Assessment of the impact of wastewater use on soil properties

Muamar AL-Jaboobi,[1,2,3] M'hamed Tijane[2], Shawqi EL-Ariqi[4], Abdellah El Housni[1], Abdelmajid Zouahri[1], Mohammed Bouksaim[1]

1. *INRA, RCAR-Rabat. P.O.Box 6570, Rabat Institute, 10101 Rabat Morocco*
2. *Laboratory of Biochemistry-Immunology, University Mohammed V-Agdal*, Rabat, Morocco
3. *Department of Plant Production, Thamar University, Thamar,Yemen.*
4. *Department of Plant Protection, Sana'a University, Sana'a, Yemen.*

Abstract
The concern of this study is to determine the quality of soil when irrigated using treated wastewater TWW in comparison with soil irrigated with groundwater. Thus, about thirty six soil's samples from area called Bani-Alharth in Sana'a Yemen were examined to assess the soil behaviour when using wastewater. Results showed decreased in pH unit from 8.16 in soil irrigated with ground water to 7.70 in soil irrigated with wastewater. Evaluation of individual parameters which have risks for human health and environment was performed. Physicochemical properties of soil irrigated with wastewater were higher, with averages of 921,591, 399, 519, 27.33 and 40.33 mg/Kg, when compared to those obtained with soil irrigated using groundwater 657,422, 474, 115, 6.22, and 15.67 mg/l; respectively; for EC, TDS, Na, K, P, and N. Also Microbiological analyzes showed higher population counts of microflora in all samples from soil irrigated using wastewater with averages of $4.6x10^7$, $1.3x10^5$, $1.2x10^3$, $2.9x10^5$, $2.5x10^4$ and $6.4x10^3$ respectively for total aerobic plate counts, total coliforms, fecal coliforms, *Staphylococcus aureus*, yeast and mould counts. Observed values of soil irrigated with groundwater were $3.5x10^6$, $5.4x10^4$, $7.8x10^2$, $5.1x10^4$, $1.0x10^4$ and $3.6x10^3$ respectively. *Salmonella*, *Shigella* and *Clostridium* bacteria were also detected in all tested samples. On the other side, this research showed that the soil irrigated with wastewater has the best concentration of organic matter, which was equal to (2.00) (%) compared to (0.74) (%) obtained for soil irrigated with groundwater. Therefore, the research exhibited that treated wastewater can effectively be used as fertility source for soil; alongside that wastewater irrigation had a high influence on microbial soil pollution.

Keywords: Soil; Wastewater; Groundwater; Bacteria

Introduction
Mankind is currently confronted with one of the greatest challenges in its history, thus how to adequately use its limited freshwater resources. In this context, the challenge is the shortage of water sources, which led to the use of wastewater for agriculture purposes. The reuse of treated wastewater for irrigation is a practical solution to overcome water scarcity, especially in arid and semiarid regions [1]. However, there are several potential environmental and health risks associated with this practices [2]. According to Kiziloglu *et al.* [3], wastewater has a high nutritive value that might improve plant growth. It had been showed that soil irrigated with wastewater contained 4.1% of organic particles by weight, but these particles harbored up to 47.8% of the total soil carbon and 41.7% of nitrogen, and thus represented an important storage of energy and nutrient for microorganisms [4]. Despite the obvious benefits of TWW irrigation, the human and environmental health recorded many concerns of this process [5]. Many reports correlated the relation between fresh vegetables and foodborne diseases outbreaks that has led to concerns about contamination of vegetables with faecal pathogenic bacteria in the agricultural environment [6]. Potential pre-harvest sources of contamination include soil, feces, green or inadequately composted manure [7]. Application of contaminated irrigation water to soil also represents possible sources of contamination. Yet, it is known that *E. coli* bacteria from various sources may persist in soil over multiple years [8]. The survival of pathogen on agricultural lands depends on a number of environmental factors and will vary according to the source of contamination [9]. However, standards are required to ensure safe use of wastewater and to avoid biological risks to the human population. Bani Al harth area, with a total surface of 269 km^2 and, situated near Sana'a city capital of Yemen, it's considered an important area to vegetables production. Scarcity and high cost of fresh water in that region caused reused effluent from Sana'a Wastewater Treatment Plant (SWTP). Although, wastewater use in the world—there is

