

## Research Article

# Characterization of Industrial Dairy Wastewater and Contribution to Reuse in Cereals Culture: Study of Phytotoxic Effect

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## Abstract

The present work aims to study the impact of dairy effluents on seed germination and seedling growth of Wheat (*Triticum aestivum*) and Maize (*Zea mays*). Dairy effluents were collected from the inlet and outlet of waste water treatment plant of two dairy industries located in Tunisia in the areas of Sousse and Mahdia. Different physical and chemical parameters were evaluated for the assessment of effluents quality before and after treatment. Total suspended solids,  $BOD_5$ , COD, detergents and Oils and fats of both Mahdia and Sousse dairy effluents decreased after treatment and were within permissible limits. While in the case of total Kjeldahl Nitrogen and total phosphorus, the reduction remained above limits. The use of treated effluent from both dairy plants enhanced seed germination and seedling growth of wheat. However, results showed inhibitory effect from the same effluents on maize seed germination and growth of roots and shoots. Observation showed that untreated effluent from Sousse dairy plant had inhibited seed germination for both wheat and maize but induced seedling growth.

**Keywords:** Dairy wastewater; Wastewater valorization; Phytotoxicity; Germination

## Introduction

Water resources in the Mediterranean are limited and very poorly distributed in space and in time. However, the demand for water has increased during the second half of the 20<sup>th</sup> century. Industrial activities (such as textiles, chemicals, food...) consume a high quantity of water and generate wastewater that increases the risk of water shortage and pollution of water resources, surface water and groundwater. Among all of these industries, dairy industry is strategically located in the Tunisian food industry and affects the country's food security as it is an important economic niche (livestock, collection, processing, self-sufficiency...). However, this branch is characterized by a high consumption of water and generates, therefore, large quantities of wastewater discharge and sludge fat. This dreadful load is a source of environmental pollution, and an economic burden in relation to the treatment processes employed by producers.

Due to the increase in demand, water management involves the application of techniques that increase natural water supply, such as reused water. In recent years, the reuse of wastewater has been considerably developed. The current daily volume of used water reaches a staggering 1.5-1.7 million m<sup>3</sup> per day in several countries, such as the U.S (California and Florida), Mexico and China [1]. The treated wastewater can be considered a «new» water resource that can be added to the overall assessment of water resources of a region. Bixio and al (2005) have classified different types of reuse into 4 categories (i) agricultural use, (ii) urban and peri-urban use and replenishing the aquifer (iii) industrial use and (iv) mixed uses [2].

In fact, agriculture consumes over 70% of water resources in

developing countries such as the Arab countries (REJEB SALOUA, 2011). Indeed, the delivery of treated water to agricultural fields would decrease the negative impact caused by the use of clean water in irrigation [2]. In addition, the treated wastewater can balance the natural cycle of water and conserve resources by reducing harmful emissions into the environment [3]. Treated wastewater can sometimes be a superior source for agriculture than some fresh water sources. Nitrogen (N) and Phosphorus (P) in the wastewater may result in higher yields than freshwater irrigation, without additional fertilizer application [4].

According to the international standards, Tunisia is a country poor in water. With a level of renewable resources per capita per year not exceeding 450 m<sup>3</sup>/capita/year, water is a barrier to social and economic development in the country. Tunisia is characterized by an arid climate and scarce water resources (an annual average of 4.8 m<sup>3</sup> of surface water and groundwater). Much of this water is salt water since 28% of surface waters have salinity greater than 1.5 g/l and 47% of the groundwater has salinity than 3 g/l (REJEB SALOUA, 2011). To cope with this water deficit the use of wastewater for irrigation has become a necessary solution and an integral part of the country's water management strategy. According to these observations, we opted in this work to study a possibility of agricultural reuse of the untreated or treated wastewater collected from two dairy industries in Tunisia for irrigation. We started our work analysing the quality of water at the inlet and outlet of wastewater treatment plant of Mahdia and Sousse. Then we studied the effect of dairy effluents on the wheat and maize germination and growth.

