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Impact of irrigation with water from the Seybouse Wadi on the biochemical quality of flat-leaf parsley

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ABSTRACT

The Seybouse Wadi is one of the most important water courses in Algeria, extending over a length of 225 km and irrigating some of the most fertile land. This wadi supplies drinking water to more than 1.5 million people (66% of the wadi water), water for industry (8%, supplied to more than 71 factories) and irrigation water (26%). Its vast drainage basin (6500 km²) is the site of major human activities (including chemical and heavy industry, agricultural and household activities). A multitude of by-products of these activities are rejected into the wadi, without pretreatment. Most of this waste material is of agricultural and domestic origin and is highly charged in nutrient salts and organic matter. The aim of this study was to carry out a physicochemical characterisation of the waters of the Seybouse Wadi, to evaluate their impact on the environment. The analyses focused on the variables likely to characterise these waters: suspended matter, BOD_5 (biological oxygen demand over five days), dissolved oxygen content, COD (chemical oxygen demand), pH, temperature, electrical conductivity and nitrate content. We found that the wastewater was highly contaminated, and that the use of this water for irrigation might have environmental repercussions.

Keywords: Seybouse Wadi, wastewater, physicochemistry, irrigation.

INTRODUCTION

Water is the most important raw material on the planet, for humans, animals, plants and micro-organisms. Almost all the vital phenomena of the biosphere are linked to the availability of water. Water thus not only provides us with unique and essential spaces, a means of transport and a vector of energy: it is also an essential component of all types of production [1].

Development, both past and present, the recommendations of the WHO (which highlights the need for universal access to water) and the effects of climate change have made water a rare and precious commodity. Algeria is a large developing country with natural resources in the form of green forests, a 1200 km-long coast in the north, and vast expanses of the Sahara desert in the south. It also has with spa towns, historical and archaeological sites, inhabited natural parks and reserves and extensive water supplies capable of meeting the needs of the inhabitants, agriculture and industry [2].

Most of the water resources come from wadis, lakes, springs and underground watercourses. The River Seybouse is one of the most important water resources in Algeria: it is the third largest river in the country in terms of its volumetric flow rate, and has abundant plant and animal species. The Seybouse wadi is also considered a vital source of water in North-East Algeria, but is faced with severe pollution problems, which have been confirmed by scientific studies and cannot be ignored; these problems are the result of the large amounts of urban and industrial waste released into this wadi on a daily basis [2].

The waters of the Seybouse wadi are polluted with solid and liquid industrial waste, and this pollution poses a threat to agricultural crops. In particular, polluted water from the drainage basin is used to irrigate agricultural land during the spring, summer and autumn, creating dangers to human health and the environment [3].

Flat-leaf parsley (*Petroselium crispum*) is a culinary and medicinal herb. It has drying, attenuating, appetite-stimulating, cleaning, diuretic and hepatic properties. As a medicinal herb, it is used principally for the treatment of obstructions of the lungs, liver, spleen, kidneys and bladder, jaundice, cachexia, large and small stones, and oliguria. Parsley leaves reduce inflammation and facilitate wound healing and are thus applied to wounds of various depths and to bruises, after crushing between the fingers. They are also applied to the breasts to release the milk of women who have recently given birth, and are used to resolve hot swellings and bruising of the eyes, in particular. Fresh leaves spread on the water of ponds or fountains are also rumoured to be beneficial to sick fish.

Numerous studies across the world have shown an impact of wastewater on agriculture [4, 5].

The waters of the various wadis in Algeria are used for irrigation, potentially leading to the penetration of pollutants into the water table, at various levels.

We report here a physicochemical analysis of the waters of the Seybouse wadi in the Annaba region, with a view to determining the extent of pollution and its impact on the biochemical quality of a medicinal plant (*Petroselinum crispum*, flat parsley) following irrigation.

EXPERIMENTAL SECTION

Study environment

The Seybouse drainage basin is part of the north-western Tell region of Algeria. This region extends to the region of Constantine in the west and the border between Algeria and Tunisia in the east. The western Tell region consists of a chain of mountains.

The Seybouse drainage basin is located in the north east of Algeria and covers a total area of 6862.39 km², corresponding to about 0.288% of the total area of Algeria. It extends from 6°48' W to 7°59' E and from 35°53' S to 36°57' N

The Seybouse drainage basin includes seven eastern Algerian provinces (wilayahs): the whole of Guelma and parts of Annaba, El-Tarf, Oum El-Baouaghi, Skikda, Souk-Ahras and Constantine. It covers 68 settlements, 30 of which are entirely encompassed by the drainage basin, and includes an estimated population of more than 1,258,710 inhabitants [6].

