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Irrigation of Romaine Lettuce (*Lactuca sativa*) Using Wastewater Treated by Non-Conventional Technologies

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Abstract

The aim of this study was to assess the capacity for reuse of wastewater treated in stabilisation ponds and subsequently reclaimed by means of different filtration systems at pilot scale. An analysis of filtered water showed turbidity values of below 5 NTU, a total suspended solids (TSS) content of 7 mg/l, and *Escherichia coli* values of up to 1.6 log CFU/100 ml. These results fall within the parameters stipulated in RD 1620/2007 Spanish Water Reuse Regulations governing the reuse of reclaimed wastewater for agricultural purposes. The water reclaimed by means of filtration systems was used to irrigate Romaine-lettuce (*Lactuca sativa longifolia*), comparing growth with that of the same variety irrigated with water from the supply network. The results showed a mean difference in lettuce growth of up to 300% in favour of the crop irrigated with reclaimed water.

Keywords

Reclamation, Reutilisation, Small Communities, Intermittent Sand Filters, Infiltration-Percolation, Stabilisation Ponds, Artificial Wetlands

1. Introduction

Water is becoming an increasingly scarce resource in certain parts of Spain, rendering it necessary to seek alternatives to traditional sources. One of these alternatives is reclaimed water, which is suitable for some but not all purposes and requires legislation governing its quality.

In recent years, legislation has been passed in Spain controlling the reuse of reclaimed wastewater. Thus, the reuse of untreated wastewater is absolutely prohibited under Spanish law, pursuant to Royal Decree-Law 11/195, RD 509/1996 and RD 1620/2007. The latter law stipulates the various uses for which reclaimed wastewater can and cannot be destined, and the minimum quality required depending on each use.

One of the permitted uses is agriculture. Wastewater has been reused for agricultural purposes for centuries, although this has caused health problems associated with water contamination, either due to direct contact with the water or to percolation into aquifers [1]. Such problems are now prevented by RD 1620/2007, Spanish Water Reuse Regulations [2].

In Spain, most of the water consumed is destined for agricultural irrigation. In 2010, 16,118 Hm^3 of water was used for this purpose, accounting for 82.6% of the total water consumed [3]. In a country that suffers recurrent drought, it is of considerable interest to mitigate this water consumption to some extent through the reuse of wastewater for this purpose.

The Royal Decree subdivides agricultural irrigation into three possible applications, which are described in **Table 1**.

The quality levels correspond to different uses. For example, **Quality 2.1** is suitable for *crop irrigation using systems that permit direct contact between reclaimed water and edible parts for human consumption in their fresh state*; **Quality 2.2** is suitable for *crop irrigation using systems that permit direct contact between reclaimed water and edible parts for human consumption, where these are not consumed in their fresh state but following subsequent industrial processing*; and **Quality 2.3** is suitable for *localised irrigation of woody crops that prevents contact between reclaimed water and the fruits consumed.*

In areas of low population density, usually characterised by dispersed or small communities, agricultural irrigation takes on a different dimension since treatment and subsequent reclamation of wastewater is limited both at the human and the technological level [4].

In Spain, more than 70% of municipalities have less than 2000 inhabitant equivalents (IE) [3]. In general, such communities have neither the economic resources nor the trained staff necessary to implement conventional wastewater treatment or reclamation technologies [4].

In these cases, extensive or non-conventional technologies may be used, some of which are suitable for wastewater treatment and reclamation. Non-conven-

Table 1. Spanish Water Reuse Regulations, RD 1620/2007.

Use	Quality	Intestinal nematodes (eggs/10l)	Escherichia coli (CFU/100 ml)	Total Suspended Solids (mg/l)	Turbidity (NTU)
Agricultural	2.1	1	100	20	10
	2.2	1	1000	35	-
	2.3	1	10000	35	-

tional treatment technologies include stabilisation ponds and wetland systems, which have received much research attention. Meanwhile, intermittent sand filters (ISA) and modified infiltration-percolation (mIP), which can be used as treatment systems, have also begun to be used as water reclamation systems [5] [6].