**Table 1:** Characteristics of sewage effluents from two Tunisian dairy plants studied in 2015.

Constituent	Unit	Mahdia Dairy Plant		Sousse Dairy Plant		TN 106.02 (1989)
		Entry station	output station	Entry station	output station	
<b>Suspended solids</b>	mg/L	4.1*10 <sup>3</sup>	6.5	1.2*10 <sup>3</sup>	13	30
<b>DBO<sub>s</sub></b>	mgO <sub>2</sub> /L	3.37*10 <sup>3</sup>	4	2.58*10 <sup>3</sup>	9	30
<b>DCO</b>	mgO <sub>2</sub> /L	9.33*10 <sup>3</sup>	<30	4.05*10 <sup>3</sup>	69	90
<b>Kjeldahl Azote</b>	mgN/L	276	5.38	68.8	6.18	1
<b>Ammoniacal Azote</b>	mgN/L	38.2	<0.068	4.38	1.61	-
<b>Nitrite</b>	mgN/L	0.046	<0.005	0.257	0.017	0.5
<b>Nitrates</b>	mgN/L	<0.45	<0.45	136	10.2	50
<b>Total Phosphorus</b>	mgP/L	47.5	8.7	14.2	10.9	0.05
<b>Chloride</b>	mg/L	361	334	384	391	600
<b>Conductivity</b>	mS/m	398	378	No data	No data	-
<b>Salinity</b>	%	2	1.9	No data	No data	-
<b>pH</b>	-	8.3 at 15.6°C	8.6 at 15.9°C	>10	8.17±0.3	>6.5 and <8.5
<b>Detergents</b>	mgABS/L	2.76	0.15	No data	No data	0.5
<b>Oils and fats</b>	mg/L	600	<5	No data	No data	10

DBO<sub>s</sub>: Biological Oxygen Demand; DCO: Chemical Oxygen Demand; TN 106.02: Tunisian Norms for release into the public domain.

## Experimental Protocols

### Wastewater samples

Wastewater samples were collected from the inlet and outlet of wastewater treatment plants of two dairy industries located in Mahdia and Sousse: Sousse Dairy Plant "SDP" and Mahdia Dairy Plant "MDP" in February, March and April 2015.

### Physico-chemical analysis of wastewater samples

The samples were analysed for various physico-chemical characteristics. Turbidity and conductivity were determined using AQUALITIC® (Dortmund, Germany), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were determined according to the method of Hur, et al (2010).

### Seedling growth

Wheat (*T. aestivum L.*) and maize (*Zea mays L.*) seeds were provided by the National Institute of Agronomical Research (INRA), Tunisia.

Seeds were raised in a soil mix of 2/3 sand and 1/3 compost. Thirty seeds were sown in each batch at 25±°C. The seeds were irrigated with treated and untreated wastewater from each dairy plant considered in this study. Controls were irrigated with distilled water. A number of seed germination was counted after six days of incubation.

**Germination percentage:** The germination percentage is the proportion of germinated seeds in relation to the total number of viable seeds that were tested by following formula:

$$\%G = (\text{Number of germinated seeds} / \text{Total number of planted seeds}) \times 100$$

**Determination of elongation:** The elongations of roots and leaves were measured with a ruler. Calculations were performed based on leaf lengths and major axial root averages.

**Length Vigour Indices (VI):** The length VI of seedling was calculated as below.

The length VI=(Mean Root Length + Mean Shoot Length). Percentage Germination [5].

**Seedling Tolerance Index (TI):** The tolerance index of seedlings was calculated by the formula:

$$TI = \text{Mean length of longest root in treatment} / \text{Mean length of longest root in control} [6]$$

**Relative Toxicity (%RT):** The relative toxicity (%RT) of each industrial wastewater, before and after treatment on the seedling growth, was calculated to determine the degree of inhibition over the control using the following formula:

$$\%RT = (X - Y) / X \times 100 [7]$$

Where X refers to the seedling length in the control after six days of incubation and Y is the seedling length in the presence of each industrial effluent (before or after treatment) after six days of incubation.

## Results

### Physicochemical analysis of dairy wastewaters

The physicochemical parameters of effluent samples were given in (Table 1). In general the physicochemical parameters of the treated effluents samples from the output station of both dairy industries fall within permissible limits set by Tunisian norms for release into the public domain (TN.106.002) except for some parameters. Total Kjeldahl Azote and total phosphorus values of untreated wastewater for both SDP and MDP exceeded the permissible values. High levels of suspended solids (4100 mg/L), total Phosphorus (47.5 mgP/L), detergents (2.76 mgABS/L) and Oils and fats (600 mg/L) were recorded in untreated wastewater from MDP. Important values of BOD<sub>s</sub>, COD and Total Kjeldahl Nitrogen, which are considered as pollution indicators, were also recorded for untreated wastewater from MDP. These parameters were higher compared to those of untreated wastewater from SDP.

**Table 2:** Effect of two Tunisian dairy Wastewaters (ww) on seed germination and early growth of Wheat (*T. aestivum L.*).

Parameters	Control	Percentage change from control (%)			
		Treated ww		Untreated ww	
		Mahdia dairy plant	Sousse dairy plant	Mahdia dairy plant	Sousse dairy plant
Seed Germination	80%	0	25	25	-12.5
Root length	5.45±1.46 cm	16.69	29.17	62.56	70.27
Shoot length	3.25±1.22 cm	23.69	36.61	19.69	31.69
Dry weight	0.17±0.03 g	23.52	11.76	29.41	35.29

**Table 3:** Effect of two Tunisian dairy Wastewaters (ww) on seed germination and early growth of maize (*Zea mays L.*).

