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Article

Disinfection of Treated Wastewater and its Reuse in the Irrigation of Golf Grass: The Case of Plant M'zar Agadir-Morocco

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Abstract: The treated wastewater of Agadir M'zar plant has a good physico-chemical quality and it contains important nutrients (NPK: nitrogen, phosphorus, potassium). However, the reuse of this water, without disinfecting it, for irrigating the golf grass in the Agadir region, revealed the presence of a bacterial load that can hinder the quality and suitability of spaces for a population that is very demanding. Among the various methods of water disinfection, chlorination with bleach is the least expensive and the most systematically simple. Its effectiveness depends only on the pH of the waters to be disinfected. This study reports the results of disinfection of M'zar plant wastewater with a solution of sodium hypochlorite (bleach), and their reuse for irrigating the golf grass. For this purpose, we carried out a monitoring protocol for germination and growth parameters (number of tillers and leaf length) in order to study and compare the effect of disinfected treated wastewater (DTWW) and groundwater (GW) on the plant turf. The obtained result showed that the disinfection with bleach increased the salinity of the treated wastewater and can affect the permeability of soils and crops.

1. Introduction

In many part of the world, wastewater reclamation and reuse have been an interesting alternative source of water for irrigation, and many researchers have confirmed its benefits. In Mediterranean countries, treated wastewater is exponentially used for irrigating ornamental plants in areas with water scarcity. It could be an economic way to decrease pollution of surface waters and provide groundwater recharge for other agricultural fields. In this context, many studies have focused on the disinfection of treated wastewater and reuse in irrigation through improving its bacteriological and chemical qualities. In Spain, Salgot *et al*. [1] studied wastewater reuse, and they concluded that the reclaimed wastewater can be reused for different applications depending on specific water quality categories. Biological and chemical parameters have to indicate all potential pathogens and chemical intoxications in relation to the origin of sewage. Therefore, it is necessary to find adequate indicators which can be performed by chemical as well as biological quantitative risk assessment. In Italy, Lubello *et al*. [2] have studied the irrigation of nursery ornamental plants with tertiary effluents (filtration and peracetic acid + UV) in comparison with nutrient enriched groundwater. They concluded that the refinery treatment by filtration and disinfection with peracetic acid and UV together were very effective in bacteria removal. It was able to maintain good plant growth as well as fertigated water for most of the tested species.

In our study, we dealt with the case of Agadir (south of Morocco: altitudes between 30 and 31° N) (Figure 1). The region of Agadir is an agricultural region that is characterized by an arid climate, very limited water resources and poor nutrient soils. The agricultural sector is the largest consumer of water. Thus, the use of treated wastewater in agriculture is a good alternative that will help preserve water resources in the region. Moreover, given the nutritional wealth of the treated wastewater, this solution will permit a recycling of these items and reduce the abusive misuse of fertilizers [3].
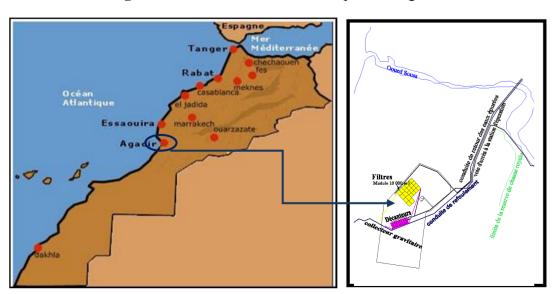
Figure 1. Localization of the M'zar plant of Agadir.

