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Assessment of Hygienic Level of Alfalfa and Wheat Plants Irrigated with Wastewater Amended with Algae and Cyanobacteria at Taif Desert Soil.

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ABSTRACT

This study was carried out to investigate the effect of different waters; tap water (control), sewage effluent (100 % & 50 %), sewage effluent with *Scenedesmus quadricauda* and sewage effluent with *Nostoc sp.* on plant growth, leaf chlorophyll and Assessment of hygienic (fungi) of wheat and alfalfa plants. The obtained results showed that plant height, herb weight (fresh and dry), total chlorophyll content of both plants were inhibited at sewage effluent while enhanced in *S. obliquus* or *Nostoc sp*. enriched water. Exceptionally, dry weight of wheat was not affected at the same trend. It exhibited its highest enhancement at tap water/sewage mixture despite the enhancement of algae-amended waters. Also, sewage effluent was not obviously inhibitory. The algal cells may sorb (absorb or adsorb) water contaminants that might inhibit plant growth. Algae, in addition, most probably killed fungal cells to invade plant stems, which have been found in control and sewage effluent.

Keywords: Wheat, alfalfa, wastewater, *Scenedesmus quadricauda*, *Nostoc sp*., Taif soil, hygienic level

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INTRODUCTION

Wastewater is that water used by either residential or nonresidential communities. About 99 % - 99.94 % of wastewater is water, and the rest is solid waste [1, 2]. Unless properly treated, wastewater can harm public health and the environment. However, it is defined as liquid wastes normally collected in a sewer system and processed in a treatment plant. Reuse of waste water is a must in arid areas including the KSA, as the country land is almost desert with all known drawbacks of desert (aridity, poor precipitation, high permeability, and high temperature). Minerals contained in sewage water and sludge might play a vital role in redistributing mineral elements, thus compensating for depletion by repeated cultivation [3, 4, 5, 6, 7]. The scarcity of water resources is expected to become more severe in the coming years, primarily because of the growing consumption of water and because of climate change [8]. Therefore, the increasing demand for clean (or at least safe) water sets the necessity of appropriate technology and management of the available and already endangered water resources. Industrial and domestic wastewater reuse is now considered a basic component of integration water management, especially in countries in the arid and semiarid regions of the world [9], like most Arab countries. In addition, estimates suggest about 1000 km^2 is lost annually due to desertification in Africa alone [10]. [11] El-Zohri et al (2014) reviewed the use of sewage in agriculture and related activities. The application of sewage (sludge and effluents) has been long practiced by many communities for fertilization and watering [12, 13, 14, 15]. Population migration to marginal lands of arid and semi-arid areas increases the problems of water shortage and worsens the situation of land degradation in the destination, and in turn causes severe problems of poverty, social instability and population health threats [16, 17].

Algae, performing oxygenic photosynthesis, play a remarkable role in the treatment of municipal wastewater. This is universal and may be much more efficient in tropical regions where temperature is warm and sunlight is optimum. Algae has indispensable role in oxygen/carbon dioxide recycling with a net sequestration of CO_2 (greenhouse gas). Algae have also a potential for nitrogen and phosphorous uptake into the cells; thus acting as fertilizers by mineral recycling. Simultaneously, they accumulate heavy metals. Moreover, algae also remove pathogens from the domestic wastewater [18, 19, 20]. Sorption of heavy metals on phytoplankton cell surfaces depends on a number of factors ranging from the concentration of inorganic ions, dissolved organic matter, pH and the nature of particulates [21, 22, 23]. In the case of KSA (Taif region is a model) algal biomass can be utilized in improving the water holding capacity, one of the major problems in sand soils. It was concluded that algae based treatment systems can significantly reduce the ecological footprint [24]. The high nutrient levels in sewage act as fertilizers and cause the number of algae to bloom. Algal blooms are rapid increases in the population of phytoplankton algae that serve as an important food source to other organisms. Oxidation ponds do not require any special skill or expertise for handling; so they are easy in operation and management than the advanced mechanized treatment facilities. Treatment of wastewater in oxidation ponds in the presence of algae is an efficient and economical way of treating wastewater. Oxidation ponds require no external source of energy i.e. aerators, pumps; rather they utilize natural source of light (the sun). Produced oxygen by algae during photosynthesis reduces the growth of fungi and bacteria; algae are thus economically feasible for treating wastewater from several aspects.

