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ORIGINAL RESEARCH

Physico-chemical and microbiological quality of M'Zar wastewater treatment plant effluents and their impact on the green irrigation of the Golf course

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Abstract

Purpose Reuse of treated wastewater (TWW) for irrigation can be an effective strategy in Morocco to overcome the pressure on freshwater resources. The M'zar wastewater plant is based on percolation infiltration treatment, allowing the purification of the wastewater of Agadir, and with its UV disinfection system, it is now possible to reuse this water for irrigation. In this sense, the aim of our study is to evaluate the microbiological and physicochemical quality of the treated wastewater of this station, used for irrigation of a Golf course as well as to determine its impact on grass and soil.

Methods A monitoring of TWW quality was carried out monthly on the level of the Ocean's Golf on water samples, grass and soil. This monitoring is related to the physicochemical (pH, temperature, conductivity, STD, COD, and BOD5) and bacteriological characteristics by counting the indicators of faecal contamination, faecal coliforms (FC), faecal streptococci (FS), *Salmonella* and *Vibrios* as well as sulphito-reducers spores (SRS).

Results The results of microbiological analysis in the three compartments confirm the presence of various organisms such as FC, FS, and SRS in a very significant number with no load in *Salmonella* and *Vibrios* during our study period. For physicochemical analyses, we observed that only the conductivity showed fairly a high value of 6.38 dS/m.

Conclusion The obtained physicochemical and bacteriological results revealed that the treated wastewater with the M'zar plant complies with national and international standards.

Keywords Treated wastewater · Treatment plant · Physicochemical quality · Microbiological quality · Wastewater reuse

Introduction

Due to the increasing competition for high-quality freshwater supplies between agricultural and urban uses, particularly in arid and semi-arid regions with high population densities, the reuse of treated wastewater has been an interesting alternative source of irrigation widespread in several mediterranean countries (Angelakis et al. 1999; Hochstrat et al. 2006). Reusing wastewater is indeed a promising option, many researchers around the globe have investigated the effects of wastewater on soil, seeds germination, and plant growth (Mekki et al. 2013) as well as the risk analysis of using them in agriculture (Ganoulis 2012). Globally, the use of this resource is growing rapidly, at a rate of 10–29% per year, and the major part of this water (70%) is used for agriculture (Aziz and Farissi 2014). Currently, the yearly volume of discharged raw wastewater in Morocco is about 700 million m^3, 60% of which is discharged to the sea, the remaining quantity is either drained off and a small part is actually reused on an area of 7000 ha (Choukr-Allah 2012). This could be a way to reduce surface water pollution and provide groundwater for other agricultural fields.

In fact, treated wastewater is often an interesting source of water containing the nutrients necessary for the proper fertilization of agricultural land and plant growth and which is always available (Jiménez-Cisneros 1995). The use of this resource in agriculture is a form of water and nutrient recycling, but can also cause serious environmental problems due to their high levels of toxic chemicals and pathogens (El

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Addouli et al. 2009) that have become resistant to antibiotics (Meloul and Hassani 1999; El Boulani et al. 2016); as a result, the use of these water in crop irrigation threatens the health of farmers and consumers products (Howard et al. 2003; Oren et al. 2004; Taylor et al. 2004).

This danger is manifested chemically by the uncontrollable rise in levels of salts, pesticides and heavy metals as well as bacteriologically by the indicators of faecal contamination, namely total coliforms, faecal coliforms, and faecal streptococci which can predict the presence of pathogenic germs presenting an increased health risk (Al-Nakshabandi et al. 1997; Toze 2006; El Addouli et al. 2009).

In the south of Morocco, the Agadir region is an agricultural zone characterized by limited water resources, especially since liquid wastewater discharges throughout the Souss basin amount to 25% million m^3/year. Consequently, the use of treated wastewater in agriculture would not only preserve water resources, but also significantly reduce the misuse of fertilizers (Mouhanni et al. 2013).

