# Evaluation of phytotoxic elements, trace elements and nutrients in a standardized crop plant, irrigated with raw wastewater treated by APT and ozone

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**Abstract** This project studied the benefits of applying Advanced Primary Treatment (APT) and ozone ($O_3$) to raw wastewater destined for reuse in agriculture. The ozone was applied directly to raw wastewater, as well as to wastewater already treated with APT, and the results compared against a control sample of potable water. The experimental conditions that reported the best results was wastewater treated with $O_3$ (at a dose of 4.8 mg/L, at pH 7, temperature 23°C, for 1 hr), given that it met standards in force in México with regard to micro-organism and heavy metal content. Under these conditions, after 15 min of ozonation, 100% destruction of the following bacteria was observed: *V. cholerae*, *S. typhi* as well as total and faecal coliforms. Destruction of helminth eggs and *Giardia* sp. took one hour. No phytotoxic elements or heavy metals were found. The balance of nutrients N:P:K (300:100:200 mg/kg) required for lettuce growth, was found in wastewater subjected to both treatment plans. However, ozone favoured the nitrification and assimilation of the nutrients, by contributing oxygen to the soil. Therefore, these conditions produced the greatest lettuce growth, the entire plant averaging 38 cm in length and 125 g. in weight. Moreover, a better appearance of the leaves was also noted.

**Keywords** APT; crop plants; garden produce; ozone; pathogens; wastewater reuse

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## Introduction

In México approximately 90% of the municipal wastewater is not treated, even though several areas along the length of the Gran Canal del Desagüe (major drainage ditch) make use of wastewaters originating in Mexico City for agricultural irrigation. This happens mainly in Chalco and Chiconautla in the State of Mexico, and in Tulancingo and the Valle del Mezquital in Hidalgo. Other districts utilizing wastewater are Valsequillo in Puebla, and Ciudad Juárez in Chihuahua (Cifuentes *et al.*, 2000).

The indiscriminate use of inadequately treated wastewaters, or worse still, untreated wastewater, represents a severe risk to public health through the possible transmission of pathogenic micro-organisms present in wastewater (WHO, 1989), given that many vegetables and garden products are eaten raw. Particularly in Mexico, concentrations of pathogenic micro-organisms found in wastewaters are very high: faecal coliforms (FC) $10^7$ to $10^9$ MPN/100 mL and *Giardia* sp. 1390 cysts/100 L (Cifuentes *et al.*, 2000); *Salmonella typhi* $10^5$ to $10^9$ MPN/100 mL and *Pseudomonas* sp. $10^4$ to $10^7$ MPN/100 mL; *Enterococci* 78/100 mL and coliphages 1350/100 mL (Juárez-Figueroa *et al.*, 2003); *Cryptosporidium* $10^3$ to $10^4$ cysts/L and helminth eggs 6–98 He/L (Rojas-Valencia, 2004). To date, technological developments for the treatment of wastewater for reuse in agriculture, have not been satisfactory. Technically and economically available technology that could be adopted, is urgently needed.

APT is a good option when the municipal wastewater is destined for agricultural reuse, because this treatment preserves a large proportion of the nitrogen (N), phosphorus (P), and organic material contained in wastewater, components which are of great benefit

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to agriculture. The APT alternative has been applied successfully in countries such as Norway, Sweden, France, Spain and the USA. In Mexico, APT technology is used in Puebla and Oaxaca, in Ciudad Juárez, Chihuahua and in Culiacán, Sinaloa (Keime, 2002).

If APT treatment is combined with chemical disinfectants such as $O_3$, the micro-biological content can be reduced, because $O_3$ has a well recognized disinfectant capacity against a wide range of micro-organisms occurring in wastewater. However, there is little information available about its effect on the productivity of crop plants, or about the toxic products that may be generated.

This project set out, therefore, to study the effect of $O_3$ on raw wastewater and on wastewater treated with APT, destined for reuse in agriculture. The aim was to maintain the levels of organic material and nutrients found in wastewater (which are beneficial to plant growth), but to destroy the micro-organisms harmful to human health, in line with the bacteriological and helminth limits laid down in Mexican Standard NOM-001-ECOL 1996. Thus, in addition to analyzing the treated wastewater, the project also measured the growth of a crop plant (i.e. lettuce) under experimental conditions, comparing the effects of irrigation with ozonated wastewater and with wastewater receiving APT plus ozone ($APT + O_3$), and looking as well at the microbiological quality and possible toxicity in the plant.