This paper presents an analysis of the results obtained for wastewater used to irrigate Romainelettuce (*Lactuca sativa*) following treatment in a complete stabilisation pond system (anaerobic, facultative and maturation ponds) and subsequent reclamation using two filtration systems; an intermittent sand filter (ISF) and a modified infiltration-percolation system (mIP) [7] [8].

2. Methodology

The experimental part of this research was carried out at the CENTA Foundation pilot plant located in the town of Carrión de los Céspedes (province of Seville, Spain) [9].

For the primary and secondary treatment of wastewater arriving at the plant, water from the plant's stabilisation pond system was used, which consists of a 200 m^3 anaerobic pond, a 3500 m^3 facultative pond and a 400 m^3 usable volume maturation pond.

The water was then reclaimed by means of an intermittent sand filter (ISF) measuring 5 m in diameter by 1.10 m high and containing a 70 cm sand filter layer aerated by shafts, and a modified infiltration-percolation (mIP) system measuring 2.5 m in diameter by 3 m high, containing a 1.5 m sand filter layer and a 45 cm layer of fine and coarse gravel and coarse sand aerated by an underlying air chamber. A detailed description of both systems is given in previous papers published by the same research team [10].

The sand used as the filtration element had an effective diameter (d_{10}) 0.27 mm and a uniformity coefficient (C_u) of 1.77.

The water was fed into both filtration systems at two different hydraulic loads; at 240 l/m^2d for two months and then at 480 l/m^2d for another two months. In both cases, the systems received 12 daily doses of wastewater (once every two hours) throughout the duration of the assay.

The effluent from the ISF and the mIP was used to irrigate two plots measuring 1 m in diameter (P2 and P3). A third plot (P1), irrigated with potable water, served as control. The cultivation substrate was the same for all three plots, as was the sowing method, which consisted of scattering seeds over the surface of the plots. The plots were manually irrigated with a single daily dose of 10 l, performed first thing in the morning to avoid evaporation processes.

The method recommended by the US Standard Methods [11] was employed to analyse the various contaminants studied and listed in RD 1620/2007 for water (turbidity, total suspended solids, helminth eggs, *E. coli* and metals, as well as COD, BOD_5, NH_4^+ and NO_3^-. The analytes referred to in the legislation on lettuces sold in the EU [12] [13], nitrates and *E. coli* were analysed using the method described in the same legislation.

3. Results and Discussion

1) Characteristics of water from the stabilisation ponds

The stabilisation pond influent and effluent water was analysed over a period of two years (103 samples). **Table 2** shows the mean results obtained from an analysis of plant influent and effluent after treatment in maturation pond II (MP II), including those for the analytes specified in RD 509/1996, and for some of those specified in RD 1620/2007.

As can be seen in **Table 2**, an analysis of the influent parameter values showed that these were characteristic of purely urban wastewater, presenting a contaminant load ranging from average (220 mg O_2/l BOD_5 and 500 mg O_2/l COD) to low (110 mg O_2/l BOD_5 and 250 mg O_2/l COD), with no apparent presence of industrial wastewater, which often has a high organic and metal load.

The influent parameter values presented very high fluctuations, with a standard deviation of the same order as the mean value or higher, confirming that the wastewater came from small communities, which are characterised by this kind of fluctuation.

Once the wastewater had passed through the stabilisation pond system, similar large variations were observed in the effluent, clearly due to the characteristics of the influent. Some parameters (turbidity, TSS, BOD_5 and nitrogen in the form of nitrate) presented a mean removal rate of over 70%, which nevertheless did not meet the requirements stipulated in the RD 509/1996 on wastewater either in percentages or in absolute values, except in the case of TSS which can be up to 150 mg/l for stabilisation pond effluent. Others presented a negative performance, as was the case of nitrogen in the form of ammonium, due to organic matter degradation and lack of nitrification, or phosphorous, as a result of dragging after deposition.

Consequently, this treated water would not be suitable for any of the uses indicated in RD 1620/2007, with the exception of use 5.4, according to which the water could be used for "other environmental applications" such as the maintenance of wetlands and environmental flows, where the minimum required quality would be studied by the competent authorities.