The objective of our work is to evaluate the quality of the treated wastewater of the M'zar station and the impact of their use in the irrigation of the ocean golf course. Such an objective remains dependent on the transition from a treatment and rejection model to a purification and reuse model.

Materials and methods

Study site

Ocean's Golf (30°21′51.1″ N, 9°34′35.0″ W) is located in the south of Agadir, Morocco (Fig. 1). This space has an exceptional view on the valley of the Souss and the mountains of the high atlas, and it is a sporting and tourist place, spread over 90 ha. The golf area is covered with grass, besides a various trees of eucalyptus, tamaris, cypresses, mimosas, and palm trees, and this vegetation is irrigated by treated wastewater provided by the station M'zar, within the framework of a convention between the water district of Agadir (RAMSA) and the Golf course since 2010.

Sampling method

Water, grass, and soil samples were collected monthly in sterile containers and transported to the laboratory under refrigeration, two sampling points were retained by our study with a distance of 683 m, the first site denoted S1 is considered as a reference site related to the sports area and is irrigated by water coming directly from the wastewater treatment plant, and the second site denoted S2 composed of plant form developed by the Agricultural and Veterinary Institute of Agadir and irrigated by the waters of the same station after storage. At each point, three compartments were sampled: for grass (G1, G2) and soil (So1, So2), samples were taken in sterile plastic bags, using, respectively, scissors and a sterile coring tool. For the water samples (W1, 2), sterile borosilicate glass bottles (1L) were filled in such a way as to keep the sample away from any source of contamination.

Physico–chemical analyses

The pH, temperature, conductivity, and total dissolved salts were determined in situ by a pH meter equipped with a multi-parameter probe-type SELECTA CD 2004. The chemical oxygen demand (COD) and 5-day biological oxygen demand (BOD5) was determined according to AFNOR standard (NF T90-101).

Fig. 1 Satellite view of sampling sites

Bacteriological analysis

Bacteriological analysis was performed on composite samples. The purpose of these analysis can be highlighted in two main objectives:

- The enumeration of faecal coliforms (FC), faecal streptococci (SF), and anaerobic sulphite-reducing spores of the genus *Clostridium* (SRS).
- The detection of pathogenic germs of the genus *Vibrio* and *Salmonella* spp. according to the Moroccan standard *NM ISO 9308-1 2007*.

Water

The enumeration of the indicator germs of faecal contamination was performed using the membrane filtration method on Slanetz and Bartley medium, Tergitol TTC, and TSC medium, respectively, for FS, FC, and SRS (Rodier et al. 2009). For FS and FC, the incubation was carried out at a temperature of 44 ± 1 °C 24 h and 48 h, respectively. For SRS, the samples were pre-heated to 80 °C for 10–15 min to destroy the vegetative forms and keep only sporulated forms, and incubated at 37 °C for 48 h. The results were expressed as number of colonies forming units (CFU) per ml.

For the detection of *Salmonella* spp., 5 l of water were filtered through 0.45 µm cellulose acetate filter, and the filter was then placed in 225 ml of pre-enrichment medium (buffered peptone water), and incubated at 37 °C for 18–24 h. A 0.1 ml enrichment of the pre-enrichment was transferred to 10 ml of RV10 Rappaport–Vassiliadis broth and incubated at 44 °C for 18–24 h. The isolation was done on a selective medium, and it consists of seeding the Hektoen and XLD medium from the enrichment broth and then incubating at 37 °C for 24–48 h. Typical colonies were selected and streaked onto nutrient agar at 37 °C for 24 h and identified biochemically by the API 20E gallery. The results are expressed in presence/absence by filtered volume.

For the detection of *Vibrio cholerae*, 450 ml of water were filtered (cellulose acetate 0.45 µm). The filter was then transferred to 225 ml of saline peptone water and incubated at 37 °C for 24 h. The surface layer is seeded on thiosulfate citrate bile sucrose (TCBS) medium and incubated at 37 °C for 24–48 h. Typical colonies were purified on 2% nutrient agar at 37 °C for 24 h. The biochemical identification of the suspicious strains was carried out by the API 20 E gallery. The results are expressed in presence/absence by filtered volume.