This work aims to utilize the available amount of sewage effluent in Taif Governorate to cultivate some plants; namely alfalfa and wheat in the desert sand soil around the city. Sewage effluent pre and post treatments by algae and cyanobacteria, in addition to being primarily a source of water, is a nutrient supplement that may replace fertilizers application in such poor sand soil, at least in part. Plants growth was assessed in terms of seed germinations, plant height, fresh weight, dry weight, and chlorophyll content. A novel work in the field is the assessment of the hygienic level of the plants after being irrigated for two months with sewage water. This hygienic level for man and animals were assessed by investigating endophytic fungi inhabiting plant tissues.

MATERIALS AND METHODS

Analysis of sewage effluent

Chemical composition, pH, BOD (biological oxygen demand), COD (chemical oxygen demand) and mineral elements (Na, Ca, K, Cl, S, N, P and organic carbon) before and after addition of algae were estimated. BOD test procedure is based on the activities of bacteria and other aerobic microorganisms (microbes), which feed on organic matter in presence of oxygen. The amount of a BOD test indicates the amount of water-dissolved oxygen consumed by microbes incubated in darkness for five days at an ambient temperature of 20 °C. Higher the BOD, higher the amount of pollution in the test sample. For the contaminants that cannot be oxidized biologically, chemical oxygen demand (COD) method is used [25]. Mineral elements were measured after filtration according the methods adopted by [26].

Soil analysis

Soil characteristics (soil texture, Na, Ca, K, Cl, S, N, P and organic carbon) and field capacity were estimated before cultivation and after plant harvesting using soil extract [26].

Purification, identification and maintenance of cyanobacteria and algae from sewage effluent

The cultivation and isolation of the cyanobacteria and algae that might be persisting in the form of spores, hormogonia, akinetes or any other perennating stages is carried out using the moist plate method recommended by [27]. Five replicate Petri-dishes (9 cm diameter) were inoculated, each with 1 ml of waste water and 20 ml of the molten medium (45°C) were added. Petri-dishes were incubated at 30 ± 1°C for blue-green algae, incubated on 16/8 hr. Light-dark cycle with a light intensity of 3000-4000 Lux; colonies are counted after about 25 day of incubation. Purified colonies or filaments of cyanobacteria and algae are picked and transferred to plates of BG-12 agar [22]. Cyanobacterial and algal species are examined by means of a binocular microscope and identified according to [28, 29]. Algae and cyanobacteria were isolated from wastewater collected from Taif sewage water treatment plant were inoculated to sewage effluent, let grown and enrich for 24 hrs.

Seeds germination

Seeds of wheat (*Triticum aestivum* L.) and alfalfa *(Medicago sativa* L.) after surface sterilizations by sodium hypochlorite were sown directly in plastic Petri dishes containing the treatments shown below. The percentage of seed germination was calculated after four and eight days.

Plant material

A pot experiment was conducted at Biology Department - Faculty of Science - Taif University - Saudi Arabia during 2015 season to investigate the effect of sewage effluent on growth and total chlorophyll of wheat and alfalfa plants. Seeds of wheat and alfalfa were used in this experiment and sown directly in plastic pots (15 cm) containing desert sand soil from Taif Governorate and irrigated with the treatments below.

Irrigation regime

The experimental pots of wheat and alfalfa plants were irrigated up to the field capacity twice a week with the following irrigation regimes:

- Tap water (TW, control)
- Sewage effluent (SE 100 %)
- Tap water + sewage effluent (1/1) (SE 50 % + TW 50 %)
- Sewage effluent enriched with the green alga *Scenedesmus quadricauda* (SE 100 % + X)
- Sewage effluent enriched with the cyanophyte *Nostoc sp.* (SE 100 % + Y)

Growth characters

The growth characters taken in this experiment were plant height (cm), fresh and dry weights/plant (g/plant), representing the mean total biomass of single plant. Fresh and dry weights of wheat and alfalfa plants were determined at the end of experiment.