## Methodology

Ozone was applied to samples of raw municipal wastewater and to wastewater already subjected to APT, in order to determine ozone's disinfection capability on helminth eggs, bacteria, and protozoa, as well as on the following biological pollution indicators, total coliform (TC), and FC bacteria.

For isolating and quantifying *V. cholerae*, two methods were employed. One was the Most Probable Number (MPN) method (with alkaline peptonated water as the culture medium), and the other was the Membrane Filter (MF) method, using Thiosulphate Citrate Bile-salts Sacarose (TCBS) selective agar. Both the MPN and MF methods were also used in the case of *S. typhi*, the medium for the MF method this time being Sulphite Bismuth Agar. The MF method was used for the quantification of TC, the medium being M-ENDO agar. The three types of bacteria were incubated for 24 hr at $35° \pm 2°C$. FC were quantified in MFC medium, and were incubated in a water bath at $44.5° \pm 2°C$ for 24 hr.

The $O_3$ experiments were carried out using a 5 L batch reactor. A 36.8 $mgO_3$/min concentration of gas-phase $O_3$ was applied to the bottom of the reactor. The concentration of dissolved $O_3$ applied in the liquid phase was 4.8 mg/L, at pH 7, temperature 23°C, for 1 hr. The dissolved $O_3$ was measured using the indigo method. Determinations were simultaneously also made of the effect of $O_3$ upon some physical–chemical parameters related to the disinfection process: alkalinity (pH), Biological Oxygen Demand ($BOD_5$), Chemical Oxygen Demand (COD).

The APT was simulated with a jar test (Phipps and Bird model 7790–400), using 80 mg/L aluminium sulphate and 1.0 mg/L of anionic polymer (Prosifloc 252) that were added under mixed conditions. After 10 min. of sedimentation, the disinfection tests were performed.

These wastewaters thus treated in the laboratory, were then transferred to crops in greenhouse conditions to study their effect when used to irrigate lettuce plants. Romaine lettuce was planted in a greenhouse, in 12 furrows, each 30 cm wide by 250 cm long. Three furrows were irrigated with raw wastewater; another three were irrigated with ozonated wastewater, and three more were irrigated with wastewater receiving $APT + O_3$. For each experiment, the furrows were irrigated with 250 mL three times a week.

The remaining three furrows were irrigated with drinking-water (these last constituting the control group). The growth of the lettuce plants was evaluated by taking measurements of root length and leaf length.

Table 1 sets out the methods employed to determine the nutrients and heavy metals in the samples of raw wastewater, ozonated wastewater, and wastewater subjected to ATP $+O_3$.

Some compounds found in wastewater are not toxic to animals, but are toxic to vegetable crop plants. This was the reason the project also carried out bio-tests on commercially important, fast-growing plants, such as the Romaine lettuce (*Lactuca sativa*), testing the seeds as well as the plants, as they are standardized test seeds (Castañeda-Sarabia, 2000).

Different dilutions of the substance to be tested, i.e. the raw wastewater or the treated wastewaters, were prepared with distilled water (20, 40, 60, 80 and 100%), in aliquots of 20 mL. The different dilutions were poured onto filter paper previously laid over Petri dishes, until the filter paper was completely soaked. Then 20 seeds per dish and per concentration were sprinkled on each piece of filter paper, the seeds being arranged in 4 rows of 5 seeds, or in 5 rows of 4 seeds. The same procedure was followed using the control sample, which was simply distilled water. All of the said dishes were covered, and placed in a humidified incubator set at 24°C for a period of 5 days.

## Results and discussion

The physical–chemical and microbiological characterization of the raw wastewater is shown in Table 2. Determinations were made of the metal concentrations and of the different treatments ($O_3$; APT; APT $+O_3$) applied to the raw wastewater and the control water sample (potable water). None of the treated wastewater samples showed the presence of metals exceeding the maximum limits permitted under NOM-001–ECOL-1996.