The sites sampled

The three sites from which wastewater samples were collected are located in the settlement of Sidi Salem. We chose to take samples from the maritime (or lower) part of the Seybouse wadi, close to the mouth of the river. The sampling stations were located more than 500 m from the sea, at the Sidi Salem road bridge on route 44.

At its mouth, the Seybouse wadi discharges large amounts of toxic waste into the sea. More than 4.5 million cubic metres of wastewater contaminated with diverse chemical products, including industrial oils, are discharged daily into this wadi[7].

Samples were taken with wide-necked bottles, in rigorously aseptic conditions, to prevent any accidental contamination during manipulation. The glass flasks designed for water sampling were first washed and carefully rinsed, to eliminate all traces of detergent, then in the time of sampling, the flask was first rinsed with the water to be analyzed, then a sample was collected manually at the surface, from a zone agitated by the current of effluent, in which there was little risk of sedimentation; finally, once completely filled, the flasks were hermetically sealed and covered with aluminium foil, to provide a double protection against possible contamination.

Industrial wastewater is rapidly modified by the diverse micro-organisms it contains. We minimised the likelihood of changes between sampling and analysis by carefully labelling the flasks, storing them at a temperature of 4°C and transporting them to the laboratory for analysis on the same day.

Irrigation with water from the Seybouse wadi

Plant material

Samples were irrigated with water from the Seybouse wadi, and are referred to as 'treated samples'. They were compared with control samples irrigated with tap water.

The trial

The experiment was carried out on two plots, each with an area of 100 m². Each plot was divided into six subplots. The six treated subplots were irrigated with water from the Seybouse wadi. The other (control) subplots were irrigated with tap water.

Plant sampling

Samples were collected from plants at least 30 days after sowing, at two developmental stages: the two-leaf stage and the seven- to eight-leaf stage. The samples collected were placed in sterile plastic bags, numbered and stored in a cool box, at 4°C, until transport and analysis.

Statistical analysis

We obtained descriptive statistics for each parameter of the biochemical metabolites studied.

Descriptive statistics

We used Statistica version 8 software to determine the various statistics considered, including the mean, minimum, maximum and standard deviation [8].

Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is generally the best type of analysis for comparing data of this type. It tests the null hypothesis that the means of several samples are equal (H_0: $\mu_1=\mu_2=\ldots\mu_n$). In other words, it assesses the homogeneity of the samples, based on their mean values. ANOVA can be used to assess the effects of one or several factors on the data studied and to compare test samples with a control. Its use is subject to several conditions: the samples must be random and independent, the data must be normally distributed and the variances must be equal.

In practical terms, ANOVA is used to determine whether the variability observed in the data is due solely to chance or whether there are significant differences between samples. Significant differences between treatments are identified by repeated-measures ANOVA, which can be performed with statistical software, such as Minitab version 14 [8].

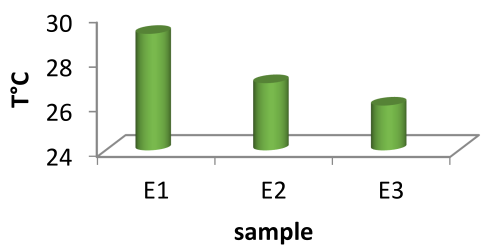
RESULTS AND DISCUSSION

Analyses of physicochemical parameters

Temperature (T)

During the study period, the waters of the Seybouse wadi had a mean temperature of 27.4°C, a maximum temperature of 29.2°C and a minimum temperature of 26°C. These values correspond to the summer period and are entirely within the normal range for this season. The air temperatures measured at the Seybouse wadi were close to the usual temperature of 30°C this for this season as shown inFigure1.

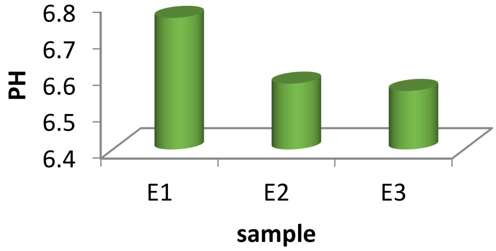
Figure 1. Temperature of the waters of the Seybouse wadi



Hydrogen Potential (pH)

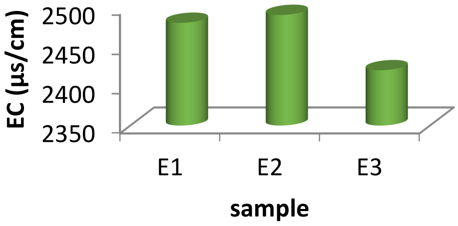
As shown in Figure 2,the mean pH values for the three sampling sites for the Seybouse wadi ranged from 6.56 to 6.76. The pH values of water discharged by the river were thus within the international norms for irrigation water (pH 6.5-8.5).