Grass and soil

The hygienic quality of the soil and grass compartments has also been the subject of this study. Samples addressed to the counting of FC and FS were prepared by mixing 10 g of each compartment with 90 ml of physiological water. Subsequently, spreading was carried out on a Bile medium with azide and esculin and Tergitol medium with TTC, respectively, for FS and F Cat 44 ± 1 °C for 24 h; the results were expressed in number colony forming units (CFU) per g.

For *Vibrio* and *Salmonella* spp., the same steps were followed by placing 25 g of each sample in 225 ml of buffered peptone water for *Salmonella* and saline alkaline peptone water for *Vibrio* spp.

Results and discussion

Physico–chemical analyses of the water samples

Table 1 highlights the obtained physico-chemical values of water samples W1 and W2. The average values of the physicochemical variables of wastewater treated and reused by the Golf Ocean show that these parameters are provided in an acceptable quantity in accordance with the standards of the irrigation water (WHO 1989; CNS 1994) and in agreement with similar studies (El Addouli et al. 2009; Mouhanni et al. 2013). Both samples W1 and W2 have the same trend in the course of the sampling year, the conductivity, COD, DBO5 increases as a function of the water temperature (Fig. 2). This can be explained by the increase of the salts in the samples in the high-temperature months (summer): the increase of salt

Table 1 Physicochemical parameters of water

Settings	W1				W2			
	Min	Max	Moy	Standard deviation	Min	Max	Moy	Standard deviation
Temperature (°C)	15	28.4	22.55	3.36	15.1	26.6	22.66	3.79
Ph	6.7	7.8	7.36	0.47	6.7	8	7.35	0.5
Conductivity (ms/cm)	2.18	4.9	3.27	0.74	2.85	6.35	3.81	0.8
Total salts dissolved (mg/l)	1113	1790	1456	205.3	1715	2450	2031	247.8
DBO5 (mg/l)	8	14	12	2	9	12	10	1
CDO (mg/l)	22	46	34	11	24	50	36	18

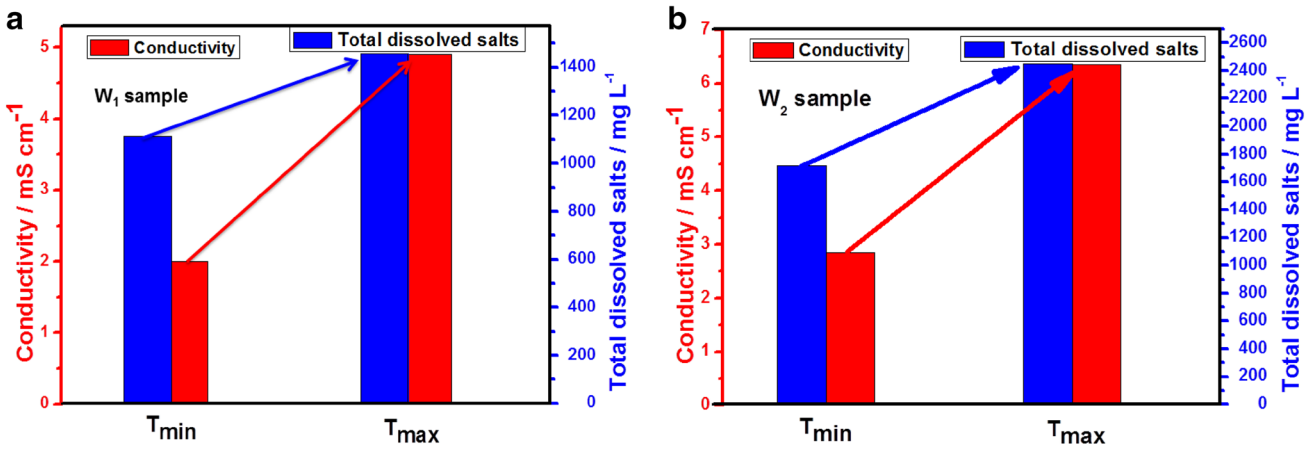

Fig. 2 Evolution of the conductivity and total dissolved salts in **a** W_1 sample and **b** W_2 sample