As can be observed in Table 2, raw wastewater was found to contain the greatest concentration of total nitrogen (44 mg/L), and a 60% removal was noted after both treatment plans. Ammoniacal nitrogen content in ozonated wastewater increased threefold over the concentration detected in raw wastewater, and also rose after the application of APT (4.1 to 13 mg/L). This can be explained by the high reactivity of ozone on the amino acids and amines which make up the live cells of the micro-organisms, resulting in ammonification (Doré, 1989). When APT $+ O_3$ was applied, up to a 5-fold increase was observed (20.2 mg/L). An increase was also observed in the case of Nitrates (from 0.01 to 1 mg/L), when wastewater was ozonated and also when receiving APT $+O_3$.

The presence of metals can be noxious to the health of consumers. In this study the presence of non-nutrient metals was not detected in any case. Thus the wastewater studied can be considered fit for reuse in agriculture. The presence of zinc was detected,

**Table 1** Physical-chemical methods applied to samples of raw and treated wastewaters

| Sample | Analysis | Method | Sample | Analysis | Method |
|---|---|---|---|---|---|
| Water | Total nitrogen | NMX-AA-026 | Soil | Total phosphorus | SM-4500 |
| | Nitrates | NMX-AA-079 | | Total nitrogen | AOCS-AC-491 |
| | Ammoniacal nitrogen | NMX-AA-026 | | Ammoniacal nitrogen | AOCS-AC-491 |
| | Organic nitrogen | NMX-AA-026 | | Organic nitrogen | AOCS-AC-491 |
| | | | | Nitrates | NMX-AA-079 Mod |
| | | | | Cd | 7000 |
| | | | | K | 7610 |
| | As, Cd, Cr, Cu, Ni, Pb and Zn | EPA 60 10B | | Zn | EPA 7950 |

**Table 2** Average physical-chemical and microbiological results of raw and treated wastewaters, compared to the limits established by NOM-001-ECOL-1996

| Parameter | Raw water | Wastewater treated with | | | Potable water | NOM-001 |
|---|---|---|---|---|---|---|
| | | $O_3$ | APT | APT + $O_3$ | | |
| Physical-chemical (mg/L) | | | | | | |
| Total nitrogen | 44 | 27 | 27 | 27 | 1.99 | 40 |
| Organic nitrogen | ND | 6 | ND | 1.2 | ND | NA |
| Ammoniacal nitrogen | 4.1 | 13 | 4.02 | 20.2 | ND | NA |
| Nitrates | 0.199 | 0.9934 | 0.1912 | 1.0 | ND | NA |
| Arsenic | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.2 |
| Cadmium | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.2 |
| Cyanide | ND | ND | ND | ND | ND | 2.0 |
| Copper | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 4 |
| Total chromium | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 1 |
| Mercury | ND | ND | ND | ND | ND | 0.01 |
| Nickel | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 2.0 |
| Lead | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.5 |
| Zinc | 2.13 | 0.58 | 0.46 | 1.265 | ND | 10 |
| $BOD_5$ | 32 | 4 | 14 | 2 | ND | 200 |
| COD | 378 | 25 | 193 | 142 | ND | NA |
| Micro-biological (MPN/mL) | | | | | | |
| Total coliforms | $5.2 \times 10^6$ | $8.5 \times 10^1$ | $5 \times 10^3$ | ND | ND | 1000 |
| Faecal coliforms | $4 \times 10^6$ | $5 \times 10^1$ | $2 \times 10^1$ | ND | ND | NA |
| *Vibrio cholerae* | $3.6 \times 10^6$ | $3.5 \times 10^1$ | ND | ND | ND | NA |
| *Salmonella typha* | $4.5 \times 10^6$ | $11 \times 10^1$ | ND | ND | ND | NA |
| Helminth eggs (He/L) | 20 | 1 | 4 | 1 | ND | 1–5 |
| *Giardia* sp. (cysts/100L) | Present | Absent | Present | ND | ND | NA |

ND: Not detected; NA: Not applicable

but in concentrations which also favour crop cultivation. The best reduction in $BOD_5$ (93%) was observed with the application of APT + $O_3$. The application of $O_3$ alone achieved an 88% reduction; and APT applied alone achieved a reduction of 43%. In the case of COD, the best reduction (93%) was achieved with the application of $O_3$ alone. On applying APT +$O_3$, a reduction of 62% was achieved, and APT alone gave a reduction of 49%.