Chlorophyll determination

The total leaf chlorophyll was determined using chlorophyll meter (SPAD-502, Minolta Co. Japan) and represented by SPAD value. Chlorophyll content was measured at 60 days old plants from the beginning of the experiment.

Assessment of hygienic level (fungal Occurrence)

Stems were sectioned 1 cm above ground level and surface sterilized using 5 % borax. After rinsing with sterilized distilled water, sections were placed above sterilized potato agar medium in sterilized Petri dishes. After incubation for 7 days, the visually grown fungi were examined microscopically and identified.

RESULTS AND DISCUSSION

The physicochemical parameters of the wastewater of Taif Treatment Stationary such as TDS (Total Dissolved Solids) , turbidity, electrical conductivity, COD, BOD, nitrate as well as phosphate were sharply reduced in the treated water by the green alga *Scenedesmus* or the cyanobacterium *Nostoc*. Among that, 50 % of TDS were removed in the sample treated by *Scenedesmus* followed by only 27 % in the sample treated by *Nostoc*. The electrical conductivity and nitrate were drastically reduced when compared with untreated water sample up to 70 % (Table 1). The domestic waste water treatment using *Scenedesmus* or *Nostoc* has shown potential results in the reduction of sodium, potassium, COD and BOD. Algae has indispensable role in oxygen/carbon dioxide recycling with a net sequestration of CO_2. Algae have also a potential for nitrogen and phosphorous uptake into the cells; thus acting as fertilizers by mineral recycling [18, 19, 20, 22, 23, 30, 31. The advantages of algae based treatment include: cost effective treatment, low energy requirement, reduction in sludge formation as well as production of algal biomass [21, 31].

Table 1: Analysis of different Physico-chemical parameters from sewage effluent pre- and post-treated by *Scendesmus obliquus* or *Nostoc sp.*

Analysis parameters	Pre-treatment	Post-treatment with *Scenedsmus*	Post-treatment with *Nostoc*
Physical Examination			
Appearance	Turbid	Turbid	Turbid
Colour (pt.co-scale)	Black	Green	Green
Odour	Foul smell	None	None
Turbidity NT Units	98.2	30	73.2
Electrical Conductivity Micro mho/cm	5300	2240	2200
Total Dissolved Solids mg.L^{-1}	3710	1568	1140
Chemical Examination			
pH	8.52	7.28	7.23
Total Alkalinity as $CaCO_3$ (mg.L^{-1})	608	340	336
Calciun Ca^{+2}	176	156	140
Magnesium as Mg^{+2}	38	29	31
Sodium as Na^{+1}	870	259	236
Potassium as K^{+1}	65	17	17
Nitrate as NO_3	56	32	26
Chloride as Cl^{-1}	1275	495	500
Sulphate as SO_4	107	36	37
Phosphate as PO_4	0.29	0.21	0.20
C.O.D (mg.L^{-1})	198	92.5	86.6
B.O.D (mg.L^{-1})	90	38	33

The used soil was characterized by sandy loam according to soil texture, and pore in nutrients, moisture content and microelements. The introduction of waste water either pretreated or post treated by the green alga *Scenedesmus* or the Cyanobacterium *Nostoc* into these soils were (Table, 2). The alkaline nature of the desert sand soils is of particular importance in the context of precipitating heavy metals and thus avoiding the toxicity for plants growing in sewage water. On average, soils with higher organic matter contents have lower pH (Cooperative Extension, solutions to soil problems ii. high pH “alkaline soil”). Accordingly, it has been advised to amend the alkalinity of the arid soil by supplementing organic matter and elemental sulfur or applying acid fertilizers such as ammonium sulfate. Subsequently, alkalinity of arid soils may become self-treated by the organic matter contained in sewage effluents (reviewed Utah State University, 2010). Algal growth, based on our findings, would be a practical alternative from easiness, and economic feasibility standpoints. Also, [32] reported that algae and cyanobacteria are able to acclimatize to extreme environments, and improve the physico-chemical properties of the soil by enriching them with carbon, nitrogen and available phosphorus, improve soil pH, texture, ion-exchange capacity and conserve moisture.