Figure 2. pH of the waters of the Seybouse wadi



Electrical conductivity (EC)
The mean electrical conductivity (EC) of the water tested for the three sampling sites ranged between 2420 and 2490 µS/cm as shown in Figure3. The EC of the river water at these sites was higher than the French norm of 1000 µS/cm. According to Gaujous[9], high EC values reflect high levels of mineralisation and an abundance of salts.

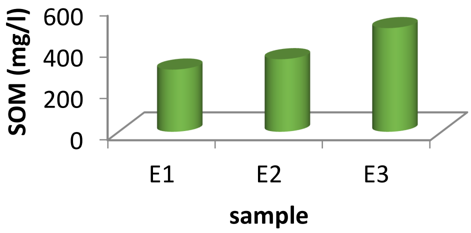
Figure 3. EC values of the sampled water



Suspended organic matter (SOM)
As shown in Figure 4, the SOM levels recorded for the samples tested ranged from 300 to 500 mg/l. These levels are very much higher than international norms, which are fixed at 60 mg/l.

The SOM content reflects the various sources of the wadi and the industrial activities taking place during the course of the study.

Figure 4. SOM content of the river water tested



Biological oxygen demand (BOD_5)
The principal parameters of quality routinely studied to obtain an indirect estimate of the total organic load of water samples are biological oxygen demand for a five-day period (BOD_5), which provides an assessment of the amount of biodegradable organic matter present in the water, and chemical oxygen demand (COD).

As shown in Figure 5, the mean BOD_5 values were 39 mg/l for site 1, 41 mg/l for site 2 and 42 mg/l for site 3.

Chemical oxygen demand (COD)
Chemical oxygen demand is the amount of oxygen consumed by the matter in the water that can be chemically oxidised. It reflects the content of the water in terms of most organic compounds and oxidisable mineral salts (sulphides, chlorides, etc.).

The mean COD values obtained at the three sites were between 148 mg/l and 150 mg/l (Figure 6). These values are higher than the international norm, which is 100 mg/l. This high COD may be accounted for by the oxidisation of industrial residues, which contain various inorganic compounds.

Figure 5. BOD_5 values for the waters of the wadi

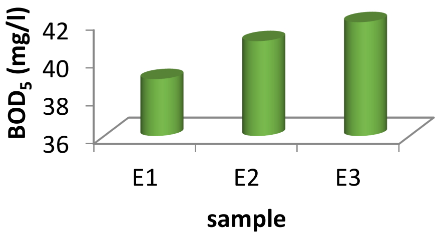
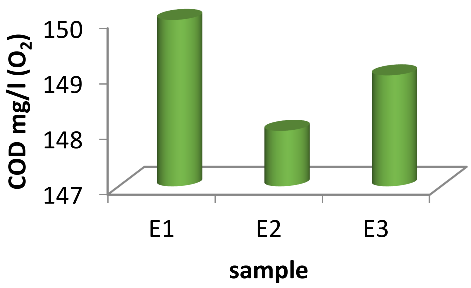


Figure 6.COD values for the water samples studied



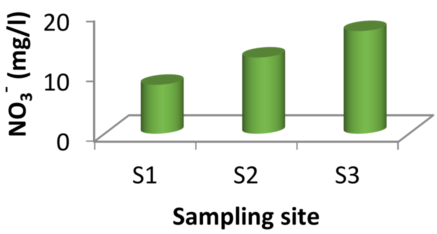
Nitrate (NO_3^-)

Ammonia is unstable and is oxidised to nitrites and then to nitrates unless oxidants are limiting, in which case ammonia accumulation may be observed.

The mean nitrate concentrations recorded at the three sites were from 8.126 to 17 mg/l as shown in Figure 7.

The nitrate contents of the samples tested were below the international norm, which is 25-50 mg/l, possibly due to dilution with other sources of water, such as runoff and washing water [10].