poor wastewater treatment in (SWTP). Thus the studied area depends upon the partially or no treatment water from (SWTP) canal in about 95%, 5% from ground water only to irrigate the cultivated areas since 10 years [10]. The study area has an arid climate, characterized by maximum humidity reaches 50% in April and November. The average temperatures ranges 22.2° in December and peak up to29.5 ° in June as maximum with average 26 °C while the minimum ranged between 11.7° in August to 2.5° in December. The lowest value reaches 34% in June. Highest wind speed 233 km/day in June. Maximum sunshine hours reach 8.9 in November and minimum is 6.3 in July. Maximum rainfall reached 68 in August [10].The aim of this research was to investigate and discuss the impact of irrigation with treated wastewater (TWW) on the physico-chemical and microbial properties of the soil.

2. Material and Methods

2.1. Soil sampling

Soil samples irrigated with wastewater and groundwater were selected to to compare the impact of water source on the soil from 3 sites across Bani Al Harth district, Bait al- kasham Location, Bait al- Alhallali Location and Bait Haroon Location(Fig. 1). The sites were a commercial vegetable farms and it is considered one of the most important site to supply vegetable markets in nearby cities. Thirty six (36) samples of soil were collected randomly from 6 farms in three times between September and December 2012 which is considered drought period. Two farms in all location were deterred. One irrigated with wastewater direct from wastewater canal of Sana'a station (Immersion method) supporting by pump machine other irrigated with shallow well with dimensions of about $50m^2$ then 5 points at depth of 20 cm in all farm were chosen to take sample. The sampling points were distributed all over the farm to ensure appropriate spatial coverage of the farm. Samples were mixed then analysed sample was taken from the mixture. The sites were of commercial vegetable farms.

Fig. 1. Map of Bani Al Harth district, location of Sana'a's wastewater treatment plants (SWTP). Point of sampling are located at Bait al- kasham, Bait al- Alhallali and Bait Haroon respectively.

2.2. Soil analysis

2.2.1 Physicochemical parameters

2.2.1.1. pH determination

20g of soil was soaked in 50 ml distilled water and mixing well until dissolved. Leave the solution 16 hours. Then the pH was determined by using a pH meter after calibration [11].

2.2.1.2. Electrical conductivity (EC) determination

50 g of soil was taken then drops from distilled water were added with stirring until reaching saturation paste. Solution was left 16 hours. Centrifuging at 1500tour/ min for 5 min was done. Then we measured from supernatant with an EC meter at 25^oC, [11].

2.2.1.3 Phosphorus (P) determination

2.5g of soil was weighted in beaker 250 ml. Then 50 ml ($NaHCO_3$, 0.5N at pH 8.5) was added and the mixture is then stirred in a reciprocating stirrer for 1 hour. The solution was filtered through filter paper $< 2\square$ m. ThenP was determined by using a UV Visible Spectrophotometer at 825 nm [12].

2.2.1.4 Sodium (Na) and Potassium (K) determination

4g of soil was dissolved in 100 ml of Ammonium acetate. Then the solution was filtered. Na and K were measured with a flame photometer [11].

2.2.1.5 Organic Matter determination

0.5-1g of dry soil was weighted in beaker 250 ml. Then 15 ml (Potassium Bichromate, $K_2Cr_2O_7$, solution 1 N) was added and 20 ml of H_2SO_4 acid. Then 50ml was titrated with Mohr's salt (0.5 N). [13].

2.2.2. Microbial Estimation

Ten grams of the soil sample was suspended in a flask containing 90 ml of peptone water stock solution. The flask was shaken then serial 6-fold dilutions of the samples in a 0.85% NaCl solution were mad in triplicate and 1ml of stock solution was transferred into 9ml serial dilutions up to 10^6 of slain solution (dilute solution). 1ml from each dilution was cultured for presence of potential pathogen using the selective media [14].

Statistical analysis

The data were statistically analyzed by SAS Institute Inc., Cary copy right © 2002. NC, USA. Software version 9.00 (TS MO).

