Effects on macronutrient contents in soil-plant irrigated with different quality waters and wastewaters

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ABSTRACT
The goals of this research were focused on investigating the effects of irrigation with untreated wastewater, ozone-enhanced primary treated wastewaters (O3EPTW), tap water and tap water + fertilizer on the macronutrient content in soil and plant tissues. The effect on plant development was evaluated by growing \textit{Lactuca sativa} in soils irrigated with these different quality waters and wastewaters, and by determining the macronutrients content in water, soil and plants. In this study, the soils irrigated with O3EPTW showed increased organic matter concentrations, which is advantageous for crop cultivation. The electric conductivity for the O3EPTW irrigated soils remained below those of the tap water + fertilizer and untreated wastewater. The soil irrigated with tap water + fertilizer showed a marked decrease in pH, and its long-term use could lead to soil acidification. Macronutrient levels in plant tissues (N, K and Mg contents) were similar for all irrigation waters, except for tap water which always remained lower than the others. It was concluded that the use of O3EPTW may become a good irrigation alternative that can be employed without the health risks associated with the use of untreated wastewaters, also reducing the adverse effects on soil’s salinity or acidification.

Key words | macronutrients, ozone-enhanced primary treated wastewaters (O3EPTW), wastewater reuse

INTRODUCTION

Sometimes wastewater is the only available alternative for agricultural irrigation, but its use represents a potential health hazard due to its elevated content of pathogenic microorganisms, including Helminth eggs. Pathogenic microorganism concentrations can be lowered by the use of an advanced primary treatment (APT), which provides an effluent beneficial to agriculture because of its nutrients content (Orta de Velásquez et al. 2008). The nutrients contained in reclaimed wastewater can contribute to crop growth, but periodic monitoring of nutrient levels is needed to avoid an imbalance in nutrient supply for the irrigated plants (Pedrero et al. 2010). Previous research has shown that the use of wastewater for crop irrigation may increase soil salinity, organic matter content, levels of exchangeable cations, phosphorus, and other microelements available for plant growth; as well as diminishing the soil’s pH (Kiziloglu et al. 2008; Adrover et al. 2012). Kiziloglu et al. (2008) also reported on the changes in chemical soil characteristics (N, P, K, Ca, Mg and Na contents) evaluated after irrigation with untreated, preliminary and primary treated wastewater. They concluded that the soils irrigated with wastewater showed an increase on the yield of the plants and in their macro and microelement content. Hence, wastewater has a high nutritive value that can improve plant growth, reduce fertilizer application and increase crop productivities of poor fertility soils. However, while untreated wastewaters can be used on agricultural land for a short period of time, primary-treated wastewaters can be used in sustainable agriculture over a long term (Kiziloglu et al. 2008).

Conversely, a study in Brazil (Herpin et al. 2007) found that the use of a secondary effluent to irrigate coffee crops had three major disadvantages: (1) an increase in soil sodicity; (2) a reduction of the soil’s organic matter level with a decrease in cationic exchange capacity; and (3) a discrepancy in the nutrient balance for the soil-plant system. That research also found that the soil’s N, P and S concentrations were insufficient for coffee cultivation. It was concluded that the macronutrients (N, P, K, Mg and Na) content in coffee plant tissues were within the normal ranges for the plant (with the exception of Ca, for which higher values were measured). On the other hand, Kiziloglu et al. (2008) established that the macronutrients content in plant tissue depends on the type and quality of water used for irrigation; the P, K, Ca, Mg and Na content in two crops (cauliflower and squash) decreased in the following order: untreated wastewater > pre-treatment > primary treatment > tap water.

Up to this date, there are few investigations regarding macronutrients content in soil-plant systems irrigated with primary treated wastewater (Kiziloglu et al. 2008), and none with ozonated primary treatment. Most of the published research in this field focuses only on irrigation with biological treatment effluents (Isea et al. 2004; Heidarpour et al. 2007; Kalavrouziotis et al. 2008; García-Delgado et al. 2012), but not on the effects that can be observed on macronutrient changes by using physical-chemical treatments such as an APT.