The current potential of wastewater treated by the Agadir M'zar plant, which might be used for unrestricted irrigation (category A WHO standards), is 10,000 m^3/day and will reach 50,000 m^3/day in the medium term. A feasibility study on the reuse of the Agadir M'zar plant wastewater was launched by RAMSA (Régie Autonome Multiservices d'Agadir; Water Supply Service of Agadir). In this context, the total surface of green spaces of Agadir city is estimated to be 878×10^4 m^2 with a need of water for irrigation reaching 8,106 m^3/year. With a daily flow of 50,000 m^3/day, the treated wastewater of the M'zar plant will completely fill this need. The golf grass alone occupy 30.5% (268×10^4 m^2) of the total area of green space in Agadir (878×10^4 m^2), with a water consumption estimated to be 3216,103 m^3/year [4,5]. This study focuses on disinfecting the treated wastewater to sodium hypochlorite for irrigating the golf courses' grass. It presents the planning, protocol and preliminary results of the tests that are carried out to evaluate the effects of the disinfected treated wastewater in the reuse for irrigating golf grass. Particular attention is given to the monitoring of the parameters of germination and growth of grass plants irrigated with disinfected treated wastewater compared to those irrigated by water from groundwater.

2. Materials and Methods

2.1. Test Protocols of Disinfected Treated Wastewater Reuse

In order to study the feasibility and assess the impact of the disinfected treated wastewater use on irrigation of golf grass, three varieties of turf golf courses V1 (Penccross), V2 (English Ray Gras) and V3 (red fescue mixed with English Ray Gras) were used on three plots (P1, P2, P3) for comparison. The same tests were reproduced under the same conditions using groundwater.

Each plot has a size of 25 m^2 and is divided into two parcels of 12.5 m^2 to ensure a repetition of the conditions of each test. The subdivision of each parcel is insured by placing a plastic insulator at a depth of 0.5 m to prevent infiltration between subdivisions. Each parcel contains a layer of 20 cm of soil composed with 75% topsoil and 25% sand and a lysimeter on two opposite corners. The lysimeters have a volume of 1 m^3 and reproduce the conditions of soil and variety of grass planted in the parcel. These lysimeters are designed with a good seal for the recovery of the leachate after irrigation [4].

In order to determine the percentage of germination, the three varieties of golf grass (V1, V2, and V3) were sown into alveolars trays on the plant site. For each variety of turf, two trays with 56 alveolars are sown (one seed per alveolars). The soil used in the trays has the same composition as in the plots. The germination of seeds in the six trays is monitored for 41 days. Counting the seedlings starts from the emergence of the first plant (first day of growth) until 41 days for each variety. The irrigation of the plots and trays is ensured by the treated wastewater that underwent disinfection before each application. The frequency and volume of irrigation is similar to the testing of irrigation by treated wastewater and groundwater: three times a day and 90 L/day per 25 m^2 plot.

2.2. Soil Characteristics and Irrigation Water

As mentioned above, soil plots are composed with 75% topsoil and 25% sand. Detailed soil analysis is presented in Table 1. From these results, the land has a loamy texture with little clay and some sand. It is slightly fitted with major nutrients and organic matter: total nitrogen, available phosphorus and

exchangeable potash. In addition, the sands are very poor in any fertilizing element. The salinity of the two types of soil is very low. Otherwise the alkalinity of the sands is higher than that of the plant earth. Therefore soil cannot provoke any risk of damaging the cultures as it does not provide them with any nutriment. Therefore, the grass can only take advantage of fertilizing elements contained in waters or brought by possible amendments.

Table 1. Tests results of soil and sand.

Parameters	Texture	pH	Organic matter (%)	Total nitrogen (%)	Total Limestone (%)	EC 1/5 (dS/m)	Soluble Salts (g/kg)	P_2O_5 Assimilable (ppm)	K_2O Exchangeable (ppm)
Topsoil	LSC *	8.70	1.85	0.15	5.20	0.12	0.41	14.56	128.70
Sand	S	9.6	0.05	0.01	36.7	0.045	0.16	1	24.9

* LSC means loamy sandy clay.

The waters used for irrigation in experimental plots are of two main types:

- the groundwater of Souss plain, drawn from the well located in the wastewater plant zone;
- the treated wastewater of the plant M'zarAit Melloul, which uses the infiltration percolation process on bed sands and disinfected with bleach.