Regarding micro-organisms, the raw wastewater samples taken from the municipal treatment plant (Cerro de la Estrella), showed 100% destruction of TC, *S. typhi* and *V. cholerae* bacteria, after a 15 min. application of 36.8 $mgO_3$/min at pH 7. The results for the application of APT +$O_3$ are given in Figure 1. As can be seen, at 10 min the FC and TC bacteria had been removed. The bacteria *V. cholerae* had been removed in less than 10 min., and *S. typhi* was not detected after 2 minutes. Table 2 shows that none of the parameters evaluated exceeded the limits permitted under NOM-001–ECOL-1996.

Table 3 shows the average physical–chemical and microbiological results of the laboratory analysis of soil irrigated with raw wastewater and with treated wastewater. As no heavy metals had been detected in the water produced as a result of either treatment plan, the soil was not tested for heavy metals either. The only exceptions were testing for zinc and cadmium. The soil irrigated with raw wastewater evidenced the presence of cadmium, but mobility was insignificant given the low concentration. The presence of zinc was considered beneficial, because a lack would lead to abnormalities in plant development such as leaf length and a shortening of the joints. Zinc is also vital to the formation of chlorophyll and growth hormones.

Table 3 demonstrates a marked increase in the quantity of nutrients in the soil over the levels detected in the water. This is mainly due to the fact that soil contains its own nutrients, even when it receives no extra nutrients apart from those supplied by irrigation with raw and treated wastewater.

Adequate concentrations in soil were registered of the macro-nutrients, nitrogen (N), phosphorus (P) and potassium (K). According to the literature, the balance of these nutrients required for good lettuce growth is N:P:K (300:100:200 $mg/m^2$). Table 3 shows that the water produced after undergoing all the treatments, contains acceptable concentrations of these elements, and that therefore the soil does not need extra fertilizer. It is important to recognize that ozone favours the nitrification and assimilation of nutrients by oxygenating the soil.

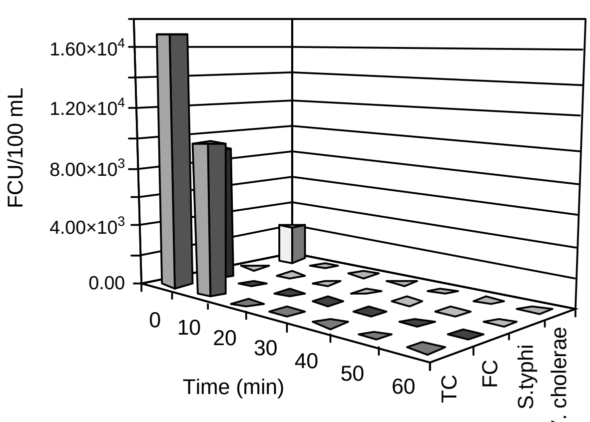


**Figure 1** Average microbiological results following the application of APT + $O_3$ at pH 7

**Table 3** Average physical-chemical and microbiological results of laboratory analysis of soil irrigated with raw and treated wastewaters, as compared to the NOM-001-ECOL-1996 limits