A previous study (Campos-Reales-Pineda et al. 2008) demonstrated that the use of an APT that combined the application of ozone at the coagulation step (ozone-enhanced primary treatment, O3EAPT), reduced the wastewater’s phytotoxicity and improved germination rates during a preliminary evaluation of the influence of the treated effluents on plant seedlings. It was mentioned that those findings could result in beneficial effects such as improved productivities for irrigated plants. Nevertheless, the relationship between the nutrient content of the water, soil and plants was not investigated. Therefore, the aim of the present research is to evaluate the effect of the irrigation with different quality waters and wastewaters (including those subjected to an ozone-enhanced primary treatment) on the macronutrients content in soil and plant tissues of Italian lettuce.

**METHODS**

In order to evaluate the effects on macronutrients content (N, P, K) and ion concentrations (Na+, Ca2+, Mg2+, and SO42−) in the soil-plant system, irrigation experiments were carried out in a greenhouse during 120 days using Italian lettuce (Lactuca sativa). For this purpose, five types of water were employed for irrigation: tap water, tap water + fertilizer, untreated wastewater and ozone-enhanced primary treated wastewaters (O3EPTW). The number of analysed samples is specified in each subsection. During the experimentation period, each type of irrigation water was prepared every time just before irrigation, according to the procedures described below.

**Types of water used for irrigation**

- Tap water: used as a control to evaluate the evolution of the original nutrients content of the soil.
- Tap water + fertilizer: used to simulate ideal nutrient conditions for growing L. sativa. It was prepared using the recommended mixture of the commercial fertilizer Ultra-sol Multipurpose, manufactured by SQM (Sociedad Quimica y Minera de Chile S.A.).
- Untreated wastewater: collected from the influent of the Cerro de la Estrella Wastewater Treatment Plant (CEWWTP), located in the southeast of Mexico City. The influent of this treatment plant mostly receives municipal discharges.
- Ozone-enhanced primary treated wastewaters (O3EPTW, A and B): these were obtained by treating in the laboratory the influent from the CEWWTP, according to Campos-Reales-Pineda et al. (2008), followed by silica sand filtration (10 μm sieve) and disinfection by either of these methods.
- O3 disinfection (O3EPTW-A); the combination of ozone followed by chlorination (O3 + NaOCl): carried out in a semi-batch reactor applying a total ozone dose of 20 mg O3/L H2O, followed by 2 mg/L of NaOCl contacted for 60 min.
- NaOCl disinfection (O3EPTW-B): dosing only 20 mg/L NaOCl and contacted for 60 min.

The chemical oxygen demand (COD), biochemical oxygen demand (BOD5), total suspended solids (TSS), turbidity
and pH were determined according to Standard Methods for the Examination of Water and Wastewater (APHA 2005). Faecal coliforms (FC) were quantified by the Membrane Filtration method (NMX-AA-102-SCFI-2006), using Difco MFC selective agar media and incubation in a water bath at 44.5 ± 2°C for 24 h. Helminth eggs (HE) were determined in accordance to the NOM-001-SEMARNAT-1996 procedures. These physicochemical and microbiological parameters were measured weekly.

Throughout the irrigation experiments, a total of 40 subsamples were collected to produce a composite sample for each type of irrigation water. Cations (K⁺, Na⁺, Ca²⁺, Mg²⁺) and anions (NO₂⁻, NO₃⁻, SO₄²⁻, PO₄³⁻) were analysed using an ion chromatography system (Model ICS-1500, Dionex, Sunnyvale, CA, USA).

Growing L. sativa

A greenhouse with 10 drawers (0.169 m³ each) was used to conduct the irrigation experiments. Each drawer was filled with 75 kg of sandy loam virgin soil suitable for agriculture. Two drawers were used for each type of water and 10 plants of L. sativa were grown in every drawer. Lettuce seedlings were planted in January 2011 and the plants were harvested in May 2011. The randomised irrigation arrangement employed in the greenhouse is shown in Figure 1. The greenhouse temperature was maintained between 10 and 25°C. Irrigation was carried out every other day: initially, a water volume of 100 mL per plant was used, until a final volume of 400 mL per plant was reached at the end of the experiments. When the plants were harvested (day 120) the crop productivities were evaluated by measuring the length and the weight of the irrigated plants.

