>6.2 ± 1.0</td>
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<tr>
<td>PO₄³⁻–P</td>
<td>mg/L</td>
<td>9.8 ± 4.7</td>
<td>4.7 ± 1.6</td>
<td>10.9 ± 2.1</td>
<td>5.0 ± 1.8</td>
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<tr>
<td>FCl</td>
<td>mg/L</td>
<td>2.2 ± 1.1</td>
<td>4.2 ± 0.8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TCl</td>
<td>mg/L</td>
<td>3.7 ± 1.0</td>
<td>8.6 ± 3.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FC</td>
<td>MPN/100 mL</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

All values are expressed as the mean ± standard deviation, *n* = 3.

DO, dissolved oxygen; COD, chemical oxygen demand (detection limit (DL): 0.01 mg/L); BOD₅, biological oxygen demand (DL: 0.5 mg/L); TSS, total suspended solids (DL: 0.05 mg/L); VSS, volatile suspended solids (DL: 0.05 mg/L); NH₄⁺–N, ammoniacal-nitrogen (DL: 0.01 mg/L); NO₃⁻–N, nitrate as nitrogen (DL: 0.5 mg/L); TN, total nitrogen (DL: 0.5 mg/L); TP, total phosphorus (DL: 0.5 mg/L); PO₄³⁻–P, phosphate as phosphorus (DL: 0.01 mg/L); FCl, free chlorine (DL: 0.01 mg/L); TCI, total chlorine (DL: 0.01 mg/L); FC, fecal coliform (1.8 NMP/100 mL); ND, not detected.
increment caused by the process of evapotranspiration that takes place in HSSF systems (Masi & Martinuzzi 2007). Moreover, these EC values were 1.9–2.4 times lower than those determined by Andreo-Martínez et al. (2017) in HSSF system where the evapotranspiration rate was 309 mm/m² compared with 191 mm/m² reported by HSSF system of this study (López et al. 2015).

For average concentrations of BOD₅, TSS and VSS, non-significant differences between different effluents (HSSF-UV, HSSF-Cl, AS-UV, AS-Cl) were observed ($p > 0.05$) and their values fluctuated between 23.7 and 27 mg/L, 6.2 and 11.6 mg/L and 6.2 and 11.6 mg/L, respectively. In the case of COD, differences of 44–45% between the effluent of HSSF-Cl and HSSF-UV with AS-Cl and AS-UV were reported ($p < 0.05$). These high concentrations of HSSF-Cl and HSSF-UV (142.6 and 148.4 mg/L, respectively) may be due to the fact that the AS technology achieves removal efficiency of COD close to 99%, as opposed to HSSF system which varied between 57% and 60% (Table 1).

Regarding $\text{NH}_4^+$-N and TN average concentrations, HSSF systems (HSSF-UV and HSSF-Cl) had 16 and 10 times higher values than AS systems (AS-UV and AS-Cl), respectively. As evidence literature, HSSF systems promote anaerobic conditions with DO and oxidation potential reduction (OPR) concentrations below 0.5 mg/L and $\sim 180$ mV, respectively (Dušek et al. 2008). These conditions do not allow nitrification which is an aerobic mechanism (DO $> 1.5$ mg/L) considered the main pathway for $\text{NH}_4^+$-N transformation to $\text{NO}_3^-$-N (Ye & Li 2009). In the HSSF system, the DO concentrations were below 0.4 mg/L. On the other hand, AS system generated aerobic conditions (DO $> 7$ mg/L) that stimulated nitrification and for this reason, they achieved removal efficiencies of $\text{NH}_4^+$-N between 52% and 96% (Limpiyakorn et al. 2011). In this study, AS-Cl and AS-UV effluents showed mean $\text{NO}_3^-$-N concentrations of 1.2 and 1.3 mg/L, respectively (Table 2). Additionally, a significant difference of 37% in $\text{NH}_4^+$-N concentration between HSSF-Cl (47.5 mg/L) and HSSF-UV (75.8 mg/L), was observed ($p < 0.05$). It is reported that during the chlorination, if the wastewater has a higher content of $\text{NH}_4^+$-N (> 20 mg/L), it can lead to the formation of disinfection byproducts (DBPs) that have been shown to be cytotoxic, genotoxic and carcinogenic (Watson et al. 2012; Du et al. 2017). In the case of HSSF systems, the average concentrations of $\text{NH}_4^+$-N in the influent were close to 60 mg/L (data no shown) and it is possible that can induce the formation of DBPs in HSSF-Cl.