ions is responsible of the increase of the conductivity in both samples; inversely, the opposite happens in the low-temperature months. High levels of salinity can also be explained by mixing domestic wastewater with industrial effluents during fish transformation process (Mouhanni et al. 2013). The specific electrical conductivity which is brought in excessive quantity and corresponds to the limit value of the direct discharge in the receiving environment. Again, relatively high values for this parameter showed that the waters studied are moderately saline (Mouhanni et al. 2013; Bourouache et al. 2019). This imposes some restrictions on their use for irrigation of sensitive crops.

There is also an increase in the values of these parameters in the water sample of the second site (W2), which could be explained by exposure of the storage tank to the sun causing evaporation and concentration of salts. Thus, the addition of fertilizers to the irrigation water of the second site can also be at the origin of this increase.

The bacteriological analysis of these waters (Table 2) indicates an average faecal coliform (FC) load of 1.14 log 10 CFU/100 ml and 1.23 log 10 CFU/100 ml, respectively for W1 and W2. Those of fecal streptococci (FS) are 0.82 log 10 CFU/100 ml for E1 and 0.97 log 10 CFU/100 ml for E2. For sulphito-reducing spores (SRS), Clostridium, the average loads are 1.37 log 10 CFU/100 ml and 1.75 log 10 CFU/100 ml for W1 and W2, respectively.

This difference in charge between W1 and W2 might be due to the fact that the storage pond which feeds the second site with irrigation water is exposed to external biotic factors. For example, faeces from animals and birds and probably from the aforementioned abiotic factors of the natural environment. In addition, water stagnation causes poor aeration, eutrophication, and, therefore, a large bacterial proliferation. The duration of water storage in the pond can also explain the difference in bacterial load.

These levels are in accordance with the Moroccan standard, WHO, and are consistent with the results of Bourouache et al. (2019) (< 1000 CFU/100 mL).

Origin of faecal pollution

To explain the presence of fecal contamination indicators, the ratio $R = \mathrm{FC/FS}$ was used as a basic element to determine the origin of faecal pollution (Fig. 3). In our case, $0.7 < R < 2.1$ showing a mixed predominantly animal pollution to a pollution of uncertain origin for the four seasons which could be explained by the presence of natural fertilizers (animal origin), besides the phenomenon of soil leaching, the urbanization of the municipality, and the exposure of the basin to different sources of contamination, including the excrement of animals and birds. In addition to animal and human origin, the physico-chemical parameters,

Table 2 Microbiological parameters of water

Microbiological setting log 10 UFC/100 ml	W1				W2			
	Min	Max	Moy	Standard deviation	Min	Max	Moy	Standard deviation
FC	0	2.29	1.14	0.85	0	2.25	1.23	0.65
FS	0	1.51	0.82	0.66	0	1.72	0.97	0.40
SRS	0	4	1.37	1.1	0	4	1.75	1.2