Macronutrients content (soil and plant tissues)

Macronutrients in soils were determined at the beginning of the experiment (virgin soil, before any irrigation) and at 40, 80 and 120 days of crop cultivation. One hundred grams of soil were taken from each plant (20 plants for each water type). In every case, the subsamples were mixed and a total of 2 kg of soil was collected to form a composite sample. Soil samples were dried, sieved (No. 10 sieve opening, 2.0 mm), and preserved at 4°C until analysis. The exchangeable cations (K⁺, Na⁺, Ca²⁺, and Mg²⁺) were analysed following the NOM-021-SEMARNAT-2000 procedures. K⁺ and Na⁺ were measured by flame atomic emission spectrometry (FAES) using a Single-Channel Digital Flame Analyzer (Model EW-02655-00, Cole-Parmer Instrument Company, Chicago, IL, USA). Ca²⁺ and Mg²⁺ were determined by inductively coupled plasma atomic absorption spectrometry (ICP-AAS) with a Model UNICAM 989 Solaar AA Spectrometer (Thermo Elemental Corp., Franklin, MA, USA). Soil physicochemical analyses (pH, electrical conductivity (EC), available P and organic matter content) were determined according to the NOM-021-SEMARNAT-2000. After soil digestion in microwave and filtration, NH₄⁺ and PO₄³⁻ were also analysed using an ion chromatography system (Model ICS-1500, Dionex, Sunnyvale, CA, USA).

For plant tissues, the N, P, K, Ca and Mg content were measured after harvesting all L. sativa plants (20 plants for each type of irrigation water). The lettuces were washed with distilled water and placed in paper bags to reduce excess moisture until analysis. In order to obtain enough biomass to perform the plant tissue analyses, composite samples were also prepared in this case. After diacid mixture digestion, N concentrations were determined by the Total
Kjeldahl Nitrogen method; P was analysed by using a Thermo Spectronic Genesys 10 UV spectrophotometer (Thermo Fischer Scientific Inc., Waltham, MA, USA); and the K, Ca and Mg content were determined by FAES and ICP-AAS as was done for soils analyses.

**Composite sampling**

Appropriate considerations on compositing were taken into account to perform the composite sampling procedures for ionic content in water; and macronutrients content in soil and plant tissues (US EPA 1995).

Temporal composite sampling was used for each of the five different quality waters and wastewaters to analyse their corresponding ionic concentrations. In every case, 40 sub-samples were collected periodically throughout the 120 days of plant irrigation. During the experiment, a volume of water equivalent to 10% of that used to irrigate the crops was collected, mixed and stored at 4°C until analysis.

Spatial composite sampling was applied in soils for each pair of irrigation drawers (Figure 1) to study the evolution of macronutrients and its characteristics during crop irrigation. Consequently, five different composite samples were taken at 40, 80 and 120 days. Each composite sample was well homogenised and formed by taking 20 evenly distributed representative subsamples.

**Statistical tests**

The crop productivities for each type of irrigation water were assessed at cultivation (day 120). The plant length and weight data (\( n = 20 \) in every case) were subjected to a one-way analysis of variance (ANOVA) to establish the similarities between samples. The statistical tests were conducted with the XLSTAT package under EXCEL.

**RESULTS AND DISCUSSION**

**Untreated and treated wastewater quality**

The quality of the untreated and treated wastewaters used in the irrigation experiments is presented in Table 1. It was corroborated that both O3EPTW (A and B) complied with the physicochemical and microbiological parameters for unrestricted irrigation (cultivation and harvesting of forage, grains, fruits and vegetables that are eaten raw, such as lettuce) as established in the local regulation (NOM-001-SEMAR-NAT-1995).