3. Results and Dissection

1. Physicochemical Estimation

Results presented in Table 1 represented Physicochemical analyzing of soil samples from two soil types; soil irrigated with wastewater SW and soil irrigated with groundwater SG.

Table 1: Physical and Chemical Characteristics of Soil Irrigated with Wastewater and Soil Irrigated with Groundwater

Parameter	Bait al kasham		Bait Alhallali		Bait Haroon		Average value of **SW**	Average value of **SG**	Significance
	SW	**SG**	**SW**	**SG**	**SW**	**SG**			
pH	7.69	8.27	7.55	8.08	7.89	8.14	7.70	8.16	P=.8900
EC μS/cm	893	667	943	600	923	705	921	657	P=0.1623
TDS mg/l	554.9	430.2	618.5	372.6	600.9	463.15	591.4	422	P<0.0001
MO%	2.17	0.83	2.13	0.70	1.70	0.69	2.00	0.74	P=0.0002
Na mg/kg	400	450	453	453	518	518	399	474	P=.7578
K mg/kg	475	107	521	121	561	117	519	115	P=0.0005
P mg/kg	27.67	8.33	29.16	5	25.33	5.33	27.33	6.22	P=0.0255
N mg/kg	38.33	22	36.33	9	46.33	16	40.33	15.67	P=0.0706

SW soil irrigated with wastewater, SG irrigated with groundwater, EC Electrical conductivity, TDS Total Dissolved Solids, and MO% Organic matter.

3.1.1. pH unit

Analyses showed that the wastewater soil pH ranged from 7.89 to 7.55 in soil irrigated with wastewater which less than from 8.27 to 8.08 in groundwater soil. Soil pH directly affects the life and growth of plants because it affects the availability of all nutrients in the soil [15]. Between pH 6.0 and 6.5, most plant nutrients are in their most available state [16]. Our result agrees with Mutengu *et al*., [17], Kiziloglu *et al*. [3]; Angin *et al*. [18] who explained that the use of TWW for irrigation can have detrimental effects on soil quality. These include decreased soil pH and increased salinity. Decreasing of soil pH is perhaps due to the included acidic components in wastewater which convert to acidic compounds which lead to reduction pH value [19]. Values in both types of soil consider ordinarily less than 8.4 of FAO, 1985 [20] recommendation.

3.1.2. Electrical conductivity (EC)

Irrigation with wastewater was resulted in an increase in EC from 893 to 943 μS/cm with an average of 921 μS/cm, in soil irrigated with wastewater while the average value of EC in the soil irrigated with ground water varied from 600 to 705 μS/cm with a mean of 657 μS/cm The EC explains the presence of salinity which is the most important indicator regarding to fields irrigated with wastewater [15]. In all, these values considered slightly normal according to the limits recommended by [16] therefore the EC of the two types of soil according to this limited, could be caused moderate salinity problem [19]. Indeed to combat this salinity is possible by applying more normal water than the plant needs to remove the salts from the root zone by leaching [21].

3.1.3. Organic matter

The Organic matter is widely regarded as a vital component of soil fertility because of its role in physical, chemical and biological processes to supply the plants with the nutrients and also helps soil to keep the moisture [22]. The best amount of organic matter was found in the soil irrigated with wastewater. It showed 2.00 %

compared to 0.74 % obtained in the case of the soil irrigated with groundwater. This implies that wastewater contains organic matter compounds. This is in agreement with several studies which have shown that TWW irrigation increases soil's organic matter [3,23].

3.1.4. Phosphorus (P)
Phosphorus is considered one from the important nutrients that has direct effect on the growth and productivity of plant [24]. Average values of Phosphorus were high in soil irrigated with wastewater, 27.33 ppm, compared to 6.22 mg/l in soil irrigated with groundwater. These results are consistent with those of Sacks and Bernstein [24] and Akponikpe *et al.*, [25] who have a sure indicator that TWW irrigation with wastewater increases soil phosphorus.