| Parameter | Soil irrigated with: | | | | |
|---|---|---|---|---|---|
| | Raw wastewater | Wastewater treated with | | | Potable water |
| | | $O_3$ | APT | APT + $O_3$ | |
| Physical-chemical (mg/kg) | | | | | |
| Total phosphorus | 482 | 261.6 | 390 | 520 | 200 |
| Total nitrogen | 1632 | 1815 | 1063 | 1355 | 1063 |
| Ammoniacal nitrogen | 147.5 | 131 | 190 | 172 | 60 |
| Nitrates | 714 | 222 | 138 | 731 | 20 |
| Potassium | 239 | 381 | 245 | 238 | 33 |
| Cadmium | 0.436 | ND | ND | 0.5855 | ND |
| Zinc | 5 | 5.8 | 4.6 | 1.2 | ND |
| Microbiological (MPN/g) | | | | | |
| Soil | | | | | |
| Total coliforms | $4 \times 10^6$ | ND | ND | ND | ND |
| Faecal coliforms | $5 \times 10^4$ | ND | ND | ND | ND |
| *Vibrio cholerae* | ND | ND | ND | ND | ND |
| *Salmonella typhi* | ND | ND | ND | ND | ND |
| Helminth eggs (He/g) | 32 | 5 | 3 | 0 | 0 |
| *Giardia* sp. (cysts/g) | Present | Absent | Absent | Present | Absent |
| Leaves | | | | | |
| Total coliforms | $6 \times 10^4$ | $1 \times 10^4$ | ND | ND | ND |
| Faecal coliforms | $1 \times 10^4$ | ND | ND | ND | ND |
| *Vibrio cholerae* | $3 \times 10^4$ | $2 \times 10^1$ | ND | ND | ND |
| *Salmonella typhi* | ND | ND | ND | ND | ND |
| Helminth eggs (He/g) | 11 | 0 | 4 | 3 | 0 |
| *Giardia* sp. (cysts/g) | Absent | Absent | Absent | Absent | Absent |
| Root | | | | | |
| Total coliforms | $4 \times 10^4$ | $2.6 \times 10^2$ | $2.6 \times 10^2$ | ND | ND |
| Faecal coliforms | ND | ND | $1 \times 10^1$ | ND | ND |
| *Vibrio cholerae* | ND | ND | ND | ND | ND |
| *Salmonella typhi* | ND | ND | ND | ND | ND |
| Helminth eggs (He/g) | 23 | 3 | 8 | 2 | 0 |
| *Giardia* sp. (cysts/g) | Present | Absent | Absent | Absent | Absent |
| Growth and weight of lettuce leaves and roots | | | | | |
| *Leaf (cm)* | 12 | 17 | 13.5 | 9 | 7 |
| *Leaf (g)* | 54 | 72 | 59 | 45 | 38 |
| *Root (cm)* | 15 | 21 | 16 | 12 | 10 |
| *Root(g)* | 13 | 53 | 4 | 4.5 | 5 |

Lettuce is a plant that demands potassium-enrichment, and it is therefore important to ensure an adequate supply of this element. Nitrogen excess needs to be avoided, in order to ensure good leaf quality, and to prevent possible phototoxicity due to an excess of salts.

As regards microbiological quality, as can be seen in Figure 2, there was evidence of raised concentrations of micro-organisms in the leaf of the lettuce plants irrigated with raw wastewater.

The lettuce plants irrigated with ozonated wastewater, presented low concentrations of the TC ($1 \times 10^1$) and *V. cholerae* ($2 \times 10^2$) bacteria, but no *S. typhi* was found. The lettuce plants irrigated with raw wastewater presented greater concentrations of helminth eggs, on both the leaves 11 He/g and roots 23 He/g, when compared to the leaves and roots of the lettuce plants irrigated with ozonated wastewater, which demonstrated a removal rate of 95% from both leaves and roots.

The soil irrigated with raw wastewater evidenced raised concentrations of TC and FC ($4 \times 10^6$ and $5 \times 10^4$ respectively) and 32 He/g; whereas the soil irrigated with ozonated

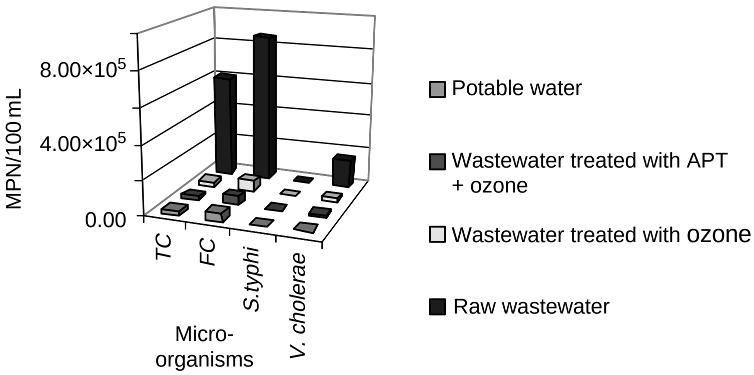


**Figure 2** Concentrations of micro-organisms on the leaves of lettuce plants irrigated with fresh water, raw wastewater and treated wastewaters



wastewater clearly showed a reduction of these micro-organisms and only 5 He/g. The presence of *Giardia* sp. and *Acanthamoeba* sp. was observed in the soil irrigated with raw wastewater, but not in the soil irrigated with ozonated wastewater. The *S. typhi* and *V. cholerae* bacteria were not detected in any case.