Parameters	Control	Percentage change from control (%)			
		Treated ww		Untreated ww	
		Mahdia dairy plant	Sousse dairy plant	Mahdia dairy plant	Sousse dairy plant
Seed Germination	70%	-14.28	0	-28.57	-14.28
Root length	2.78±0.53 cm	-79.85	-17.98	-46.76	11.51
Shoot length	1.95±0.17 cm	-73.84	-29.23	-78.46	11.79
Dry weight	0.3±0.01 g	-16.66	-23.33	-13.33	3.33

However, the values of nitrites, nitrates and chlorides in untreated MDP ww did not exceed permissible limits. Concerning untreated ww from SDP, we noted high level of nitrates (136 mgN/L) which exceeded the value recorded for untreated MDP ww (<0.45 mgN/L). It was observed that pH value of untreated ww from SDP (>10) exceeded the permissible limits (>6.5 and <8.5) nonetheless, pH of untreated ww from MDP (8.3) was in the norms.

#### Reuse of dairy wastewaters for seed irrigation

The use of treated effluent from both dairy plants enhanced seed germination and seedling growth of wheat. Yet, results showed inhibitory effect from the same effluents on maize seed germination and growth of roots and shoots. It was observed that untreated effluent from Sousse dairy plant had inhibited seed germination for both wheat and maize but induced seedling growth.

Laboratory experiments on wheat showed inducer effect of ww on seed germination, root length, shoot length and dry weight for both dairies, except of untreated ww from (SDP) which has a low Relative Toxicity (RT) (12.5%) on seed germination (Table 2). A high level of root length induction, as compared to control, was marked in the case of irrigation with untreated ww from SDP and Mahdia Dairy Plant (MDP) (70.27% and 62.56% respectively). By irrigating with treated ww, inductions were only 29.17% (SDP) and 16.69% (MDP) (Table 2). VI (Figure 1) of seedlings in untreated and treated ww of both dairies was higher than control VI (764). Maximum VI (Figure 1) and TI (Figure 3) of seedlings were recorded in untreated ww of MDP (1275 and 1.65 respectively). These indices decreased to 830.4 and 1.54 in treated ww (Figures 1-4). While, VI of seedlings in ww of SDP was lower in untreated ww (949.2) and it increases to 1148 in treated ww. TI (Figure 3) was 1.45 in untreated ww and decreases to 1.07 in treated ww (Figure 1).

However, in maize an inhibitor effect of MDP ww was observed on seed germination, root length, shoot length and dry weight. Treated ww have a high RT on root length (79.85%) and Shoot length (73.84%). RT was lower on seed germination (14.28%) and dry weight (16.66%) (Table 3). Untreated ww has also significant inhibitor

effect on shoot length (78.46% RT). On SDP the inhibitory effect is less attenuated. The higher RT (29.23%) was recorded for treated ww on shoot length. On seed germination the RT was null (Table 3). An enhancement of untreated ww was recorded on root length (11.51%), shoot length (11.79%) and dry weight (3.33%) (Table 3). Untreated and treated ww from MDP, showed a low VI (95 and 64.2 respectively) compared to control (331.1) and to SDP (316.8 and 256.2 respectively) (Figure 2). TI of maize was low if irrigated with untreated and treated ww from MDP (0.6 and 0.26 respectively), compared to SDP ww (1.03 and 0.88 respectively).

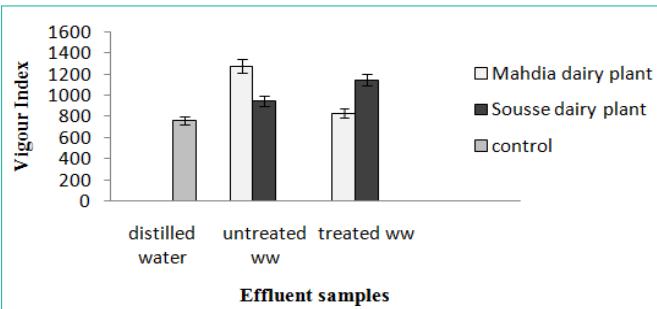
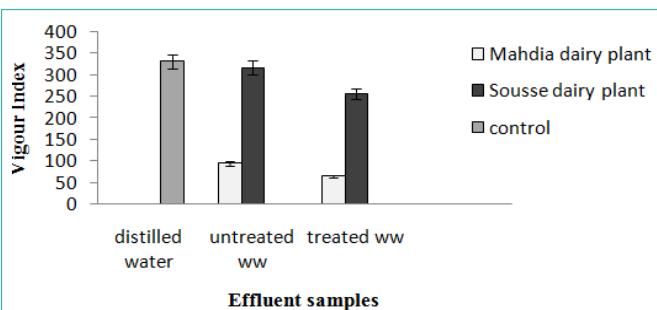


Figure 1: Effect of wastewaters on length vigour index of wheat.