The main characteristics of treated wastewater before disinfection are shown in Table 2. The assessment of water analysis of the groundwater and those treated allow their classification as irrigation water according to the USDA (U.S. Department of Agriculture). The USDA classification is based on the values of electrical conductivity (EC) and those of the index SAR (Sodium Adsorption Ratio) [6].

- The groundwaters are classified as C2S1, they can be used on any type of soil with minimal risk of sodium accumulation. Water salinity (EC = 0.520 ds/m) is close to the limit of turf growth without applying special treatments for salinity (EC limit located = 0.750 ds/m).
- The treated wastewaters are classified as C4S1, they are very saline (EC at 25 °C is of 3.15 ds/m). However, they can be used for any type of soil as the value of the SAR is less than 10.

Table 2. Results of ionic balance of treated wastewater before disinfecting and groundwater used for irrigation. (Please check)

	Groundwater		Treated wastewater	
	mg/L	($\times 10^{-3}$) mole/L	mg/L	($\times 10^{-3}$) mole/L
pH		7.4		7.1
EC at 25 °C dS/m		0.58		3.15
Sodium Adsorption Ratio (SAR)		0.84		6.66
Chloride Cl^-	128	3.61	720	20.31
Potassium K^+	2.89	0.07	43	1.1
Sodium Na^+	30.52	1.32	487	21.21
Calcium Ca^{2+}	47.8	1.19	294.8	7.35
Magnesium Mg^{2+}	32.1	1.32	65.9	2.71
Total Nitrogen	1.5	0.1	44.6	3.18

Table 2. *Cont.*

	Groundwater		Treated wastewater	
	mg/L	$(\times 10^{-3})$ mole/L	mg/L	$(\times 10^{-3})$ mole/L
Phosphorus	0.39	0.01	8.85	0.28
Bicarbonate HCO_3^-	191	3.13	317	5.19
Sulphate SO^{42-}	10	0.1	85	0.88
Nitrate $N^- NO3$	5.2	0.08	350	5.64

3. Results and Discussion

3.1. Disinfection Tests Protocol

Before applying the disinfected of the treated wastewater, we should first determine the chlorometric degree, Chlorine demand and break point for the bleach.

3.1.1. Determination of the Chlorometric Degree

The bleach solution is titrated with sodium thiosulfate 0.1 N and potassium iodide 10%. The bleach used for disinfection of treated wastewater is titrated with a volume (V = 69.2 mL) of thiosulfate for a sample (v = 10 mL). Henceforth, the quantity of bleach is 24.56 Cl_2 g/L, so the bleach chlorometric degree is 7.74° Cl.

3.1.2. Chlorine Demand

In order to determine the amount of chlorine that can be consumed by the water for its disinfection and destruction of organic matter, we followed the bacteriological analysis of the purified water throughout a week in order to select the maximum bacteriological load (Table 3). The samples are prepared in 10 numbered and corked bottles of 250 mL. In each 100 mL of treated wastewater that were analyzed, increasing amounts of chlorine solutions (0.1 g Cl_2/L) were introduced. The bottles were kept in the dark for 30 minutes at an ambient temperature of 20.6 °C. Then, the residual chlorine was determined by the method of comparison in the range of the 10 bottles and pH as shown in Table 4.

Table 3. Treated wastewater bacteriological load for disinfection tests.

Designation	Total Coliform (TC) (37° 24 h) UFC/100 mL	Feacal Coliforms (FC) (44° 24h) UFC/100 mL
Stock solution (SM)	20×10^4	9×10^3

Table 4. Range to determine the treated wastewater demand in chlorine

Sample	1	2	3	4	5	6	7	8	9	10
Volume of Chlorine Solution (mL)	5.2	11.1	25	42.8	66.6	81.8	150	233.3	400	900
Active Concentration of Cl_2 (mg/L)	5	10	20	30	40	45	60	70	80	90
Residual Chlorine mg/L	1	6	10	20	50	60	40	30	20	70
pH of Chlorine Solution	8.09	8.13	8.06	7.90	7.91	7.77	7.87	7.97	8.38	8.73