Table 2 shows the cationic and anionic content for the composite sample analysis of the different types of water used for irrigation as an average of the 120-day trial. Between the treated and untreated wastewaters, the cationic concentrations (NH₄⁺, K⁺, Ca²⁺, Mg²⁺ and Na⁺) are in the same order of magnitude. This result suggests that the soils irrigated with O3EPTW (A, B) can maintain the nutritional content (except PO₄³⁻) for its reuse in agricultural irrigation. This is in accordance with previous results for

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated Wastewater (Mean ± S.D.)</th>
<th>O₃EPTW-A (Mean ± S.D.)</th>
<th>O₃EPTW-B (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg/L)</td>
<td>322.4 ± 44.6</td>
<td>82.6 ± 28.1</td>
<td>82.5 ± 38.0</td>
</tr>
<tr>
<td>BOD₅ (mg/L)</td>
<td>172.0 ± 12.2</td>
<td>31.3 ± 11.3</td>
<td>34.2 ± 11.1</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>56.3 ± 8.7</td>
<td>16.2 ± 14.3</td>
<td>41.6 ± 12.1</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>234.0 ± 40.3</td>
<td>84.5 ± 0.7</td>
<td>87.3 ± 3.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 ± 0.03</td>
<td>7.6 ± 0.06</td>
<td>7.6 ± 0.01</td>
</tr>
<tr>
<td>FC (CFU/100 mL)</td>
<td>1.14 × 10⁷</td>
<td>0</td>
<td>1 × 10²</td>
</tr>
<tr>
<td>Helminth eggs/L</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ion</th>
<th>Tap water fertiliser</th>
<th>Tap water + O₃EPTW-A</th>
<th>Tap water + O₃EPTW-B</th>
<th>Untreated wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺</td>
<td>0.20</td>
<td>24.41</td>
<td>27.81</td>
<td>27.62</td>
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<tr>
<td>K⁺</td>
<td>5.19</td>
<td>147.90</td>
<td>14.58</td>
<td>12.05</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>21.72</td>
<td>24.31</td>
<td>27.85</td>
<td>27.11</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>20.09</td>
<td>25.40</td>
<td>18.95</td>
<td>18.19</td>
</tr>
<tr>
<td>Na⁺</td>
<td>31.01</td>
<td>63.58</td>
<td>60.09</td>
<td>63.60</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>0.68</td>
<td>11.49</td>
<td>4.87</td>
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<tr>
<td>SO₄²⁻</td>
<td>34.48</td>
<td>71.73</td>
<td>76.14</td>
<td>78.13</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>2.06</td>
<td>18.10</td>
<td>14.23</td>
<td>11.08</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>0.04</td>
<td>0.03</td>
<td>0.58</td>
<td>0.32</td>
</tr>
</tbody>
</table>

**Table 1 | Physicochemical and microbiological characterization of wastewaters**

**Table 2 | Ionic concentrations in waters and wastewaters used for irrigation**
conventional APT effluents (Orta de Velásquez et al. 2008). With regard to the tap water + fertilizer, it can be seen that the NH$_4^+$ concentration is around three times that of the wastewaters, while the K$^+$ content is almost 10-fold. As expected, tap water does not show a considerable nutritional value in N, P, K ions (NH$_4^+$, K$^+$, PO$_4^{3-}$, NO$_3^-$, NO$_2^-$). The concentrations of these ions are low for the tap water, which is consistent with previously reported values (Jiménez-Cisneros 2000); and its concentrations comply with the regulations for drinking water (NOM-127-SSA1-1994).

However, with regard to the untreated wastewater, the O$_3$EPTW (A and B) exhibited a decrease in the phosphate content and an increase of sulphates. Tap water + fertilizer contained the highest concentrations of phosphate (PO$_4^{3-}$: 11.49 mg/L) and nitrate (NO$_3^-$: 18.10 mg/L) anions. This indicates that for the tap water + fertilizer, there are more ions available for plant growth and development.