Table 2. Analysis of plant influent and effluent after treatment in maturation pond II.

	pH	O_2 mg/l	Turbidity NTU	TSS mg/l	BOD_5 mg/l
Influent	7.57 ± 0.52	2.32 ± 2.08	191 ± 237	261 ± 505	198 ± 262
Effluent	8.34 ± 0.67	5.14 ± 4.88	45.4 ± 31.4	69.2 ± 44.4	51.49 ± 23.5
yield	−10.1	−121.5	76.2	73.5	74.0
	COD mg/l	**$N-NH_4$ mg/l**	**$N-NO_3$ mg/l**	**total P mg/l**	**E. Coli CFU/100 ml (log u)**
Inluent	279 ± 411	15.3 ± 16.1	10.4 ± 12.9	4.42 ± 4.58	6.52 ± 6.73
Effluent	176 ± 50.0	19.3 ± 9.88	0.41 ± 0.26	5.47 ± 1.21	3.7 ± 3.79
yield	37.1	−20.5	96.1	−23.7	2.82

2) Characteristics of filtered water

Before using water from stabilisation ponds for agricultural irrigation (**Table 1**), it must be subjected to reclamation treatment. In the present study, this consisted of feeding it into two filtration systems: an intermittent sand filter (ISF) and a modified filtration-percolation (mIP) system.

Table 3 shows the mean values obtained for the influent (water from MP II) and the effluent from both types of filtration system, during a 100-day assay in each of them.

First, it can be seen that the values obtained for the filtration system influent during the 100-day assay were very similar to those obtained for the effluent from the stabilisation pond system shown in **Table 2** which, as previously mentioned, corresponded to a period of two years. In addition, almost half the influent parameters analysed presented high fluctuations, and in some cases the standard deviation was of the same order as the mean value. This was consistent with the findings discussed in the previous section on the characteristics of the effluent from the stabilisation pond system.

With regard to the values obtained for effluent from both filtration systems (ISF and mIP), these were lower and generally more stable than influent values, and complied with wastewater legislation and many of the stipulations in the RD on reuse.

Table 3. Mean values obtained for the influent. ISF and mIP.

	pH			E.C. (μS/cm)			Temperature (°C)		
	Influent	ISF	mIP	Influent	ISF	mIP	Influent	ISF	mIP
values	7.64 ± 0.34	7.12 ± 0.32	7.48 ± 0.40	1227 ± 155	1170 ± 152	1074 ± 326	13.5 ± 4.14	14.3 ± 4.70	13.8 ± 4.46
yield		6.19 ± 5.60	2.43 ± 3.76		5.89 ± 3.74	13.6 ± 23.1		−5.81 ± 9.31	0.42 ± 6.20
	O_2 (mg/l O_2)			Turbidity (NTU)			TSS (mg/l)		
	Influent	ISF	mIP	Influent	ISF	mIP	Influent	ISF	mIP
values	5.42 ± 4.73	4.78 ± 2.14	8.23 ± 2.14	54.6 ± 61.9	4.60 ± 6.09	3.41 ± 3.14	76.3 ± 65.2	6.00 ± 9.30	3.13 ± 3.28
yield		−32.7 ± 77.1	−103 ± 113		88.5 ± 12.7	90.7 ± 8.78		87.8 ± 17.1	93.6 ± 6.73
	BOD_5 (mg/l O_2)			COD (mg/l O_2)			Total N (mg/l N)		
	Influent	ISF	mIP	Influent	ISF	mIP	Influent	ISF	mIP
values	65.4 ± 44.0	10.1 ± 8.36	3.80 ± 5.88	174.7 ± 71.7	42.9 ± 28.5	40.75 ± 17.6	34.9 ± 3.88	32.2 ± 4.27	31.7 ± 4.02
yield		82.5 ± 16.3	91.7 ± 11.5		73.7 ± 18.9	73.6 ± 13.9		7.55 ± 15.8	8.18 ± 15.5
	NH_4 (mg/l N)			NO_3 (mg/l N)			total P (mg/l P)		
	Influent	ISF	mIP	Influent	ISF	mIP	Influent	ISF	mIP
values	27.0 ± 4.94	5.77 ± 8.31	4.69 ± 6.58	0.49 ± 0.43	24.8 ± 10.9	25.4 ± 8.4	6.03 ± 0.69	5.63 ± 1.25	5.38 ± 1.25
yield		80.7 ± 26.4	84.5 ± 21.2		6896 ± 9739	5188 ± 4667		13.1 ± 28.2	16.0 ± 27.2
	E. coli (CFU/100 ml; log. u.)			Nematodes (egg/10l)					
	Influent	ISF	mIP	Influent	ISF	mIP			
values	3.25 ± 3.35	2.68 ± 3.05	1.77 ± 2.08	0 ± 0	0 ± 0	0 ± 0			
yield		84.6 ± 20.1	94.6 ± 8.1		-	-			