3.1.5. Nitrogen (N)
In parallel, using wastewater led to improve total nitrogen in soil which was significantly high in SW with average of 40.33 mg/kg compared to those irrigated with groundwater 16 mg/kg. Similar results were found by Akponikpe *et al.,* [25] and Mutengu *et al.*, [17]. It is known that N and P are considered as the important macro nutrients that are required by crops for ample growth. We noted that both types of soils had less than 0.1 of % total N. However, FAO Guidelines, [20] considered soil that has Less than 0.1 % of total N is poor soil.

3.1.6. Sodium (Na)
Results showed that the amount of sodium (Na+) in soil irrigated with wastewater was 399 compared to 474 ppm recorded in the soil irrigated with groundwater. Minimum concentration of sodium was obtained in the case of Bait al kasham. Such result was found by [26]. Sodium is one of most concern among the specific toxic ions. It is reported that sodium directly affects the availability of crop water and causes adverse physico-chemical changes in the soil, particularly to soil structure. It has the ability to disperse soil thus leading to decreased permeability, lowered shear strength and increased compressibility [1,16,20,27]. In our case the concentration of sodium in the soil obtained in the different locations still below the toxic levels.

3.1.7. Potassium (K)
Potassium is considered the second important macro element for soil and crop productivity. It is said that potassium normally required for agricultural crop production would be supplied by the effluent [16, 28]. Results showed that irrigated soil with wastewater contains large amount of Potassium. It was observed that there is increase in value of potassium in the soil irrigated with wastewater (519 ppm) than the other type of soil (115 ppm).

3.2. Microbial Estimation
Data in table 2 cleared that TWW induced changes in microbial activity and community composition. It showed a high number in the most WS when compared to GS microbes in the studied area (three locations).There were significant differences between types of soil in microbial load. This need to pay more attention to the role of WWT in spreading out of bacterial pollution [23, 29]. A total aerobic plate counts (APC) incidence level was observed from 1.5×10^6 to 6.0×10^7 CFU/g with a main of 2.5×10^7 CFU/g. WS in Bait Al-Hallali exhibited the highest contamination among all samples. The levels of APC bacteria met values obtained in other studies where the bacteriological quality of soil has been assessed in the long-term irrigated soil with wastewater [4, 30]. It is reported that the APC contamination can be considered as an efficient indicator of pollution [31].
Both soils watered with TWW and GW presented a rise contamination in total coliforms and faecal coliforms numbers. This was because of a poor quality of irrigation water source, which exposed soil to different microbial pollution which are a favorable environment for spread the reliably risk related to wastewater application because they assemble high concentrations of bacteria's nutrients [32, 33]. Concentration of faecal coliform indicators an observed increase of 1.3×10^3 CFU/g in soil irrigated with wastewater and 3.7×10^2 CFU/g in soil irrigated with groundwater. The average of the faecal coliforms found in the present research are at rate over the FAO and WHO recommended limits for vegetable to be eaten uncooked and to those detected in soil irrigated with wastewater, by Unc *et al.*, [34], Akponikpè *et al.*, [35] and Gatica & Cytryn [2]. Soils irrigated with TWW are characterized by a certain tendency for higher microbial activity. Also it is noted that addition of sterile municipal sewage biosolids to clay soil increases the numbers of *Escherichia coli* which had detected considerably in assays carried out on the soil/sludge mixtures by Estrada*, et al.*, [36] and Allen *et al.*, [37].

Table 2: Estimated counts of pathogens contamination of soil Irrigated with Wastewater and soil Irrigated with Groundwater samples

Parameter	Bait al kasham		Bait Alhallali		Bait Haroon		Total /Average	Significance
	WS	GS	WS	GS	WS	GS		
APC CFU/g	4.1×10^7	5.6×10^6	6.0×10^7	3.5×10^6	3.6×10^7	1.5×10^6	2.5×10^7	P=.0416
TC CFU/g	1.8×10^5	3.8×10^4	1.1×10^5	6.4×10^4	9.6×10^4	5.8×10^4	9.1×10^4	P=0.3803
FC CFU/g	1.3×10^3	8.8×10^2	1.0×10^3	1.1×10^3	1.1×10^3	3.7×10^2	7.9×10^2	P=0>.0001
Sal/Cl	+	+	+	+	+	+		
STF CFU/g	5.9×10^5	7.0×10^4	1.7×10^5	3.1×10^4	9.8×10^4	5.2×10^4	1.7×10^5	P=0.0001
Y CFU/g	5.5×10^4	1.0×10^4	1.2×10^4	8.1×10^3	9.1×10^3	1.3×10^4	1.8×10^4	P=0.5056
M CFU/g	5.8×10^3	5.1×10^3	6.8×10^3	1.5×10^3	6.5×10^3	4.2×10^3	5.0×10^3	5.0×10^3