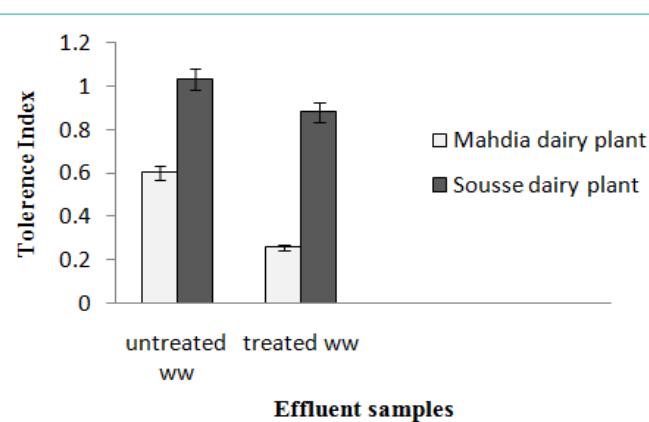


Notes: **ww: wastewater**

Figure 2: Effect of dairy wastewaters on length vigour index of maize.



**Figure 3:** Effect of dairy wastewaters on tolerance index of wheat.



**Figure 4:** Effect of dairy wastewaters on tolerance index of maize.

## Discussion

Untreated ww, from both studied dairies, contains high rates of various micro nutrients (N, P...), essential for plant growth (Table 1), hence their positive effect on wheat VI and TI. However, it cannot be exploited for agricultural irrigation because of their impacts on crops and soil. Indeed, excess N has the potential to cause excessive vegetative growth, delayed growing season and maturity and consequently economic loss to farmer. Furthermore, high DBO and DCO values with important levels of suspended solids lead to the development of septic conditions, humus build-up and the development of sludge deposits causing anaerobic conditions [8]. The low amount of oxygen in dissolved form reduces the energy supply through anaerobic respiration causing retardation of growth and development of seedling [9].

In this study, dairy plants used three levels of treatment including physical and biological operations which allow removal of organic and inorganic solids, greases and oils and convert of the organic content of the effluent to more stable forms. These processes were resulting in ww with acceptable levels of DBO<sub>5</sub> and DCO, permissible pH value and low nitrite concentrations. These values are encouraging for possible reuse of treated ww studied, in agricultural irrigation with more attention to the amount of nitrogen which slightly exceeds the permissible values. It was reported that excess nitrogen will leach and contaminate the groundwater resources [8]. Though, plants can control the N loss in the soil-plant system by minimizing N leaching [10].

In this context, we suggest a possible reuse of treated ww studied in irrigation of crops with high water and N demands on heavy textured soils (plants and soil will play the role of natural filters of nitrogen). The choice of crops for treated ww irrigation was found to be the principal factor for the sustainability of effluent irrigation [11].

In this work, we studied the effect of dairy ww on germination and early growth of two cereals (wheat and maize). Treated ww from both dairies studied had positive effect on germination and growth of wheat, in laboratory conditions. Indeed, these effluents were a rich source of wheat nutrients such as N and P. The nitrogen is involved in the synthesis of amino acids. It is an important factor of yield by promoting the growth of plant tissue [12]. Phosphorus is a cellular component and an energy carrier [13]. It participates with the nitrogen to plant growth, especially root growth. It also promotes flowering, fruit set and seed formation. Untreated ww from MDP had greater positive effect on wheat VI and TI than SDP ww.

Furthermore, it promoted considerably root length. This is due to their composition encompassing higher level on ammoniacal nitrogen (38.2 mgN/L). However, treated ww had toxic impact on maize germination and growth. Maize (a C4 plant) is more responsive to N than C3 species [14,15]. Indeed, treated ww continuous nitrogen in this study was not enough for the needs of maize. Treated ww from MDP had the greater RT on both maize germination and growth. In fact, MDP treated ww is deficient in nitrate compared to SDP ww. It has been reported that deficiency of nitrate in agricultural soils leads to growth retardation and yield losses [16].

## Conclusion

The impact of irrigating with dairy treated ww on germination and growth varies from crop to crop and also from species to species. In this study, dairy ww were suitable only for wheat irrigation. It may act as a supplemental source of fertilizer thus increasing crop germination and growth, delivering savings in chemical fertilizer cost and reducing pollution risks to the ground water. This study represents a strong argument for possible reuse of treated dairy ww in wheat irrigation.

It suggests further field trial, impact of long-term irrigation on soil physicochemical properties and further experiments on suitable plant species used for dairy effluent irrigation.

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