Thus, almost all parameters presented high removal rates in the filtration systems, with values close to 90% for most of them, although values for COD were lower, at close to 75%. In general, the mIP system yielded better results than the ISF for practically all the parameters analysed, and it also yielded less fluctuation, most probably due to the different thickness of the filter layer. With regard to microbiology and *E. coli* removal, there was an almost 10% difference in removal rates between the two systems in favour of the mIP, which also yielded practically 50% less fluctuation. In the case of nematodes, these were never detected in either the influent or effluent, and thus nothing can be concluded about the behaviour of either system.

The biggest difference between the parameters analysed was observed for dissolved oxygen, where the mIP yielded higher effluent values and lower standard deviations since its design permitted greater contact with the air, favouring oxygenation of the water.

Of note was the low or non-existent removal of phosphorus, due to the process being mainly carried out by adsorption. This occurs because the sand is not renewed. Therefore, once the sand has become saturated by adsorption, an adsorption-desorption balance is established and the sand's effective retention capacity is decreased over time.

With regard to nitrogen, it should be noted that ammonia was almost completely removed by nitrification (80.7% in the ISF and 84.5% in the mIP), but the same was not the case for nitrate, the concentration of which increased more than 50 fold in both filtration systems because the conditions for nitrate removal (anaerobic conditions and a carbon source) were not established. Consequently, total nitrogen removal was low, and on average did not reach 10%.

Figure 1 shows the evolution of the turbidity, TSS, BOD_5, COD, NH_4 and *E. coli* parameters over the 17-weeks assay. A steep reduction in all the parameters analysed was observed at the beginning of the assays. Subsequently, both filtration systems presented worse performance by the end of the assays due to silting up of their surfaces and the generation of preferential channels. Besides worsening performance, the standard deviation of parameter values was also observed to increase.

The results reported in this section thus indicate that stabilisation pond effluent cannot be used directly for any of the applications established in RD 1620/2007 except use 5.4, mainly due to the high value obtained for TSS. In contrast, they show that effluent from the filtration systems was of sufficiently high quality to permit its use for most applications described in RD 1620/2007, the exceptions being those that would be restricted due to the concentration of nitrogen (5.1 and 5.2) and phosphorus (4.2, for stagnant water) and the microbiology (uses 1.1, 3.2 and 5.2), since values for the *E. coli* parameter barely reached compliance. As explained previously, the failure to achieve stipulated values for these parameters may be due to silting up of the filtration systems, since *E. coli* values prior to this stage, for example, barely exceeded 1.5 log units.