3.1.3. Break Point Determination

The break point is determined by the second concave curvature of the curve of the residual chlorine as a function of Cl_2 concentration of the different concentrations (Figure 2). Therefore, here, the ninth bottle is the breaking point, where there is a concentration of 80 mg/L of active chlorine which corresponds to 30% of HOCl and 70% of CLO^- a pH of 8.38.
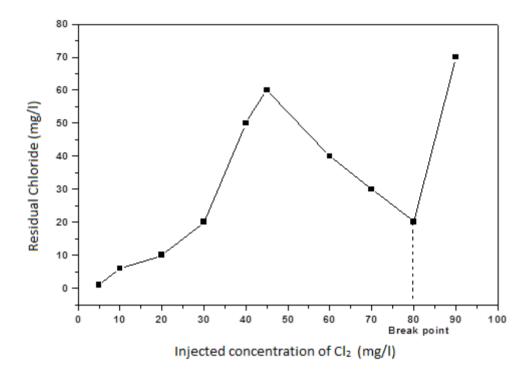
Figure 2. Disinfection break point determining curve of treated wastewater.

3.1.4. Bacteriological Tests Results

In order to evaluate fecal pollution indicators before and after the break point of the treated wastewater, we have shown the water in the bottles 4, 9 and in the parent strain on the selective medium of total coliforms (TC) and fecal coliforms (FC). The results recorded on the plates after incubation are shown in Table 5.

Table 5. Results of the bacteriological tests of the disinfected treated wastewater.

Designation	Total Coliform (TC) (37° 24 h) UFC/100 mL	Feacal Coliforms (FC) (44° 24h) UFC/100 mL
Stock solution (SM)	20×10^4	9×10^3
SM for dilution 10^1	9×10^4	0
SM for dilution 10^2	10^5	0
4th bottle	4×10^3	0
4th bottle for dilution 10^1	0	0
4th bottle for dilution 10^2	0	0
Break point (9th bottle)	0	0

We note an absence of fecal and total coliforms at the 9th bottle that matches the break point. This confirms the effect of disinfectant bleach to this concentration of chlorine (80 mg/L) for the treated wastewater of the M'zar plant. However, the disinfection by chlorination requires mastering many parameters (pH, temperature, turbidity, contact time, residual chlorine) that influence its effectiveness against the microorganisms.

3.2. Growth Parameters

The irrigation test results with disinfected treated wastewater and groundwater are presented in Figures 3, 4 and 5. These figures show the evolution of germination percentage, the number of tillers and leaf length for each variety of grass, respectively.

Figure 3. Evolution of the germination percentage of the different seed varieties irrigated with the two kinds of water: well water and disinfected treated wastewater.
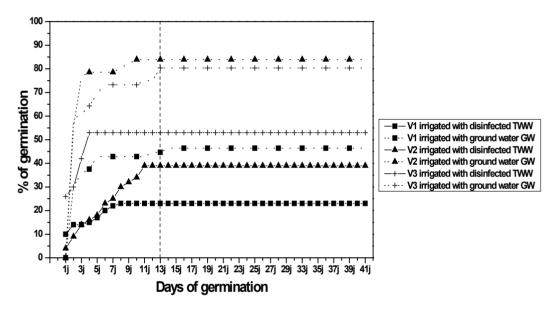

Figure 4. Evolution of the number of tillers in the irrigated plots with the two qualities of water: groundwater and disinfected treated wastewater.
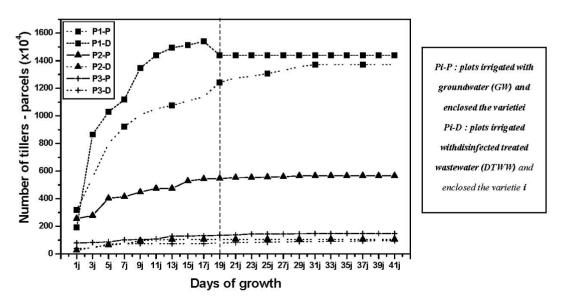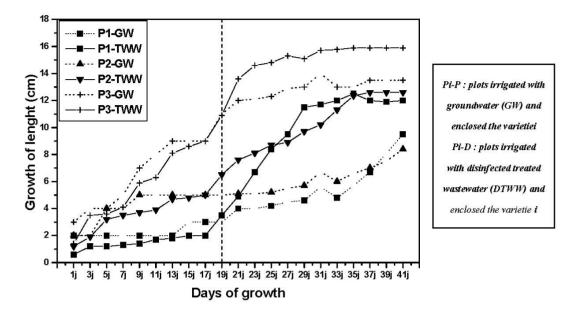
Figure 5. Evolution of the length of leaves in the irrigated plots with the two qualities of water: groundwater and disinfected treated wastewater.