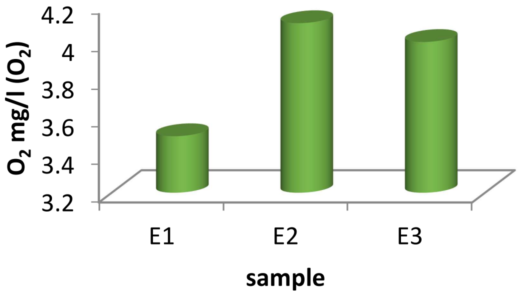
Figure 7. Nitrate levels in the water samples studied



Dissolved oxygen (O_2)

Dissolved oxygen (O_2) is very important because it may modify the state of several mineral salts and condition the degradation of organic matter and the life of aquatic animals. The dissolved oxygen content of natural waters is determined principally by the respiration of living organisms, photosynthetic activity, the oxidation and degradation of pollutants and exchanges between the air and the water. Dissolved oxygen levels varied considerably between the sites: taking into account all the samples collected, the values ranged between 3.5 and 4.1 mg/l. These values indicate that the waters of the wadi are slightly under oxygenated (Figure 8). The dissolved oxygen concentration is an important indicator that provides information about the state of the wadi and it favours the growth of the micro-organisms responsible for breaking down organic matter. In general, low dissolved oxygen concentrations favour the development of pathogenic microbes. In all cases, the oxygen contents observed may decrease further once the degradation of organic matter and the multiplication of aerobic micro-organisms have been primed.

Figure 8. Dissolved oxygen concentrations in the waters of the Seybouse



Statistical analysis

Descriptive statistics

We calculated the following elementary statistics for each of the variables studied, for each stage of development: arithmetic mean (M), standard deviation (SD), maximum (Max) and minimum (Min). The results of this statistical description are presented in table 1. Mean chlorophyll a concentrations, for both developmental stages, were between 8.95 and 10.26 µg/g FW. Mean values for chlorophyll b concentration were between 3.19 and 3.73 µg/g FW. For chlorophylls a and b, mean concentrations were between 16.14 and 52.52 µg/g FW. However, total carbohydrate concentrations were of the order of 143.05 to 484.05 µg/g FW.

Table 1. Statistical description of the biochemical species determined for each stage of development

Stage	*N*	Mean	Min	Max	Standard deviation (SD)
Stage 1 Chlorophyll a	6	8.95500	6.170000	12.99000	2.427408
		10.26000	6.900000	15.50000	3.862999
Stage 2 Chlorophyll a	6	24.97833	23.65000	25.99000	0.794768
		25.28500	23.60000	26.80000	1.179318
Stage 1 Chlorophyll b	6	3.186667	1.780000	4.700000	1.312428
		3.733333	1.350000	6.460000	2.008160
Stage 2 Chlorophyll b	6	27.02167	23.45000	30.32000	2.701358
		28.61500	23.60000	32.33000	3.132620
Stage 1 Chlorophylls a and b	6	13.62000	10.52000	17.38000	2.685018
		16.14167	11.37000	21.97000	4.380427
Stage 2 Chlorophylls a and b	6	52.25500	48.91000	56.96000	3.206847
		53.89000	49.24000	56.07000	2.748440
Stage 1 Total carbohydrates	6	56.8850	50.0000	69.6600	7.17797
		143.0533	115.6600	169.0000	22.06053
Stage 2 Total carbohydrates	6	484.0517	308.6600	610.0000	109.3924
		541.9700	393.5000	649.6600	107.5785
Stage 1 Total protein	6	30.34833	26.31000	33.55000	3.161369
		35.58833	30.27000	40.12000	3.705560
Stage 2 Total protein	6	23.16667	13.03000	30.96000	6.546170
		23.10000	14.93000	31.58000	6.592053
Stage 1 Proline	6	3.670000	1.510000	5.870000	1.966652
		4.643333	2.500000	7.060000	2.134054
Stage 2 Proline	6	7.38167	5.32000	9.33000	1.459197
		13.04333	10.46000	17.72000	2.769597

Analysis of variance

The mean chlorophyll a concentration in the control plants was 4.70 µg/g FW at stage 1 and 25.9 µg/g FW at stage 2. The leaves of plants treated or irrigated with water from the Seybouse wadi contained slightly more chlorophyll a (26.80 µg/g FW). Chlorophyll b concentrations were also higher for treated than for control plants, and similar results were obtained when chlorophylls a and b were considered together as shown in table 2. The higher concentrations of chlorophyll may reflect the leaves being a site not only of photosynthesis, but also of other types of metabolism. According to Rawls [11] high chlorophyll concentrations may reflect high concentrations of metallic elements in polluted water, resulting in a stimulation of photosynthesis.