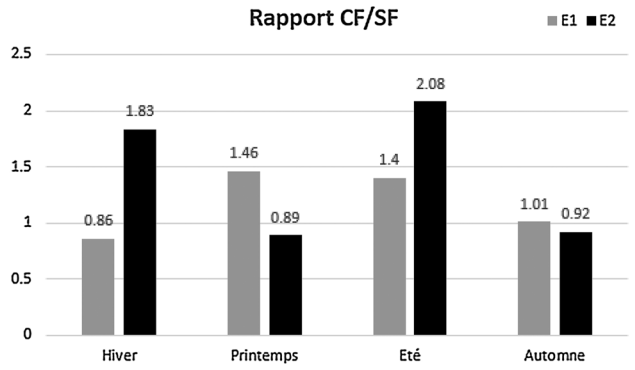

Fig. 3 Origin of pollution according to the report "faecal coliforms/faecal streptococci" (R = CF/SF). $R < 0.7$ mainly or entirely of animal origin, R between 0.7 and 1 mixed predominantly animal, R between 1 and 2 uncertain origin, R between 2 and 4 mixed predominantly human, $R > 4$ source exclusively human

as temperature and pH, can also contribute to variations in bacterial activity (Chigbu et al. 2004).

Characterization of the soil and the grass studied

The bacteriological analyses of soil and grass are presented in Tables 3 and 4. We have presented the data of the minimum and maximum faecal pollution to better understand the trend of its presence in both media. Thus, we have observed that the bacteriological quality of soil and grass is moderately normal: the average number of faecal coliforms obtained is 1.94 log 10 CFU/g and 2.45 log 10 CFU/g for G1 and G2, with 2.27 log 10 CFU/g and 2.3 log 10 CFU/g for So1 and So2, respectively. It is the same for fecal streptococci, and the average values obtained are 2.28 log 10 CFU/g and 2.54 log 10 CFU/g for G1 and G2, and 1.85 log 10 CFU/g and 2.46 log 10 CFU/g,, respectively for So1 and So2. According to these results, the faecal contamination varies largely depending on the nature of the irrigated substrate (soil or grass) and the type of water used (water coming directly from the station or water from the reservoir).

The bacterial load is higher in the compartments of site 2 than those of the first site, and this could be explained by the fact that the water (E2) used in the irrigation of these compartments is more loaded with bacteria, since it is water stored in basins exposed to several sources of contamination. Some samples have zero charge, the influence of climatic conditions could be the origin, since the plots are open air and exposure to the sun can destroy the fungi contamination germs (FGIC) by UV to which they are exposed. In addition, stopping irrigation during periods of rain can cause a decrease in bacterial load.

The absence of germs indicating a faecal contamination (GIFC) in both compartments and during different periods can be explained by the lack of favorable conditions for their survival in these substrates, since they are very demanding in terms of alkalinity. There is also the possibility of competition between soil or turf bacteria and exogenous bacteria (GIFC), which can induce the decrease of the number of these microorganisms in the soil, on the other hand, the existence of predators of these microorganisms, especially in the first centimeters of soil (Sou 2009). The presence of grass covering the ground could also act as a filter that prevents the passage of germs. The GIFC load is higher in the turf than in the soil because of its exposure. (Al-Lahlam et al. 2003)

Results of *Salmonella* and *Vibrios*

Pathogen analysis showed negative results for all samples analyzed for water, grass, and soil at both sampling sites despite the presence of fecal contamination indicator

Table 3 Microbiological parameters of the grass

Microbiological setting log 10 UFC/g	G1				G2			
	Min	Max	Moy	Standard deviation	Min	Max	Moy	Standard deviation
FC	0	4.38	1.94	2.14	0	5.82	2.45	1.83
FS	0	5.19	2.28	1.93	0	5.87	2.54	2

Table 4 Microbiological parameters of the soil

Microbiological setting log 10 UFC/g	So1				So2			
	Min	Max	Moy	Standard deviation	Min	Max	Moy	Standard deviation
CF	0	2.3	2.27	1.94	0	4.2	2.3	1.63
SF	0	4	1.85	1.7	0	4.6	2.46	1.51