For PT and $\text{PO}_4^{3-}$-P concentrations, the behavior was similar to $\text{NH}_4^+$-N and TN. In this case, the average concentrations were two times higher in HSSF systems (HSSF-UV and HSSF-Cl) than in AS systems (AS-UV and AS-Cl), for both parameters evaluated. HSSF and AS systems have similar removal efficiencies of phosphorus which are close to 10% (Table 1). However, some research reported that the phosphorus adsorption capacity of CWs support medium (gravel) decreased over 5 years and became saturated (Vohla et al. 2011). HSSF system was implemented in July 2011 (López et al. 2013). In the case of FC, for all effluents evaluated (HSSF-UV, HSSF-Cl, AS-UV, AS-Cl), the concentrations of pathogens were below the detection limit that, in the analysis used (9221-E), was 1.8 MPN/100 mL.

According to the guidelines determined by USEPA (2022b), there exist three categories for water reuse in agriculture. For the first category, which involved the use of reclaimed water for surface or spray irrigation of food crops which are intended for human consumption and consumed raw, the effluents of HSSF-Cl, HSSF-UV, AS-Cl and AS-UV did not accomplish with concentrations of BOD₅ ($\leq 10$ mg/L) and FC (1 mg/L). For the second and third category, only effluents of HSSF-UV and AS-UV can be used for surface irrigation of food crops commercially processed and non-food crops. The chlorination provides effluents (HSSF-Cl and AS-Cl) with concentrations of FC above the limit set by USEPA (2022b) guidelines (1 mg/L).

**Phytotoxicity effects of HSSF and AS effluents evaluated in Petri dishes bioassays**

Table 3 shows the phytotoxicity effects of different wastewater and disinfection treatments on *R. sativus* and *T. aestivum* in Petri dishes bioassays. In the case of *R. sativus*, the PGI achieved negative values for all effluents evaluated (HSSF-UV, HSSF-Cl, AS-Cl, AS-UV) that fluctuated between $-3$% and $-18$%. This result indicates that effluents have a positive effect on seeds germination. However, positive values of PGI were observed in AS-UV effluent, specifically for 6.25% and 50% concentrations with percentage of 15% and 18%, respectively. For PEE values that varied from $-7$% to $-77$% in all effluents, the behavior was similar to PGI and they showed that the epicotyl elongation of this species was stimulated. Likewise, differences between effluents (HSSF-UV, HSSF-Cl, AS-Cl, AS-UV) in phytotoxicity indicators (PGI and PEE) were not detected ($p > 0.05$). For *T. aestivum*, the PGI values differed between wastewater and disinfection treatments. For HSSF-Cl effluent, the PGI increased between 3% and 24% from 6.25% to 100% concentration showing an inhibition of seeds germination. Different results were observed for HSSF-UV effluent.
Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R. sativus</th>
<th>T. aestivum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wastewater Treatment</td>
<td>Disinfection System</td>
<td></td>
</tr>
<tr>
<td>GI (PGI %)</td>
<td>6.25% 12.5% 25% 50% 100%</td>
<td>6.25% 12.5% 25% 50% 100%</td>
</tr>
<tr>
<td>UV</td>
<td>9 ± 3</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>HSSF</td>
<td>14 ± 5</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>AS</td>
<td>7 ± 3</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>PEE (%)</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
</tr>
</tbody>
</table>

All values are expressed as the mean ± standard deviation. n = 3.

PGI, percentage of germination inhibition; PEE, percentage of epicotyl elongation.

where the PGI values were negatives (between −4% and −18%) without germination inhibition (p > 0.05). In this case, significant difference between HSSF-Cl and HSSF-UV was detected (p < 0.05). In the case of AS-UV, positive effect on germination was only observed for 100% concentration with PGI value of −31%. Furthermore, the PEE values were significantly different between HSSF-Cl and AS-Cl and between HSSF-UV and AS-UV (p < 0.05). For HSSF effluents (HSSF-Cl and HSSF-UV), a negative effect on the epicotyl elongation was observed with values that fluctuated between 5% and 10%. However, when seeds were irrigated with AS effluents (AS-Cl and AS-UV), the epicotyl elongation stimulation was observed (PEE > −48%).

Figure 2 shows the GI of R. sativus and T. aestivum seeds determined on Petri dishes for different wastewater and disinfection treatments. For seeds of R. sativus irrigated with HSSF-Cl (Figure 2(a1)) and AS-Cl (Figure 2(a2)), the GI decreased between 164% to 92% and between 179% to 90%, respectively, when the effluent concentration increased (p < 0.05). Despite this behavior, in both cases the GI were above 80% which indicate that germination was not inhibited. The same results were observed for AS-Cl effluent where GI values were over of 100% for all concentrations. Furthermore, for AS-UV effluent, positive effect was also detected with GI values above to 82%, except for 6.25% concentration (GI = 76%). In this case, the germination was mildly inhibited. In general, the germination of T. aestivum seeds was promoted when they were irrigated with HSSF-Cl, HSSF-UV, AS-Cl and HSSF-Cl (GI > 80%) (Figure 2(b)). However, in some cases, the germination was mild inhibited with GI values between 60% and 80%. This was the case of HSSF-Cl (50% and 100% concentrations) and AS-Cl effluents (50% concentration). A similar tendency was observed by Ravindran et al. (2016) where they evaluated the phytotoxicity of untreated and treated wastewater. The results of this study showed that GI values were above 80% for seeds of L. esculentum, R. sativus, D. carota and A. cepa when they were irrigated with treated wastewater.