The evolution of the germination percentage shows that the germinating variety V1, V2 and V3 is favored by groundwater until the 13th day. Otherwise, the germination of these varieties is delayed by the salts in solution-treated wastewater that was disinfected during this phase. The salts caused the reported increase in the osmotic pressure of soil solution which has prevented the seed absorption and subsequently a poor port and then a reduction in performance [3,7-9].

After the 13th day, the germination percentage remained stable and reached a maximum of 23, 39 and 53% for varieties V1, V2 and V3, respectively. However, the maximum percentage of these varieties is doubled when they are irrigated with groundwater (46, 84 and 80% respectively) and similarly with treated wastewater (50, 79 and 88% respectively).

Thus, the irrigation with disinfected treated wastewater has led to a reduction in the maximum germination percentage that reached more than 50% for all varieties compared to irrigation with groundwater.

Regarding the evolution of the number of tillers (Figure 4), we note that their increase before the maximum rate of germination reached is due to the contribution of the phenomenon of germination and tillering. The germination is reached after 13 days of sowing which is the 19th day of growth because we have a germination time of six days. The increased number of tillers after the 19th day of growth is limited to the phenomenon of tillering [10,11].

The irrigation using groundwater promotes tillering for the varieties V2 and V3 throughout the monitoring period. Variety V1 is the only one that has a better adaptation to the quality of disinfected treated wastewater which promotes tillering compared with groundwater. The observed variation compared to irrigation with disinfected treated wastewater is reduced only for varieties V1 and V3 after the 19th day of growth. Following this trend, we can conclude that tillering is stimulated by irrigation with groundwater, the treated wastewater and the disinfected treated wastewater in descending order.

In order to monitor the growth of the aerial part of plant grass, a leaf sample is taken every two days. The average leaf length of each sample is measured on graph paper. The evolution of this length for different varieties irrigated by the two water qualities over 41 days is shown in Figure 5.

Before the maximum germination rate is reached (before the 19th day of growth, shown in Figure 3), we cannot observe the effect of irrigation with disinfected treated wastewater on the evolution of the leaf length. However, after the 19th day of growth, it is obviously the case that the irrigation by disinfected treated wastewater promotes the increased leaf length compared to the irrigation with groundwater for all varieties.

Irrigation by the disinfected treated wastewater affects the germination percentage and the tillering phenomenon of the leaf length. This is due to the concentration of sodium and chloride ions which influence the turf plant growth according to whether or not it is resistant to salinity. These results are consistent with [4,5] in which it is reported that the variety Ray Grass English accumulates more Na^+ and Cl^- in leaves than in roots, whereas, according to [12,13], it is classified as a species that is moderately sensitive to salinity.

3.3. Microbiological Parameters

At the end of the monitoring, samples of the aerial part of three varieties of turf were collected in plots and irrigated with groundwater and with disinfected treated wastewater for microbiological tests. These tests involve only the enumeration of *Faecal Coliforms* (FC) and *Faecal Streptococci* (FS) which are signs of germs and fecal contamination [14,15].

