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Potential for Bacterial Load and Contamination of Wastewater Vegetables Irrigation in N'djamena Along 1st and 6th districts, Chad

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ABSTRACT

In global, microorganisms-mediated water pollution is one of the major concerns for the aquatic environment. The fecal matter waste from hospitals, industry, and cattle farms increases the bacterial load in a water system. Hence, the main objective of this work is to investigate the validity of water crop irrigation that may be polluted with bacterial microbes and represents a source of microbiological health risk if not treated. In the present study water samples were collected from 1st and 6th districts in N'Djamena city according to the procedure recommended by American Public Health Association (APHA). The results were evaluated in logarithm as colony forming units per milligram (CFUs/mg) and also, were quoted at $P < 0.05$ levels of significance by using the statistical analysis program Rrecomander (R×643.2.5. Lnk), then, were compared with the values obtained from The World Health Organization (WHO) in Nigeria and other health, environmental institutions. Consequently, it was found that the microbial load of all collected irrigation water samples was estimated as 7.02×10^2, 9.85×10^2 and 3.89×10^2 log cfu/mg for total aerobic mesophilic flora (FAMT), total fecal bacteria and for *Staphylococcus aureus*, respectively. The observed counts in water irrigation samples were higher than the recommended standards of $1x10^3$ CFUs/mg. This experiment was performed in Bacteriology Laboratory of the livestock Research Institute for development of the Ministry of livestock and Animal Production.

INTRODUCTION

N'djamena like most developing countries is facing water pollution, having point sources of water contamination with pathogens such as industrial effluents, municipal/ domestic wastewater, oil wells, and underground coal mines close to water bodies, while, non-point sources include wild animal defecation, roads, construction sites, and runoff water from the field (Odjadjare *et al.,* 2010). This widespread problem of water pollution (or aquatic pollution) is jeopardizing our health.

Unsafe drinking and irrigation water that contains pathogens (disease-causing microorganisms) has received high attention from scientists because these pathogenic microbes kill more people each year than war and all other forms of violence combined by penetrating the bodies of living organisms through the food chain (Abdul Khaleq *et al.*, 2019).

Once water is polluted, it is difficult, costly, and often impossible to remove the pollutants and this may have serious public health implications as about 80% of South Africans are reported to depend on surface water bodies for drinking, domestic and agricultural purposes (Mackintosh and Colvin, 2002; Venter, 2001). Although, various studies have reported that the existence of pathogens as free-living or plankton-associated cells, is critical to their survival in the environment as well as their transmission from one host to another, there are also several investigations warning about health risks and environmental impacts (Dolan and Costerton, 2002; Sou *et al.*, 2011). The World Health Organization (WHO, 2009) recorded 200, 000 deaths from food-borne pathogens due to the unavailability of safe irrigation wastewater in Nigeria. So, many investigators worldwide have explained a very close correlation between the consumption of fruits and vegetables irrigated with raw wastewater and dangerous diseases like gastroenteritis, cholera, typhoid fever and chemical toxicity (Sou *et al.*, 2011; Mustafa, 2017). In N'Djamena capital, some edible African leafy vegetables, especially watercress and lettuce are precious for people whereas their leaves are a good source of calcium, magnesium, beta carotene, iron and vitamin C, as well as, the roots and seeds are rich in proteins. Being these crops sometimes near sanitary sewers in an unhealthy way causes many foodborne diseases to in the population (Gamar, 2017).

Reduction in potable water in arid countries gave rise to the use of treated wastewater for crop production. However, among the damages caused by using unsuitable irrigation techniques was severe contamination of plants, soils, and freshwater ecosystems (groundwater, drinking and irrigation crops water), which subsequently affected the habitat and quality of fish life. Therefore, wastewater treatment has become essential to protect humans, animals and plants from health hazards (El-Essawi, 2014). Some known contaminants of concern in freshwater include: fecal coliforms (FC) i.e., Escherichia coli (Jeddah, 2022; Talib, 2014), total aerobic mesophilic flora (FAMT) (Ababouch, 1995), *Staphylococcus aureus* (Yasuda, 2010), *Salmonella*, viruses and protozoa are considered an indicator of water microbial pollution, their existence in water points to disease-causing organisms (pathogens) (Halablab *et al.*, 2010; Kouassi *et al.*, 2019). Thus, contamination of freshwater resources with fecal bacteria or viruses from wastewater poses a threat to the agricultural fields and other wildlife directly or indirectly (Talib, 2011), for that, these microbes must be examined and tested in the laboratory (Jameh, 2013).