When APT $+O_3$ was applied, 100% of the bacteria were destroyed by 6 min, and helminth eggs were destroyed by 15 minutes. In the control group, no bacterial growth was observed on any of the lettuce plants.

The lettuce plants irrigated with ozonated wastewater, showed the greatest growth in both leaves (17 cm) and roots (21 cm). The lettuce irrigated with raw wastewater showed lesser growth (leaves 12 cm and roots 15 cm). The plants irrigated with wastewater subjected to APT $+O_3$, showed the following growth: leaf (9 cm) and root (12 cm). The plants irrigated with drinking water demonstrated the least growth: leaf (7 cm) and root (10 cm).

The reason why APT + $O_3$ gives a smaller crop may be explained from the fact that the soil that was irrigated with such treated wastewater is the one that shows the highest nitrates and phosphorus concentration of all (see Table 3). These macronutrients, essential for good plant growth and development are, when in excess, not favorable for plants and have the potential to damage entire crops.

This effect, where very high (or low) nitrogen levels reduced the growth of plants such as lettuce, has been previously reported (Huett and Dettmann, 1991). According to Walworth *et al.* (1994), high rates of nitrogen application to soils reduce head lettuce yields. In addition, Hochmuth *et al.* (1994) suggests that excess nitrogen can reduce the head quality of lettuces and may even cause the rotting of plant bottoms. Also, it has been shown (Chavan and Karadge, 1980) that sodium chloride and sodium sulphate salts suppress the growth of peanut plants, because of the hindering influence they have on the uptake of some nutrients (as potassium) by the plants. In the present study, the increased nitrate concentrations of the soil irrigated with wastewater treated with APT + $O_3$ may have made the soil contain higher salinity levels which resulted in a phytotoxic effect that decreased nutrient assimilation, hindering the growth of lettuce. It is also worth noting (Table 3) that this soil, in addition to having the highest levels of (probably in excess) nitrates and phosphorus, also had the lowest levels of potassium, which is necessary to utilize nitrogen and water efficiently and to produce and synthesize the proteins required for plant growth.

Very little has been published in the literature about the use and phytotoxicity of aqueous ozone; and more specifically, on the irrigation of crops with wastewater treated by ozone. However, a study evaluating the effect of ozonated tap water on the growth and development of golf-course grass (Sloan and Engelke, 2005) showed that the bentgrass irrigated with ozonated water had higher crown weights, but without any effect on root mass. Focusing more on the soil effects, it has also been mentioned (Raub *et al.*, 2001) that the presence of ozone in irrigation water can increase crop vigor, reduce pests or diseases, reduce fertilizer needs and also improve water penetration. These related studies agree with the findings obtained in this research.



## Conclusions

The experimental condition reporting the best results was wastewater treated with ozone (at a dose of 4.8 mg/L, at pH 7, 23°C for 1 hr), because this process produced water complying with norms in force in México regarding micro-organisms and heavy metals content.

Under these conditions, after 15 min ozonation, 100% destruction was observed of the following of bacteria: *V. cholerae*, *S. typhi* and TC and FC. Complete destruction of Helminth eggs and *Giardia* sp. took one hour.

The results for the application of APT $+O_3$ showed that at 10 min the FC and TC bacteria had been removed. The bacteria *V. cholerae* was removed in less than 10 min, and *S. typhi* was not detected after 2 minutes.

Wastewater treated with APT could be employed in agricultural irrigation, because the treatment preserves the original nutrients in the water. Nevertheless, even though APT results in a noticeable removal of FC and other pathogenic micro-organisms, the water does not comply with NOM-001.

No phytotoxic elements were found, nor any metals which might affect plant productivity, or affect public health due to consumption of plants that had been irrigated for three months.

In the germination of the *Lactuca sativa* lettuce seed, an increase in root length was noted in 75% of the samples that had been irrigated either with ozonated wastewater or with wastewater subjected to APT $+O_3$.