Table 2. The effect of chlorophyll on the biochemical quality of the plant

Chlorophyll a concentration at stage 1						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	5.4	5.4			
Error	10	103.0	10.3	0.52	0.487	NS
Total	11	108.4				
Chlorophyll a concentration at stage 2						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	3.9	3.9			
Error	22	2030.3	92.3	0.04	0.839	NS
Total	23	2034.2				
Chlorophyll b concentration at stage 1						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	5	5			
Error	34	3427	101	0.05	0.831	NS
Total	35	3432				
Chlorophyll b concentration at stage 2						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	11	11			
Error	46	5716	124	008	0.772	NS
Total	47	5727				
Chlorophylls a and b, concentration at stage 1						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	24	24			
Error	58	5868	101			NS
Total	59	5892		0.23	0.631	
Chlorophylls a and b, concentration at stage 2						
Source	**DL**	**SC**	**CM**	**F**	**P**	**Significance**
Factor	1	31	31			
Error	70	19816	283	0.11	0.741	NS
Total	71	19847				

As shown in table 3, total carbohydrate concentrations were also higher in treated than in control plants. Indeed, carbohydrates are synthesized by photosynthesis, which involves the electron-trapping molecules chlorophyll a, chlorophyll b and carotenoids, which capture the energy of light, transforming it into chemical energy, which is then used for the synthesis of organic molecules.

Table 3. The effect of carbohydrate on the biochemical quality of the plant

Total carbohydrate concentration at stage 1						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	3296	3296			
Error	82	94252	1149	2.87	0.094	NS
Total	83	97549				
Total carbohydrate concentration at stage 2						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	2339	2339			
Error	94	2227744	23699	0.1	0.754	NS
Total	95	2230083				

Protein levels were lower at stage 2 than at stage 1. Mean protein concentrations were 35.19 µg/g FW for the treated plants and 29.93 µg/g FW for the control plants at the first stage of development as shown in table 4, but the opposite pattern was observed at the second stage of development considered. Our results for protein concentration are consistent with those of Amerhein [12], who reported that wastewater may contain compounds that inhibit shikimic acid, leading to a blockade of protein synthesis and a decrease in the amount of carbon entering the shikimic acid pathway.

The statistical analysis confirmed that there was a significant difference in protein concentration between the control and treated plants, at both stages of plant development considered.

Table 4. The effect of protein on the biochemical quality of the plant

Protein concentration at stage 1						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	848.6	848.6	10.69	0.003	*
Error	28	2223.1	79.4			
Total	29	3071.7				
Protein concentration at stage 2						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	846.9	846.9	11.77	0.002	*
Error	34	2446.9	72.0			
Total	35	3293.8				

The proline concentrations recorded were lower in control plants than in treated plants as shown in table 5. Proline is an amino acid that accumulates in plants in which the metabolic balance has been disturbed by unfavourable environmental conditions. The proline concentration may differ between plants and between biotopes.

As reported for other compounds, such as glutathione and phytol, the proline concentration may change if the plant is subjected to stress (climatic factors, pollution). Indeed, for this and other reasons, proline is a good bioindicator of pollution.

Table 5. The effect of proline on the biochemical quality of the plant

Proline concentration at stage 1						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	0.67	0.67	0.12	0.734	NS
Error	10	55.01	5.50			
Total	11	55.68				
Proline concentration at stage 2						
Source	**DL**	**SC**	**CM**	***F***	***P***	**Significance**
Factor	1	96.16	96.16	19.63	0.001	*
Error	10	49.00	4.90			
Total	11	145.16				

An ANOVA for two controlled factors revealed a significant treatment effect for only two biochemical metabolites protein and proline.

CONCLUSION

The results of this analysis of the physicochemical properties of the waters of the Seybouse wadi in the Annaba region of eastern Algeria indicate that these waters contain large amounts of organic matter and chemical compounds, some exceeding the reference thresholds. The pH and temperature values recorded were within acceptable norms, whereas dissolved oxygen levels were lower than the accepted norms. Biodegradable organic matter content, as evaluated by calculating BOD_5 and COD, was variable and slightly exceeded the recommended thresholds. This evaluation of the degree of physicochemical pollution indicates that, for the parameters measured (particularly BOD_5, COD and SOM), the waters of the Seybouse wadi can be classified in the low to medium concentration category, as defined by Metcalf & Eddy [13].

We observed higher levels of total chlorophyll, soluble carbohydrates and proline in the leaves of the plants irrigated with river water than in the control plants, at both stages of development. Our results indicate that the plants studied may be considered to have developed a biochemical mechanism of adaptation to irrigation with polluted water.

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