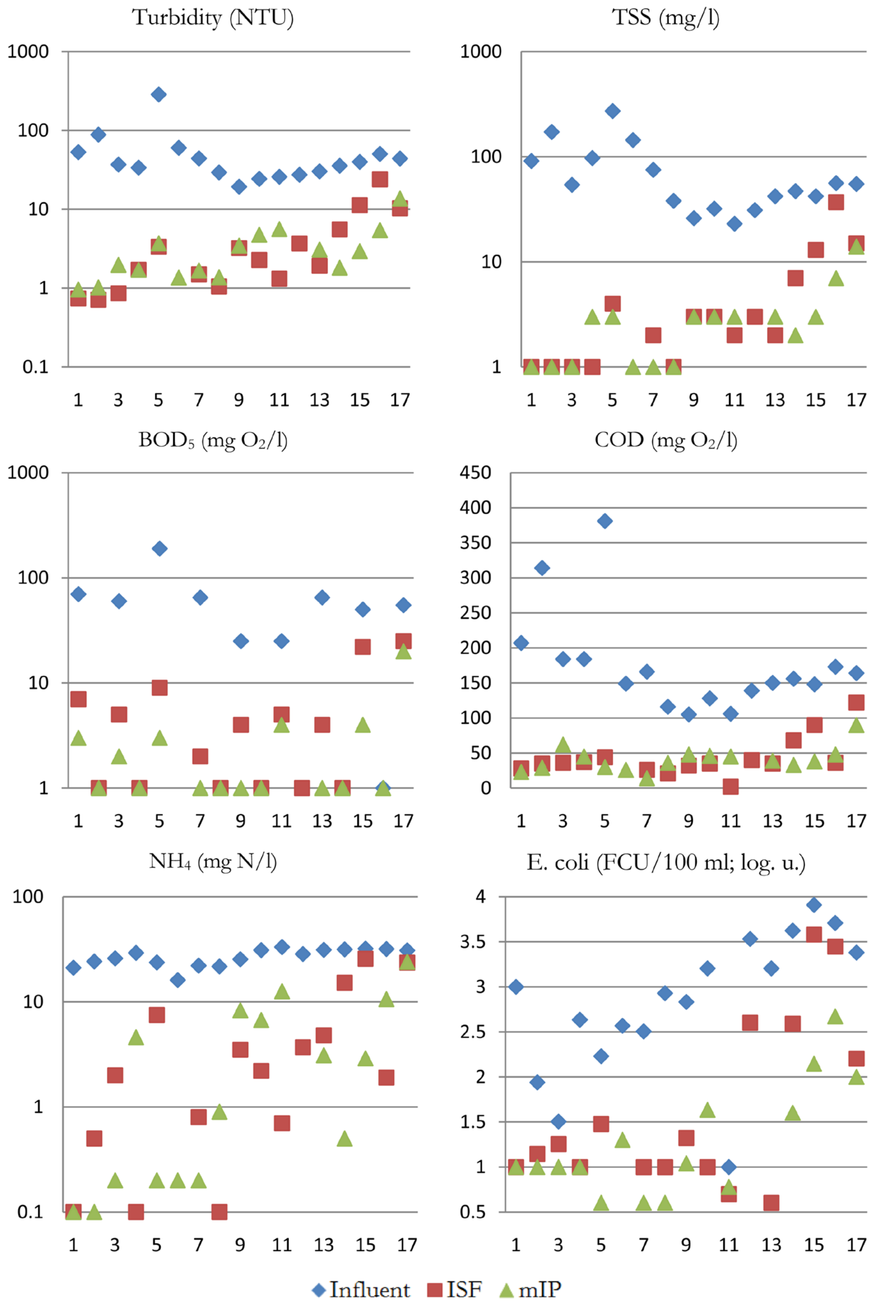

Figure 1. Evolution of parameters in 17 weeks.

3) Metals analysis

In the specific case of reuse to irrigate Romaine lettuce, water must comply with the requirements of use 2.1 (**Table 1**). This particular use also requires an analysis of metals. **Table 4** shows the results of the metals analysis performed on the experimental plant influent, as well as the maximum values given in the RD for use 2.1. These results were as expected for purely urban wastewater.

Based on these influent values, and given that sand does not contain any of these metals, effluent from the filtration systems will not contain them either. It can therefore be concluded that as regards metal content, reclaimed water

Table 4. Metals analysis performed on the experimental plant influent.

	SAR (meq/l)	As(mg/l)	B (mg/l)	Cd (mg/l)	Co (mg/l)	Cu (mg/l)	Mn (mg/l)
Use 2.1	6	0.1	0.5	0.01	0.05	0.2	0.2
yield	3.05 ± 0.53	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.014 ± 0.038	0.00 ± 0.00
	Ni(mg/l)	**Se(mg/l)**	**Be(mg/l)**	**Cr(mg/l)**	**Mo(mg/l)**	**V (mg/l)**	
Use 2.1	0.2	0.2	0.1	0.1	0.01	0.1	
yield	0.029 ± 0.076	0.00 ± 0.00	0.00 ± 0.00	0.014 ± 0.038	0.00 ± 0.00	0.00 ± 0.00	

obtained with these technologies (stabilization ponds and intermittent filters) can be used for agricultural irrigation in all circumstances.