Wastewater subjected to $O_3$ contains sufficient concentrations of nutrients effectively to constitute a sizeable on-going supplement and eliminate much of the need for fertilizer.

The continuous aeration resulting from irrigation with ozonated wastewater, was found to generate an effluent rich in nitrates. This gives better crop plant productivity with growths up to 38 cm and weights up to 125 g. The appearance of the lettuce was also better than that of lettuces grown in soil irrigated with wastewater receiving the other treatments.

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## References

Castañeda-Sarabia, O. (2000). Determinación de la Toxicidad de los Lodos generados por una Planta Potabilizadora,Utilizando Bioensayos. Tesis maestría, DEFI, UNAM. pp. 1-200.

Cifuentes, E., Gómez, M., Blumenthal, U., Tellez-Rojo, M., Romieu, I., Ruíz-Palacios, G. and Ruiz-Velazco, S. (2000). Risk factors for *Giardia intestinalis* infection in agricultural villages practicing wastewater irrigation in Mexico. *Am. J. Trop. Med. Hyg.*, **62**(3), 388–392.

Chavan, P.D. and Karadge, B.A. (1980). Influence of salinity on mineral nutrition of peanut (*Arachis hypogea* L.). *Plant and Soil*, **54**(1), 5–13.

Doré, M. (1989). "L' ozone sur les composés organiques" *Chimie des Oxydants et Traitement des Eaux*, Paris Lavosier, Technique et Documentation, 1989, Chap. 1, 6: pp. 44, 328.

Hochmuth, G.J., Hanlon, E., Nagata, R., Snyder, G. and Schueneman, T. (1994). Fertilization recommendations for crisphead lettuce grown on organic soils in Florida. *Horticultural Sciences Department Document SP153*, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, pp. 1–10.

Huett, D.O. and Dettmann, E.B. (1991). Nitrogen Response Surface Models of Zucchini Squash Head Lettuce and Potato. I. Effect of N on growth, dry matter partitioning and on fresh yield and quality. *Plant and Soil*, **134**(2), 243–254.

Juárez-Figueroa, L., Silva-Sánchez, J., Uribe-Salas, F. and Cifuentes-García, E. (2003). "Microbiological indicators of water quality in the Xochimilco canals, Mexico City" *Salud Pública de México* **45**, No.5, Septiembre-Octubre, pp. 389–395.

Keime, M.P. (2002). Esquema inovador para el tratamiento de aguas negras y su reúso en México. *XXVIII Congreso Interamericano de Ingeniería Sanitaria y Ambiental Cancun*. México, 27 al 31 de Octubre, pp. 1–7 (In Spanish).

Norma Oficial Mexicana (NOM-001-ECOL-1996) (1997). Que establece los límites máximos permisibles de contaminantes en las descargas de aguas residuales en aguas y bienes nacionales. *Diario Oficial de la Federación*, Enero 6 de 1997. México. pp 67–81 (In Spanish).

Raub, L., Amrhein, C. and Matsumoto, M. (2001). The effects of ozonated irrigation water on soil physical and chemical properties. *Ozone: Science & Engineering*, **23**(1), 65–76.

Rojas-Valencia M.N. (2004). "Tratamiento avanzado de desinfección con ozono para eliminar *Vibrio cholerae, Salmonella typhi,* amebas y huevos de helmintos en aguas residuales tratadas para reúso. División de Ciencias Básicas e Ingeniería. Doctorado en Ciencias e Ingeniería Ambiental. Universidad Autónoma Metropolitana. México, pp. 1–200 (In Spanish).

Sloan, J.J. and Engelke, M.C. (2005). Effect of ozonated water on creeping bentgrass growth in a sand medium. *American Society for Horticultural Science, Hort Technology*, **45**(1), 148–152.

Walworth, J.L., Carling, D.E. and Gavlak, R. (1994). Rates and methods of application of nitrogen and phosphorus for commercial field production of head lettuce in Southcentral Alaska. *University of Alaska Fairbanks, Bulletin 100*, 1–8.

WHO (1989). *Health Guidelines for the Safe Use of Wastewater in Agriculture and Aquaculture*, Technical Report Series 778. World Health Organization, Geneva, pp. 1–20.