**Macronutrients content in soil**

According to the properties for soil fertility (NOM-021-SEMARNAT-2000), the virgin soil displayed ‘very low’ concentrations of NH$_4^+$ (21.7 mg/kg) and K$^+$ (110.0 mg/kg); while its P (13.1 mg/kg) and Mg$^{2+}$ (473.0 mg/kg) contents were classified as ‘low’, and the levels of Ca$^{2+}$ (1448.0 mg/kg) as ‘medium’. Additionally, the soil contained a high percentage of organic matter (6.59%, important for moisture-retention). Its pH of 6.60 was considered neutral. Figure 2 shows the evolution of macronutrients and soil characteristics for the five types of irrigation water during crop cultivation.

A large percentage of nitrogen was accumulated in the soil irrigated with tap water + fertilizer (Figure 2(a)). The irrigation with tap water did not provide a significant supply of NH$_4^+$, so its availability in the soil was almost completely consumed. The untreated wastewater showed a slight increase in nitrogen concentration at 80 and 120 days. The O$_3$EPTW irrigation showed a slight increase in soil NH$_4^+$ at 40 days, but then it showed a decrease in its concentration, being more pronounced for the O$_3$EPTW at the end of the cultivation period.

In all cases, soil concentrations of P decreased at 120 days (Figure 2(b)). This suggests that there is an important demand for this element by the crops (CPHA 2002). K$^+$ accumulated in all soils (except O$_3$EPTW-A); the largest increase in K$^+$ content occurred for the tap water + fertilizer irrigation (Figure 2(c)). Parameters such as Na$^+$, Ca$^{2+}$, SO$_4^{2-}$, EC and organic matter, had a similar behaviour and their contents in all soils tended to increase during the crop cultivation (Figure 2(d), (e), (g), (h), (j)).

In spite of the increasing exchangeable ionic concentrations in soils, it was found that the irrigation with O$_3$EPTW did not change the soil’s salinity classification (NOM-021-SEMARNAT-2000) as determined by its electrical conductivity, EC (Figure 2(h)). During the 120 days of crop irrigation, the EC of the soils irrigated with O$_3$EPTW (A and B), remained consistently lower than the values measured in the soils irrigated with tap water + fertilizer (between 33 and 58% lower) and untreated wastewater (around 65% lower). These results are consistent with the findings of Kizilolu et al. (2008), where the salinity of the soil irrigated with primary treated wastewater was about half of that contained by the soils irrigated with untreated wastewater.

For the current research, this can be explained by a buffer effect that may be promoted by the ozone-oxidised organic matter. The organic matter of the two irrigation waters that were subjected to ozonation (O$_3$EPTW) will contain higher levels of COOH groups. The formation of these groups can increase the soil’s cation exchange capacity (Bot & Benites 2005) and, consequently, the soils irrigated with O$_3$EPTW will exhibit a lower EC than that for untreated wastewater and tap water + fertilizer throughout all cultivation. According to the NOM-021-SEMARNAT-2000 classification, at 120 days of crop cultivation the soil irrigated with untreated wastewater was the only case that started to show signs of salinisation.

On the other hand, the soil irrigated with tap water + fertilizer showed a marked decrease in its pH (pH: 5.52; Figure 2(i)), so its long-term use can lead to soil acidification. In contrast, the soil samples irrigated with untreated wastewater and O$_3$EPTW (A and B) maintained a pH between 6.1 and 6.2, which is similar to that of the untreated wastewater. The acidification of the soil irrigated with tap water + fertilizer can be explained by a variety of factors, including the nitrification of ammonium (Vázquez-Montiel et al. 1996), oxidation of sulphites and the production of organic acids, which are a consequence of the mineralization of organic matter (Oliveira et al. 2002).
Figure 2 | Evolution of macronutrients and soil characteristics during crop irrigation.
Lastly, it was observed that the content in organic matter increased in all soil samples (Figure 2(j)). For the untreated wastewater and treated wastewaters soils, the increase is due to the organic content of these waters, in addition to the presence of crop residues. In the case of tap water, this was attributed to the presence and degradation of plant debris and humus.