As previously mentioned, this project was designed to detect the common species of pathogenic bacteria in the wastewater vegetables and fruit irrigation at two main districts (first and sixth) of the capital, N'Djamena. Also, determining the influence of these polluted crops on the health of their consumers.

MATERIALS AND METHODS

1. Study Area and Sampling Procedure:

A total of 36 wastewater irrigation samples were taken from four quarters at two basic districts: 1st district (12.211°N 15.039°E) 6th district (12.112°N 15.083°E), N'Djamena capital of Chad (Fig. 1) in three periods (from March to May, 2023). The sampling sites were located at a distance of around 4m from the Chari River which is the meeting point of wastewater of both El-Slakhana in 1st district and Radisson Blu, Hilton Ndjamena hotels as well as a compound containing 60 villas. All specimens were sampled aseptically using sterile plastic bottles with adding chloroform to limit any microbial activity. Then, these

bottles of wastewater were labeled, preserved under the icebox and transmitted within 1h of collection to the Bacteriology Laboratory of the livestock Research Institute for development of the Ministry of livestock and Animal Production. Water samples were collected according to the procedure recommended by American Public Health Association (APHA, 1998).

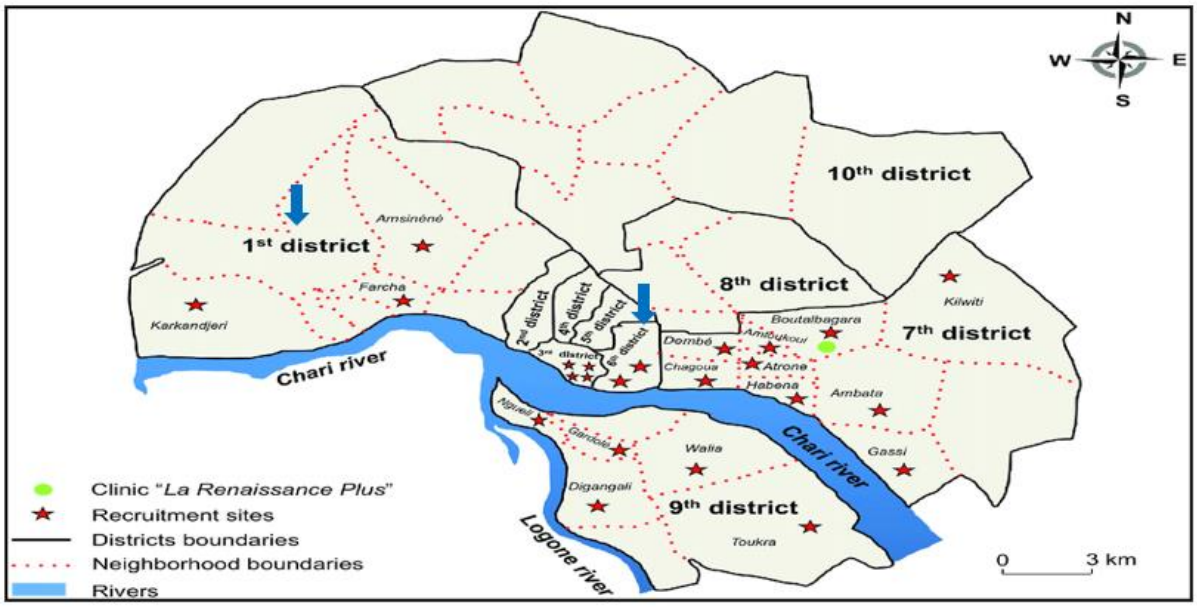

Fig. 1. Description map of **studied sites** (1st and 6th districts) in N'Djamena, Chad.