Table 2: Soil analysis before plant cultivation and after plant harvesting at various wastewater treatments.

Analysis	Before cultivation	After harvesting				
		Control (tap water)	Waste water	Wastewater/ tap water 1:1	Waste water + *Scenedesmus*	Waste water + *Nostoc*
Soil texture						
Sand	55.5	55.2	51.3	50.7	49.7	50.1
Silt	22.7	21.6	22.4	23.8	21.8	22.8
Clay	21.8	23.2	26.3	25.5	28.5	27.1
EC mhocm^{-1}	230	152	390	287	350	330
Mixture content (%)	22	23	25	27	26	32
pH	8.5	8.1	8.3	8.1	7.3	7.2
Total Alkalinity as $CaCO_3$ (mg.g^{-1})	411	409	490	440	380	388
Calcium	0.5	0.5	1.2	0.56	0.74	0.97
Magnesium	0.44	0.45	0.99	0.7	0.87	0.86
Sodium	9.7	10.1	25.9	15.5	21.2	19.7
Potassium	6.8	6.1	9.6	4.5	5.6	5.9
NO_3	0.19	0.21	3.6	2.1	1.1	1.7
Cl^{-1}	0.7	0.81	20.3	18.7	15.8	14.9
SO_4	10.5	12.3	52.7	32.5	15.6	20.3
PO_4	0.22	0.23	1.9	0.9	0.72	0.77
Organic Matter %	0.7	0.71	1.9	1.1	2.1	3.2

The percentage of seed germination of wheat and alfalfa seeds soaked in waste water pre- and post-treated with *Scenedesmus* or *Nostoc* in comparison with the control (tap water) for 4 and 8 days were illustrated in Table (3). In general, sewage effluent (100 % SE) suppressed germination of seeds of both plants, whatever the time elapsed. In the case of waste water amended with *Scenedesmus* or *Nostoc*, however, the percentage of seed germination were to somewhat similar to the control (TW). Algae and Cyanobacteria represent valuable bio-resources, which have been utilized mainly as a bio-fertilizer in agriculture due to their well established role as diazotrophs, establishing proficiency in diverse soil ecologies, and ability to compete with native flora and fauna [33, 34].

Table 3: Seed germination (after 4 and 8 days) of wheat and alfalfa plants grown under tap water, sewage water and sewage water amended with algae treatments

Treatments	Wheat		Alfalfa	
	(%) after 4 days	(%) after 8 days	(%) after 4 days	(%) after 8 days
Control	45±0.57	96±0.71	63±0.38	99±0.13
50 % SE + 50 % TW	43±0.42	91±0.55	61±0.60	98±0.23
100% SE	40±0.42	87±0.30	58±0.27	95±0.30
100% SE + *S. obliquus*	44±0.42	93±0.40	61±0.71	97±0.19
100% SE+ *Nostoc* sp.	46±0.43	98±0.44	64±0.48	99±0.19

TW, (tap water), 100 % SE (100 % sewage effluent), TW/SE (mixture of tap water with sewage effluent in a ratio of 1/1). Presented values are means of 25 plants of five replicate cultures ± SE (*n=25*). Percentage changes are relative to the height of control plants.

The imposed treatments variably altered plant height (Table 4). Sewage effluent (100 % SE) noticeably impaired the stature of both plants, down to 70 % relative to the height of the control irrigated with 100 % tap

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water (100 % TW). Mixing TW/SE (1/1) further reduced plant height of both plants. However, enrichment of sewage effluent (100 % SE) with the alga *Scenedesmus quadricauda* or with the cyanobacterium *Nostoc sp*. overcome the inhibitory effect of sewage water i.e. enhanced plant height to increase up to values surpassing the control plants. The magnitude of enhancement was more pronounced in alfalfa than in wheat. In addition, *Nostoc sp.* was more stimulatory than *Scenedesmus quadricauda*in. [35] stated that algae and cyanobacteria are employed as inoculants for enhancing soil fertility and improving soil structure, besides enhancing crop yields [34]. The estimated amount of nitrogen fixed by cyanobacterial inoculation is in the range of 20-30 kg/ha. Moreover, the nutrient fixed by the cyanobacteria is made available to rice as well as other plants or microbial life present in the soil [35, 36].