**Macronutrients content in plant tissues**

With regard to the macronutrients content in plant tissues (Figure 3), it was noted that for all irrigation waters the N, K and Mg content in plant tissues were similar. However, the plants irrigated with tap water + fertilizer exhibited the highest P and Ca content.

In plant tissues, the nutrient content for the treated and the untreated wastewaters appeared to be within the reported ranges for *L. sativa* (Alzate & Loaiza 2008). Lettuces, like many other crops, can easily assimilate nutrients that are provided by reclaimed municipal wastewaters; because most of them exist as free ionic species, which are readily available for plant uptake (Pereira et al. 2012).

Another interesting result is that the content of nutrients and ions (N, P, K, Ca and Mg) in plant tissues irrigated with treated and untreated wastewater was broadly similar to that of the plants that were irrigated with tap water supplemented with fertilizer, despite the differing concentrations found in soils and irrigation waters. This shows that the nutrient assimilation by the crops is not affected by the O3EPTW irrigation.

**L. sativa crop productivity**

Figure 4 shows the length and weight for the lettuce plants at harvest (day 120). The statistical analysis (ANOVA) of the plant productivities allowed confirmation that there are statistical differences between the growths of lettuce plants irrigated by the five types of water.

As expected, the plants irrigated by the tap water + fertilizer displayed the largest productivities ($p = 0.000$ for lengths and weights) due to the highest macronutrients content in the corresponding irrigation water and soil (Table 2 and Figure 2).

The lengths of the plants irrigated by untreated wastewater showed a smaller length than those that were irrigated with O3EPTW (A and B), and the tap water ($p = 0.003$). With regard to the lettuce weights, no statistical difference was found between the plants irrigated with O3EPTW (A and B), tap water and the untreated wastewater ($p = 0.0712$).

Therefore, the O3EPTW irrigation can be employed as an option to the use of tap water wherever water resources are limited, or as an alternative to the untreated wastewater irrigation without the microbiological risks that its use represents.

A factor to be considered is that the original soil already contained sufficient nutritional components necessary for the single-season crop cultivation like the one carried out in this research. However, the benefits of irrigating soils with O3EPTW treated waters (such as the increase in organic matter content) could become more evident if depleted soils are used.
For this purpose, in future studies it is recommended to use a depleted or an infertile soil (without any nutrients, like for example, sand; or tezontle, a type of volcanic-rock), in order to minimize interferences from nutrients that are already present in the original (starting) soil. Additionally, it is necessary to consider that there are other factors (such as biological ones) that may also affect the plant growths.

CONCLUSIONS

O$_3$EPTW irrigation can provide an alternative to untreated wastewater (without the microbiological risks that its use represents) or replace the need of fresh water for irrigation (wherever water resources are limited).

According to Mexican legislation (NOM-001-SEMARNAT-1996), the quality of the O$_3$EPTW waters allows their use for unrestricted irrigation (for watering vegetables and agricultural products that are eaten raw).

The increase in organic matter content with lower electrical conductivity values for O$_3$EPTW soils can provide positive effects in the long term that could lead to enhanced crop productivities, and a better management of wastewater and agricultural resources.

The soil irrigated with untreated wastewater started to show signs of salinisation, while soil acidification occurred with the tap water + fertilizer. These issues did not occur with O$_3$EPTW water irrigation due to a buffer effect on the electrical conductivity of the ozone-oxidised organic matter.

The irrigation of _L. sativa_ with treated wastewaters maintained the macronutrient content in the cultivated plant’s tissues. For plant tissues, the N, K and Mg contents were similar, except for tap water, which always remained lower.

For the crop productivities, between the treated and the untreated wastewaters the plant lengths showed statistically significant differences; no differences were found with regard to plant weights. Due to an abundance of macronutrients in ionic form, plant growth was the highest for tap water + fertilizer for this single-season crop cultivation experiment.

The results of this study confirm that the use of O$_3$EPTW (A and B) represents a good alternative for agricultural irrigation because it maintains adequate levels of nutrients content in the water, soil and plant tissues.

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