2. Isolation and Enumeration of Wastewater Pathogens:

The common pathogens that colonized water samples were cultivated on Plate Count Agar (PCA) medium for total aerobic mesophilic flora (FAMT), Chapman's Agar or Mannitol Salt Agar (MSA) medium for *S. aureus* and Eosin Methylene Blue (EMB) medium for total fecal coliforms (TFC). By autoclaving (121°C/15minutes), these culture media were sterilized. To isolate these microbes from the collected water samples, , the serial dilution method was used in order to avoid overlapping colonies. All samples were diluted up to (10^{-5}). Sterile petri plates filled with 15-20 ml sterile media were inoculated with 0.1 ml of different dilutions (10^{-2}-10^{-5}) in duplicates, which were spread on agar media by using sterile L-shaped glass rods then plates were incubated an inverted position at 37°C for FAMT, *S. aureus* and TFC but were at 44ºC for thermo-tolerant coliforms.

The colonies of isolated pathogens were counted within 24 to 48hrs of incubation *via* International Standard methods: ISO 4833 (2003), while, thermo-tolerant coliforms were counted according to ISO 4832 (2006). Isolates were identified by their morphological aspects and by microscopic observation.

3. Statistical Analysis:

Data from the current study were analyzed using the statistical analysis program Recomander (R×643.2.5. Lnk), and the values were considered significant the probability at probability level ($p \leq 0.05$).

RESULTS AND DISCUSSION

During the study period, many pathogenic bacterial species have been isolated from collected wastewater samples as aerobic mesophiles, *S. aureus*, fecal coliforms and *Salmonella*. *Staph*. colonies appeared as yellow with yellow zones on MSA medium and the cells were found to be cocci, gram-positive and non-spore formers.

Total aerobic mesophilic flora (FAMT), *S. aureus*, total fecal coliforms (TFC) and *Salmonella* counts were determined and analyzed for water quality

indicators. Within wastewater samples of 1st district, the bacterial load ranged from 5.65×10^3 log cfu/mg to 5.72×10^3log cfu/mg, from 5.53×10^3 log cfu/mg to 5.35×10^3log cfu/mg and from 5.84×10^3 log cfu/mg to 6.14×10^3log cfu/mg for *Salmonella* counts during April, May and June, respectively. Regarding log counts (cfu/mg) of FAMT, TFC and *S. aureus* in water samples that were taken from two quarters in 1st district, values were presented in Table (1). Data showed that there was a higher count of total aerobic mesophilic flora (FAMT), S. aureus, total fecal coliforms (TFC) and *Salmonella* as 7.02×10^2, 3.89×10^2, 9.85×10^2 and $6{,}14\times10^3$ log cfu/mg respectively, thus, it indicated that the crops irrigation wastewater in the first district is more polluted with fecal coliforms (FC) (Fig. 2A). On the other hand, counts of total pathogenic bacteria in wastewater that were collected from two quarters in 6th district during the same three periods of time in 2022, were also recorded in Table (1). In the wastewater samples of 6th district, data detected that high counts of *staphylococci* were (3.76×10^2 log cfu/mg), *Salmonella* was (6.19×10^3 log cfu/mg), total aerobic mesophilic flora (FAMT) were (7.18×10^2 log cfu/mg) and total fecal coliforms (TFC) were (9.58×10^2 log cfu/mg). Also, data revealed that the crops irrigation wastewater in the sixth district had the highest values of faecal coliforms counts (FCC) (Fig. 2B), as well as, significant differences ($P\leq0.05$) were observed in FAMT, TFC, *Salmonella* and *S. aureus* counts of irrigation wastewater among the two sampling areas (1st and 6th districts).

Table 1. Comparison of Log counts (CFU/mg) of total pathogenic bacteria in both 1st and 6th districts wastewater during March, April, May 2023.