Table 4: Plant height (cm) of wheat and alfalfa plants grown under tap water, sewage water and sewage water amended with algae treatments

Treatments	Wheat		Alfalfa	
	Plant height			
	(cm)	(%) change	(cm)	(%) change
Control	24.71±1.45	100	6.08±0.23	100
50 % SE + 50 % TW	15.00±0.76	60.69	3.71±0.27	61.09
100% SE	17.2±063	69.60	4.28±0.26	70.39
100% SE + Alga X	28.44±1.17	115.08	8.80±0.38	144.74
100% SE+ Cyanobacteria	28.00±0.99	113.29	10.12±0.61	166.45

TW, (tap water), 100 % SE (100 % sewage effluent), TW/SE (mixture of tap water with sewage effluent in a ratio of 1/1). Presented values are means of 25 plants of five replicate cultures ± SE (*n=25*). Percentage changes are relative to the height of control plants.

Table 5: Fresh and dry weights (g/plant) of wheat and alfalfa plants grown under tap water, sewage water and sewage water amended with algae treatments

Treatments	Wheat				Alfalfa			
	Fresh weight (g/plant)	% change	Dry weight (g/plant)	% change	Fresh weight (g/plant)	% change	Dry weight (g/plant)	% change
Control	23.88±0.34	100.00	6.20±1.95	100.00	15.95±1.40	100.00	3.13±0.80	100.00
50 % SE + 50 % TW	23.00±1.36	96.29	6.73±2.45	108.56	13.52±4.35	84.73	2.33±1.15	74.46
100% SE	18.60±1.03	77.88	4.65±1.45	75.00	11.50±2.75	72.10	1.71±0.50	54.62
100% SE + Alga X	24.09±0.79	100.88	6.21±1.20	100.24	13.44±1.00	84.22	1.06±1.05	33.70
100% SE+ Cyanobacteria +	25.68±0.37	107.52	4.51±1.40	72.71	22.19±4.00	139.08	3.24±1.45	103.46

TW, (tap water), 100 % SE (100 % sewage effluent), TW/SE (mixture of tap water with sewage effluent in a ratio of 1/1). Presented values are means of 25 plants of five replicate cultures ± SE (*n=25*). Percentage changes are relative to the height of control plants.

Table (5) displays the values of fresh weight; it shows that sewage water (100 % SE) inhibited fresh weight of both wheat and alfalfa plants, relative to the control values (100 % tap water). Mixing sewage water with tap water (1/1) relatively enhanced fresh weight of both plants. Amendment of sewage water with *Scenedesmus quadricauda* highly enhanced fresh weight of wheat plants, recovering their control values and somewhat more enhancement upon amendments with *Nostoc sp*. In the case of alfalfa plants, the highest enhancement of fresh weight was induced in *Nostoc* amended sewage water (39 % higher than the control) whereas amendment with *Scenedesmus quadricauda* was comparatively much less (16 % less than the control but higher than sewage effluent). Dry mass was obviously different in response to the applied treatments compared with that of the fresh weight. Amendment of sewage water with *S. quadricauda* maintained 100 % dry mass relative to that of wheat control plants whereas severely dropped in the case of alfalfa to the least dry mass in absolute terms among all the treatments. The opposite was recorded at *Nostoc sp.* amended sewage effluent. The above data was also calculated per plant, sewage effluent also inhibited fresh and dry mass of both plants to be the least weights (Table 5). However, the stimulatory impact of amendment with algae was more pronounced than per pot (total biomass); *Nostoc sp*. was more stimulatory than *Scenedesmus quadricauda* (data not shown). Algae and cyanobacteria have been known to produce an impressive array of biologically active compounds or metabolites which encompass a wide range of chemical classes, including a diversity of nitrogen-rich alkaloids and peptides [36, 37].