The bacteriological tests of the treated wastewater before disinfection are of about 1.32×10^4, 7.06×10^4 and 4.29×10^3 CFU/100 mL, which are the annual average respectively for FC, TC and FS. The tests of the turf irrigated by these waters gave results that varied between (190,000 and 590,000 CFU/g) and (160 and 510 CFU/g) respectively for CT and CF such as SF, which varies between 100 and 500 CFU/gr. Salmonella is absent for all varieties irrigated by treated wastewater (Table 6). These values are close to those of the turf irrigated with well water which shows no *Faecal* contamination. CF, CT, FS and *Salmonella* were absent in 25 g.

Table 6. Bacteriological tests of irrigated golf grass by treated wastewater and the groundwater at the end of 41 days and a turf irrigated by groundwater of an outer golf course.

	Grass irrigated by TWW			Grass irrigated	Outer golf Grass
	EV1	**EV2**	**EV3**	**by GW**	**irrigated by GW**
Total Coliforms (UFC/gr)	1.90E + 05	2.20E + 05	5.90E + 05	3.80E + 04	1.00E + 03
Faecal Coliforms UFC/gr	2.10E + 02	1.60E + 02	5.10E + 02	5.00E + 02	1.00E + 03
Faecal Streptococcus UFC/gr	1.2E + 02	2.00E + 02	5.00E + 02	2.20E + 02	1.00E + 02
Salmonella UFC/25 gr	0	0	0	0	0

These results lead us to decide that the grass plant has had an external contamination originating from environmental pollution. Moreover, the treatment plant is installed in a reserve, and the plots of turf are installed in the next plant close to the sand filters and stock sludge. Therefore, we cannot exclude the possibility of an accidental contamination by the movement of wind, animal activity or the trampling of workers in the processing of the grass.

In order to confirm this hypothesis, tests of a sample turf taken from the outer golf course is approximately 10^3 CFU/g, 10^2 CFU/g for CF, TB and SF, with an absence of *Salmonella*. These results confirm the results obtained from the irrigated grass by disinfected treated wastewater which has a concentration of CT between 8.2×10^4 and 4.3×10^5 CFU/g. However, CF and anaerobic sulfite-reducers are below than 10 CFU/g as well as *Salmonella* that are absent in 25 grams, but the aerobic microorganisms are greater than 3×10^7 CFU/g (Table 7). This is consistent with the results of [8] in which it was announced that lettuce plants irrigated with the treated wastewater are more altered toward the end of the harvest than plants irrigated with fertilized water. This alteration may be due to the abundance of total mesophilic aerobic bacteria (5.1×10^5 CFU/g) as the lettuce grows at ground level and can be easily contaminated by the bacterial group. Therefore, we can conclude that the disinfection has been successful from a microbiological point of view. However, it affects the germination and growth of turf plants by their intake of sodium and chloride ions, which increases the salinity of the treated wastewater.

Table 7. Bacteriological tests of irrigated golf grass by disinfected treated wastewater at the end of the monitored 41 days.

	Irrigated grass by disinfected TWW		
	EV1	EV2	EV3
Total Coliforms (UFC/gr)	4.3E + 05	8.2E + 04	3.2E + 05
Faecal Coliforms UFC/gr	<10	<10	<10
Faecal Streptococcus UFC/gr	<100	<100	<100
Anaerobic Sulfite-Reduction UFC/gr	<10	<10	<10
AerobicMicroorganism at 30°C UFC/gr	>3.107	>3.107	>3.107
Salmonella UFC/25 gr	0	0	0

4. Conclusions

The bacteriological tests of the irrigated turf by treated wastewater showed that concentrations of faecal and total coliforms and faecal streptococci are very high, while there is an absence of salmonella. Thus, the disinfection of these waters was prompted primarily by the sodium hypochlorite which gave satisfactory results in the treated waters and that has marked an absence of faecal contamination.

In general, the disinfection of sodium hypochlorite increased the salinity of the treated wastewater. As a result, these waters are suitable for irrigation only under some conditions: good soil leaching, salt-tolerant plant and highly permeable soils. As a result, care must be taken first in the management of irrigation doses to prevent accumulation of salts in the root zone and then in controlling their transfer into the waters of the aquifer.

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