C.T.	S.N	*Salmonella*		*S. aureus*		TFC		FAMT	
		1st dist.	6th dist.	1st dist.	6th dist.	1st dist.	6th dist.	1st dist.	6th dist.
March	E_1	5.65×10^3	6.19×10^3	3.49×10^2	3.76×10^2	8.07×10^2	7.90×10^2	4.60×10^2	4.32×10^2
	E_2	6.01×10^3	5.91×10^3	3.89×10^2	2.89×10^2	9.24×10^2	8.94×10^2	5.35×10^2	4.90×10^2
	E_3	5.72×10^3	5.42×10^3	2.92×10^2	3.12×10^2	9.85×10^2	9.10×10^2	5.83×10^2	5.70×10^2
April	E_1	$5{,}53\times10^3$	5.63×10^3	3.76×10^2	3.03×10^2	9.15×10^2	9.58×10^2	6.01×10^2	6.25×10^2
	E_2	$4{,}91\times10^4$	4.61×10^4	2.95×10^2	3.48×10^2	9.05×10^2	8.99×10^2	5.50×10^2	5.31×10^2
	E_3	$5{,}35\times10^3$	5.59×10^3	3.38×10^3	2.98×10^3	8.46×10^2	9.02×10^2	5.14×10^2	6.14×10^2
May	E_1	$5{,}84\times10^3$	6.04×10^3	2.75×10^2	3.15×10^2	9.12×10^2	9.32×10^2	6.85×10^3	6.25×10^3
	E_2	$5{,}28\times10^4$	5.78×10^4	3.47×10^2	2.97×10^2	8.69×10^2	9.05×10^2	7.02×10^2	7.18×10^2
	E_3	$6{,}14\times10^3$	5.74×10^3	2.97×10^2	3.02×10^2	9.09×10^2	8.89×10^2	5.99×10^2	5.75×10^2

***Key*:** C.T. = Count Time, S.N. = Samples Number, 1st / 6th dist. = first / sixth districts, *S. aureus* =*Staphylococcus aureus*, TFC = Total Fecal Coliforms and FAMT = Total Aerobic Mesophilic Flora.

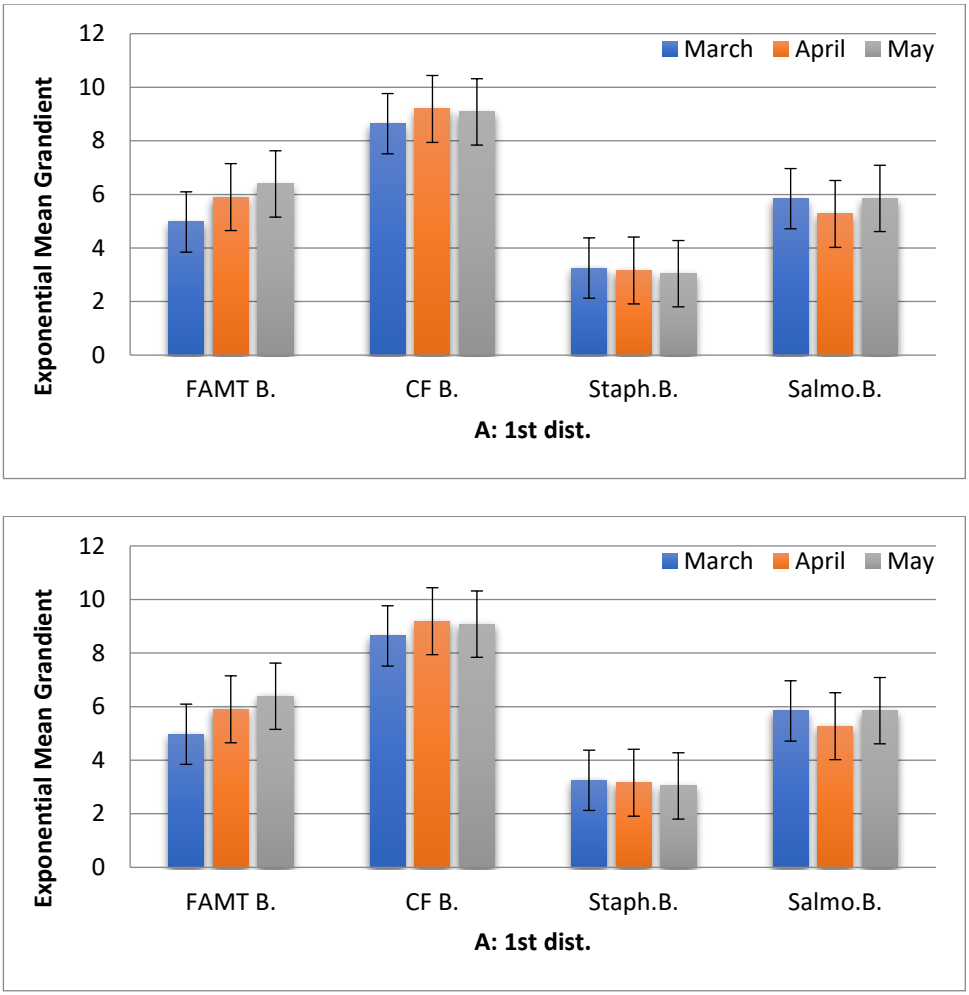

Fig. 2A, B. Diagrams illustrating bacterial load count in wastewater samples obtained from 1st and 6th districts.