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Chlorophyll contents (Table 6) of both wheat and alfalfa plants exhibited obvious enhancement by algae-amended sewage effluent compared with sewage without amendment (*Nostoc sp.* more than *S. quadricauda*). Growth promoting substances are produced by algae and cyanobacteria may be hormones like auxins, gibberellins, cytokinins or abscisic acid, vitamins or amino acids. Antibiotic or toxic compounds and molecules with pharmacological, immune-suppressive or enzyme inhibiting activities have also been reported by [38].

Table 6: Total chlorophyll (SPAD value) of wheat and alfalfa plants grown under tap water, sewage water and sewage water amended with algae treatments

Treatments	Wheat	Alfalfa
	Total chlorophyll (SPAD value)	
Control	28.28± 2.41	52.6±2.89
50 % SE + 50 % TW	24.26±3.49	42.92±5.05
100% SE	21.74±1.64	35.00±2.63
100% SE + Alga X	31.62±2.46	56.98±3.80
100% SE+ Cyanobacteria +	33.28±1.99	55.98±2.93

TW, (tap water), 100 % SE (100 % sewage effluent), TW/SE (mixture of tap water with sewage effluent in a ratio of 1/1). Presented values are means of 25 plants of five replicate cultures ± SE (*n=25*). Percentage changes are relative to the height of control plants.

In this work, none of the plants irrigated with algae-treated sewage water displayed any considerable fungal infection (or association), indicating the test plants are hygiene to be used for crop production (wheat) or animal fodder (alfalfa). Algae or cyanobacteria, most probably killed fungal cells, which have been found in control and sewage effluent, thus inhibited fungal infections characteristic and preventing them from invading both plant stems. In this respect, a lot of active substances with antibacterial, antiviral, fungicide, cytotoxic and algicidal activity have been isolated from cyanobacterial biomass [39, 40]. The fungi, *Armillaria sp*., *Penicillium expansum*, *Rosellinia sp.*, and *Sclerotinia sclerotiorum*, were inhibited by the biomass extract of the filamentous cyanobacterium *Nostoc* at a concentration of 0.25 g of dry biomass/liter of inoculated medium [41]. In addition, [42] showed in vitro inhibition of the growth of fungal plant pathogens *Sclerotinia sclerotiorum* causing cottony rot of vegetables and flowers and *Rhizoctonia solani* causing root and stem rots upon treatment with extracts from the cyanobacterium *Nostoc muscorum*, and further concluded that seed treatment with cyanobacterial extract is the most cost effective and easier approach for many crops, however, root drenches should be applied to high value transplanted crops.

Water content of both plants (wheat and alfalfa) exhibited its highest values in plants irrigated with Nostoc-amended sewage effluent, followed by those irrigated with *S. obliquus* (Table 7). Higher water content provided relevant medium for higher growth. In contrast, the lowest water contents have been found in raw sewage effluent without algal growth; so was growth at its lowest growth magnitudes.

CONCLUSIONS

Wheat and alfalfa plants can be cultivated using sewage effluents; the green alga *Sceneesmus* and the cyanobacterium *Nostoc sp.* efficiently validated sewage water for better growth of the test plants. Furthermore, sewage water has become hygienically safe after treatment with *Scenedesmus obliquus* and *Nostoc sp*.; no fungal colonies have been detected in the test plants and as such can be used for food and feed.

Table 7: Water content of wheat and alfalfa plants grown for two months under tap water, sewage water and sewage water amended with algae treatments

Treatments	Wheat		Alfalfa	
	g water/g plant	% (change)	g water /g plant	% (change)
Control	0.14	100.00	0.18	100
50 % SE + 50 % TW	0.15	107.95	0.17	91.09
100% SE	0.14	103.71	0.15	84.50
100% SE + Alga X	0.17	122.53	0.28	154.42
100% SE+ Cyanobacteria	0.26	191.33	0.28	156.05

TW, (tap water), 100 % SE (100 % sewage effluent), TW/SE (mixture of tap water with sewage effluent in a ratio of 1/1). Presented values are means of 25 plants of five replicate cultures ± SE (*n=25*). Percentage changes are relative to the height of control plants.

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