APC: Aerobic Plate Count, TC: total coliforms, FC: fecal coliforms, STF: *Staphylococcus aureus*., , Sal: Salmonella, CL: Clostridium, Y: Yeasts,and M: Molds

Highest *Staphylococcus aureus* count value of 5.9×10^5 CFU/g was observed in WS of Bait al kasham but the 9.8×10^4 CFU/g as lowest value was found in WS of Bait Haroon. Indeed, soil contamination with staphylococci has recognized as a soil-born bacterium related of wastewater contact [38,39]. It's reported that soil is one potentially rich of resistance bacteria and largely unstudied environmental reservoir [37]. Many studies have reported that *S. aureus* is a major cause of food intoxications, also it is the most frequently occurring bacterial pathogen among clinical isolates from hospital inpatients and is the second most prevalent bacterial pathogen among clinical isolates from outpatients [40,41**]. *Salmonella spp.* was found in almost all the tested samples. The results oscillated from time to time in the present study. This supports the recognized investigations in many parts of the world of transfer possibility of Salmonella spp. from water to soil [42]. Moreover Salmonella spp is most likely to be survived in soil in a great number [42,43].
In Clostridium case, results showed higher incidence of *Clostridium prefergeces* in SW than SG which illustrates the bad impact of using wastewater over the soil. Study in Costa Rica was found similar results by Gamboa *et al*., [44] who showed that *C. perfringens* was the more frequently bacterial strains isolated in soils. Also in Colombia Ortega *et al*., [45] and in Tunisie Mekki *et al.,* [29] reported that this bacteria genus is often present when the soil was analyzed.
Clostridium is very important due to their capacity to adapt to different environmental and their pathogenic effect .It can be responsible for huge economical losses in animal production because they can produce diverse disease, acute and sudden mortality in animals which causes by the toxin effects delivered by Clostridium after they have been ingested by the animals or in infectious process [45, 46].
In parallel, yeasts incidence of soil when irrigated with wastewater were between 5.5×10^4 and 9.1×10^3 CFU/g while in soil irrigated with groundwater, its value was from 1.3×10^4 to 8.1×10^3 . Such result was obtained by Yurkov *et al*., [46]. Also yeasts were found in soils worldwide at different levels, in China by Pan *et al*., [47] and in Germany by Yurkov *et al*., [46]. Yeasts were prevalent organisms found in a broad range of pH values and temperatures [48]. It was said that yeasts influence soil aggregation [49].
Our results showed that the concern of mould exhibited a narrow variance in comparison between tested soil types. At the overall, high CFU of fungi found in the soil irrigated with wastewater from 5.1×10^3 CFU/g (Bait al kasham) to 6.8×10^3 CFU/g (Bait al Hellali). We noted in all dates of sampling, the fungal CFU number increased when using wastewater but remained in approach with CFU in GS. Further study in Tunisie revealed that fungi were much higher in soil when treated with wastewater than the control soil [29]. Some genera of moulds are common detected in soil irrigated with wastewater [37]. Recent research by Al- Jaboobi *et al*., [50] showed many species of fungi have ability to grow at rich in organic matter such as the wastewater.

Conclusion
Considering these results, we can concluded that using wastewater or polluted water sources without adequate safeguards draw attention to several issues. There is existing of optimize in soil properties with raising of Organic Matter and decries of pH unit but on the other hand there is persistence of the contamination in local environment with several bacterial such as in the soil irrigated with wastewater which led to potential health risks for farmers and consumers alongside environmental actual risks. Thus there is need to draw as future goal to go on the study on pathogen bacteria and idea around the efficient way in order to develop efficient and sensitive process for removing bacteria population from certain interesting matrices related to foods chain.

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