The phytotoxicity indicators (PGI, PEE and GI) evaluated in Petri dishes determined that R. sativus showed a higher level of tolerance to different effluents as compared to T. aestivum during the early growth phase of the seedlings. This finding was similar to those reported by Bhateria & Dhaka (2017) which evaluated the effect of electroplating industry effluent on germination and indicated that Hordeum vulgare (H. vulgare) had better results in phytotoxicity indicators than T. aestivum. Regarding the effect of different disinfection system on T. aestivum seeds, the germination and the epicotyl
Elongation were more affected by chlorination than by UV system. It is known that the FCI is toxic for sensitive crops already at concentration of 0.05 mg/L (USEPA 2012b). In HSSF-Cl and AS-Cl, the concentration of FCI was 2.2 mg/L and 4.2 mg/L, respectively. Likewise, the effect of different wastewater treatments on phytotoxicity indicators of *T. aestivum* seeds were also noted, showing that the germination and epicotyl elongation were more stimulated in AS systems. As mentioned in the section ‘Physicochemical and microbiological characterization of HSSF and AS effluents’, the high concentration of NH$_4^+$–N (47.5 mg/L) in the influent can promote the formation of DPSs which can influence the germination and epicotyl elongation inhibition (Akande et al. 2010).

Phytotoxicity effects of HSSF and AS effluent evaluated in soil bioassays

Table 4 shows the phytotoxicity effects of different wastewater and disinfection treatments on *R. sativus* and *T. aestivum* in soil bioassays. As opposed to results determined in Petri dishes, the PGI and PEE achieved by seeds of *R. sativus* and *T. aestivum* were positive. These values, which fluctuated from 7% to 67%, indicate that the germination and the epicotyl elongation were affected by HSSF-Cl, HSSF-UV, AS-Cl and AS-UV effluents. Only for 6.25% concentration, negative values (from −1% to −11%) of PGI (AS-UV) and PEE (HSSF-Cl, HSSF-UV, AS-UV) were detected in both species (*p* < 0.05). Moreover, few significant differences between wastewater and disinfection system were observed. For seeds of *T. aestivum*, significant differences between HSSF-Cl and AS-Cl were reported (*p* < 0.05).

Figure 3 shows the GI of plant seeds determined on soil for HSSF-Cl, HSSF-UV, AS-Cl and AS-UV effluents. In this case, the responses of irrigated seeds depended on treatments (wastewater and disinfection) and different concentrations evaluated. For *R. sativus* seeds irrigated with HSSF-Cl, the germination was mildly and strongly inhibited (55% < GI < 67%), except for 12.5% concentration which achieved a GI value above 80% (Figure 3(a1)). Similar results were observed for HSSF-UV effluent where the germination was affected strongly in 6.25% and 12.5% concentrations (GI = 54% and 56%, respectively), but it was mild and not affected in 25%, 50% and 100% concentrations (GI > 72%). In contrast, seeds irrigated with AS-Cl and AS-UV effluents have GI above 82% (Figure 3(a2)). Only for 6.25% and 50%
Concentrations of AS-Cl effluent, the germination of *R. sativus* seeds was strong (GI = 50%) and mildly inhibited (GI = 62%), respectively. For seeds of *T. aestivum* irrigated with HSSF-Cl and HSSF-UV, the GI values fluctuated between 50% and 77% (Figure 3(b1)).

In these cases, these effluents have negative effects on seeds germinations (mild and strong), except for 6.25% concentration in both effluents (GI > 80%). Moreover, only for 50% and 100% concentrations of AS-Cl, the germination of *T. aestivum* was stimulated (GI > 96%). As opposed to the results achieved by seeds of *R. sativus* irrigated with AS-UV, the germination was mildly inhibited (68% < GI < 79%).