4) Irrigation of Romainelettuce

The lettuce was not cultivated in traditional rows, nor was the recommended space (20 - 30 cm) left between plants; instead, seeds were scattered over the plots and seedlings were then partially thinned in order to obtain a higher number of albeit smaller plants and better determine the influence of water on growth. Not any kind of fertilizer or phytosanitary was employed at any time.

The lettuces in each of the plots were manually irrigated with a daily single dose of 10 l of each type of water. At 100 days from sowing, the lettuces were weighed (**Table 5**).

There was a clearly evident difference in lettuce weight between the three plots, whereby those irrigated with water from the ISF filtration system weighed most. This may have been due to varying concentrations of nutrients and organic matter in the different types of water used for lettuce irrigation. The values for these parameters are shown in **Table 6**.

Figure 2 shows the evolution of lettuce weight over time for the three plots.

The best fit corresponds to an exponential equation of the type $y = A\,e^{bx}$, with R^2 values superior to 0.9 in all cases. The results obtained for lettuce irrigated with the three types of water were as follows: with potable water, $y = 3.6703e^{0.0479x}$ and $R^2 = 0.9439$; with ISF water, $y = 12.434e^{0.049x}$ and $R^2 = 0.895$; and with mIP water, $y = 6.4714e^{0.052x}$ and $R^2 = 0.9298$.

Figure 3 depicts the development of the lettuce crops over the 14-week experiment. The differences in growth between the three plots are clearly evident.

Subsequently, to determine whether the lettuces irrigated with reclaimed water were commercially viable, three commercially produced lettuces were purchased. One was the product of organic farming and the other two were bought in supermarkets. All the lettuces were analysed in accordance with EC Regulations 1881/2006 and 2073/2005, which establish the limits for nitrate and *E. coli* in lettuces.

Table 7 shows the results obtained and the maximum admissible values stipulated in current legislation. It also shows the dry weight in each case; although there is no need to control this parameter, it is indicative of saline influence on the plant.

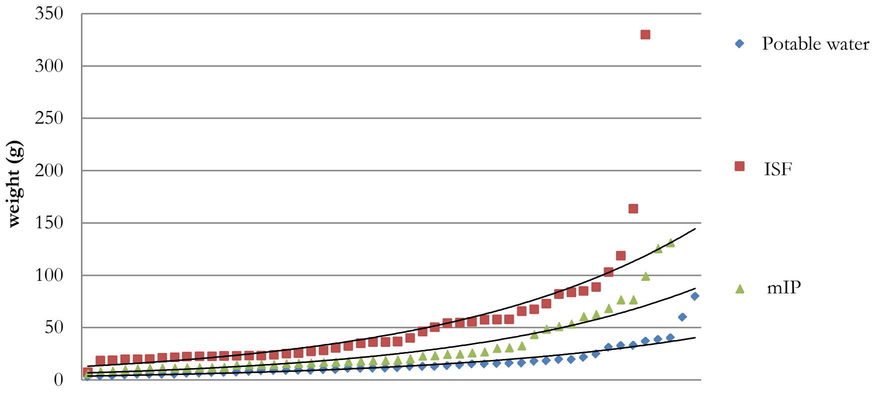

Figure 2. Evolution of lettuce weight.