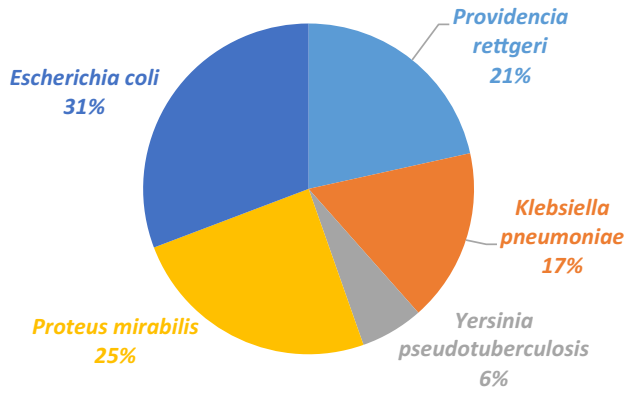

Fig. 4 Percentage of different bacterial species revealed in the three compartments

bacteria. The probable existence of these viable non-cultivable organisms would call into question the conventional culture techniques used. However, the absence of these germs would probably be related to the presence of antimicrobial substances (polyphenols, tannins, and fatty acids) (El Addouli et al. 2009).

These results are in agreement with the Moroccan water standards for irrigation and are consistent with the previous reports (El Addouli et al. 2009). However, a report published earlier showed the presence different strains of salmonella even after the installation of the tertiary UV treatment (El Boulani et al. 2016). This can be explained by the difference in the sampling period.

It is also noted that biochemical tests have revealed the presence of other pathogenic species for humans such as *E. Coli*, *Providencia rettgeri*, *Klebsiella pneumonia*, *Proteus mirabilis,* and *Yersinia pseudotuberculosis* (Fig. 4). These germs belong to the family Enterobactereacea, colonizing the digestive tract of humans; they can cause gastrointestinal, pulmonary or urinary infections, and could also be involved in nosocomial infections (Podschun and Ullmann 1998). They can be isolated from wastewater or soil and considered as an indicator of faecal contamination. Other studies have confirmed the presence of this family in the soil after sewage irrigation (Ahmed and Müller 1984).

Valorisation of spent wastewater

Based on our results, treated wastewater parameters (pH, BOD5 and faecal coliforms) of M'zar station analyzed comply with WHO and USEPA standards (also based on a goal of zero pathogen in reused waters) (Table 5), and therefore, the sanitary quality of these waters would be acceptable in the case of reuse for irrigation. It should, therefore, be noted that other factors may directly or indirectly affect the efficiency and effectiveness of the treated wastewater reuse such as irrigation type (surface irrigation or sprinkler irrigation), the nature of the soil to irrigate and the type of crop to be grown (FAO 2003).

Conclusion

The concern of this study is to determine the reusability of the treated wastewater for the irrigation of the Golf course by monitoring the physicochemical and bacteriological quality of the M'zar-treated wastewater and hence reused in the irrigation of green areas of the Ocean Golf course. The results of microbiological analysis confirm the presence of various germs such as faecal coliforms, faecal streptococci, and clostridia sulphito-reducers in appreciable numbers with an absence total of Salmonella and vibrio. The comparison of the physicochemical and microbiological quality of these waters with WHO and USEPA standards has shown the conformity of these waters to irrigate without major negative impacts on the environment. The salinity levels in the treated wastewater indicate high values, which should be considered for the selected species to be grown. This also indicates that the M'zar system based on sand infiltration–percolation and UV disinfection has a significantly higher treatment performance: the effluent produced contains very low suspended solids, bacteria and far less organic compounds.

Considering these results, we can conclude that using treated wastewater from the Agadir's M'zar Plant station

Table 5 Biological parameters of treated water compared to WHO (1989) and USEPA (USEPA 2004) standards

Settings	W1	W2	USEPA	WHO
CF	1.14 log 10 UFC/100 ml	1.23 log 10 UFC/100 ml	< 2.30 log 10 UFC/100 ml	3 log 10 UFC/100 ml
DBO5	11 mg/l	10 mg/l	< 30 mg/l	–
Ph	7.36	7.35	6–9	–

is safe but adequate safeguards draw attention to monitor salinity in the soil as the conductivity of the wastewater is over 3.5 dS/m.

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