In general, the phytotoxicity indicators (PGI, PEE and GI) showed that chlorination disinfection (HSSF-Cl and AS-Cl) had a toxic effect on both seeds species growing in soil. It is reported that the FCl can react with the organic matter of the soil, leading to the formation of organo-halogenated byproducts (OX) that may affect physiological plant parameters such as photosynthesis and plant growth (Crebelli *et al.* 2005; Akande *et al.* 2010). Lonigro *et al.* (2017) evaluated the use of chlorinated water for irrigating lettuce crops grown in pots with different doses of FCl (0.2, 10 and 40 mg/L). This study evidenced that the presence of FCl and combined Cl in treated wastewater for agriculture irrigation results in accumulation of OX compounds in the soil with consequent loss of soil fertility and bioaccumulation into edible parts of the crops. In HSSF-Cl and AS-Cl effluents, the concentration of FCl were 2.2 and 4.2 mg/L, respectively. Moreover, negative effects on the germination and the epicotyl elongation of HSSF effluents (HSSF-Cl and HSSF-UV) were also observed. In this case, the phytotoxicity can be related with the high EC (1,275–1,278 μS/cm) values and NH₄⁺–N concentrations of the influent. Rengasamy (2010) reported that high EC inhibits the water uptake by plants due to osmotic pressure of saline soil solution. In this study, plants cease to take up water (18% water content of the total water) when solution which fed the soil had EC values above 1,000 μS/cm.

In spite of non-significant differences on PGI, PEE and GI between different wastewater and disinfection treatments, the AS-UV effluent has a positive effect on *R. sativus* seeds germination. This result was similar to those found by Libutti *et al.* (2018) which studied the reuse of tertiary treated wastewater (AS process and UV disinfection) to irrigate *Lycopersicon esculentum* Mill. (*L. esculentum*) and *Brassica oleracea* L. var. *italic* (*B. oleracea*). These findings indicate that the irrigation with treated wastewater were not influenced the yield of crops.

### Table 4

<table>
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<tr>
<th>Parameters</th>
<th>Wastewater Treatment</th>
<th>Disinfection System</th>
<th>6.25%</th>
<th>12.5%</th>
<th>25%</th>
<th>50%</th>
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<tbody>
<tr>
<td>PGI (%)</td>
<td></td>
<td>AS</td>
<td>67.38</td>
<td>67.20</td>
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<td>49.62</td>
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<td>67.20</td>
<td>47.27</td>
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<tr>
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<td></td>
<td>UV</td>
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<td>67.20</td>
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<td>49.62</td>
<td>19.2</td>
</tr>
<tr>
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<td></td>
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<td>0.0</td>
<td>0.0</td>
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</table>

All values are expressed as the mean ± standard deviation. n = 3.
CONCLUSIONS

As mentioned previously, this study is the first research that compared the phytotoxicity between effluents from AS and HSSF using different disinfection such chlorination and UV. According to the three categories for water reuse in agriculture determined by USEPA \(^{1}\), the effluents of HSSF-Cl, HSSF-UV, AS-Cl and AS-UV did not accomplish with concentrations of BOD\(_5\) (\(\leq 10\) mg/L) and FCl (1 mg/L) established by the first category. Only effluents of HSSF-UV and AS-UV can be used for surface irrigation of food crops commercially processed and non-food crops as determined in the second and third categories.

Regarding phytotoxicity tests evaluated in Petri dishes, \(R.\ sativus\) shows higher level of tolerance to different effluents as compared to \(T.\ aestivum\) during the early growth phase of the seedlings. In fact, seeds of \(R.\ sativus\) achieved negative PGI and PEE values for all effluents evaluated which fluctuated between \(-3\%\) and \(-18\%\) and between \(2\%\) and \(-77\%\), respectively. Moreover, differences between disinfection systems on \(T.\ aestivum\) were observed and the germination and the epicotyl elongation were more affected by chlorination than by UV system.

Finally, the phytotoxicity of HSSF-Cl, HSSF-UV, AS-Cl and AS-UV effluents on soil determined positive PGI and PEE values that indicate that the germination and epicotyl elongation of \(R.\ sativus\) and \(T.\ aestivum\) were affected. The same behavior was observed for GI values of both plant species (GI <80%). GI values above 86% in \(R.\ sativus\) seeds and the good physicochemical and microbiological parameters achieved by AS-UV, indicate that in this study, this effluent has a positive effect on the seeds germination and can be recommended for treated wastewater reuse in agricultural irrigation. Despite these results, more research in this area is needed because of the safe reuse of treated wastewater on agriculture depends on several factors such as the type of the crops, the different properties of the soil and the presence of micropolutants in the wastewater. This study is the first approach to face this new challenge and for this, the combination of AS and HSSF with other disinfection technologies must be studied for having a different alternative for wastewater reuse.

ACKNOWLEDGEMENTS

This work was supported by INNOVA BIO BIO Proyect N° 13.3327-543 IN.IIP and CONICYT/FONDAP/15130015.
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