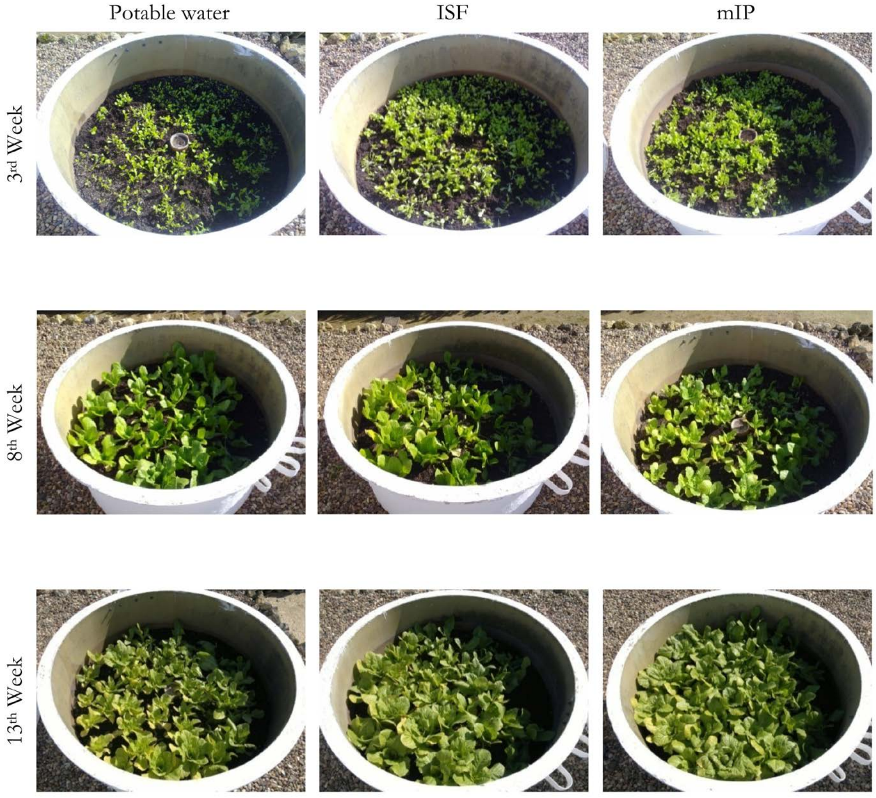

Figure 3. Development of the lettuce crops.

Table 5. Weight lettuce.

	Potable water	ISF	mIP
Lettuces	50	46	48
Average weight (gr)	16.4	51.9	31.7
Deviation	14.62	52.25	29.51
Max weight (gr)	79.8	330.3	131.3
Min weight (gr)	3	7.6	6.2

Table 6. ISF, mIP and potable water analysis of nutrients and organic matter.

	BOD_5 (mg/l)	COD (mg/l)	NH_4^+ (mg/l)	NO_3^- (mg/l)	Total P (mg/l)
ISF	10.1 ± 8.36	42.9 ± 28.5	5.77 ± 8.31	24.8 ± 10.9	5.63 ± 1.25
mIP	3.80 ± 5.88	40.75 ± 17.6	4.69 ± 6.58	25.4 ± 8.4	5.38 ± 1.25
Potable water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 7. Aollutants analysis required by current regulations.

	Nitrate (mg/kg)	E. coli CFU/g	dry weight (%)
Regulation (max. value)	2.500 - 4.500	100	-
Potable water	164.5	<20	13.64
ISF	274.3	<20	7.86
mIP	402.3	<20	10.55
Ecological	493.7	<20	6.18
Commercial I	4.810	<20	4.22
Commercial II	4.705	<20	5.71

The data in this table indicate that the lettuces irrigated with reclaimed water were perfectly viable; furthermore, they demonstrate the excess of nitrates in lettuces sold to the large supermarkets.

4. Conclusions

Based on the results obtained, it can be concluded that:

- Intermittent sand filters and modified infiltration-percolation are both effective systems for reclaiming wastewater treated with non-conventional technologies. This results in a high quality water for reuse at very low cost.
- The greater the influent hydraulic load with water from ponds, the faster the filters silt up; consequently, the recommended hydraulic load is 240 l/m^2d.
- Depending on the primary goal, whether higher quality water (mIP) or a higher concentration of nutrients in water (FIA), one or the other filtration system should be used.
- Reclaimed water contains a certain amount of nutrients, mainly as nitrate nitrogen and phosphorus, and these could replace some of the fertiliser normally added to crops, a 25% of the nitrate and 100% of the phosphorus in the case of the Romaine lettuce
- Crops irrigated with this water comply with current legislation on contaminants. The abusive use of fertilizer can cause excessive nitrate levels in lettuce, above what European legislation dictates

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