In recent years, a lot of diseases spread due to the use of untreated wastewater irrigation, which causes soil and crops contamination. Wherefore, most studies that discussed wastewater irrigation have been interested in pathogenic microbiological contaminants and assessment of the resulting health risks (Alegbeleye *et al.*, 2018). Irrigation with wastewater is widely used all over the world but major obstacles to wastewater irrigation include the presence of disease-causing bacterial pathogens (Alemu *et al.*, 2018). The presence of these pathogens was indicated in all wastewater samples of our study. For further demonstration, microbiological analysis was performed and depicted that wastewater-irrigated vegetables and crops that were collected from four quarters in the 1st and 6th districts at the river Chari were highly contaminated with pathogenic bacterial species specifically with fecal coliforms as 9.85×10^2, 9.58×10^2 log cfu/mg in first and sixth districts, respectively. Furthermore, total counts of all bacterial pathogens such as Total aerobic mesophilic flora (FAMT), *S. aureus*, total fecal coliforms (TFC) and *Salmonella* that were isolated in this work exceeded the standard values recommended by the World Health Organization (7052/2005) and (WHO) in Nigeria as described in Table (2), and as a result, none of the water samples from the studied sites were suitable for irrigating crops, vegetables and human use. The presence of fecal coliforms is the main indicator of the bacteriological quality of

water (Chigor *et al.*, 2012). In addition, Goueu (2006) and Campose *et al.* (2009) mentioned that the presence of coliforms in food products is evidence of contamination and transferee to the product by means of many mechanisms through dust from the surrounding environment, or even from the materials in which the product was packaged. Therefore, the administrators should maintain basic hygiene rules and standards during the production process otherwise; this may cause harmful effects to people.

Table 2. Guidance on the standards of microbiological analysis of wastewater and groundwater (WHO 7052 / 2005).

Pathogenic bacteria	(Colony-Forming Unit (cfu)/mg)		
	Satisfactory	Borderline	Unsatisfactory
Total aerobic mesophilic flora (FAMT)	≤ 10/ml	≤ 100/ml	≤ 1000/ml
Fecal/Tolerant coliforms (FC/TC)	0 /100ml	≤ 103/ml	≤ 1000/ml
S. aureus	0 /100ml	≤ 103/ml	≤ 1000/ml
Salmonella	0 /100ml	≤ 100/ml	≤ 1000/ml

Note: Borderline, is test results that are not unsatisfactory but are also not satisfactory.

Conclusion

Based on the results obtained in this study, it was found that the wastewater of El-Slakhana in 1st district and the big hotels located at the banks of the river Chari had high counts of pathogenic bacteria such as coliforms, aerobic mesophiles, and golden *Staph*. bacteria and *Salmonella*. In irrigation wastewater samples total counts of pathogenic bacteria exceeded the convenient limits according to the standards of the World Health Organization (7052/2005) and (WHO) standards in Nigeria. It could be concluded that these pathogens can cause food-borne diseases to consumers when are transmitted to the people through the consumption of crops that were irrigated with this polluted wastewater. So, this study is considered as a notification to induce the public for cleaning vegetables and fruits well with disinfectants before use.

Recommendations

According to the results stated by this study, we recommend the following suggestions:

- The necessity of wastewater treatment in the four studied quarters of first and sixth districts by applying advanced techniques before using it in crop irrigation to avoid health risks.
- Performing microbiological analysis to detect the species of microbes that weren't included in this study to check whether or not were present in the wastewater and crops.
- Making farmers stop irrigating their farms with water sources close to El-Slakhana and hotels that are at the river Chari.
- The concerned entities in N'Djamena capital should set fines for users of untreated wastewater irrigation, which damages human health.
- It is important to conduct such a study in other seasons and compare the results.
- Permanent monitoring of bacteriological contamination in the treated wastewater.

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ARABIC SUMMARY

احتمالية الحمل الميكروبي وتلوث الخضروات المروية بمياه الصرف الصحي بالدائرتين الأولى والسادسة بمدينة أنجمينا، تشاد

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على المستوى العالمي، يعد تلوث المياه عن طريق الكائنات الحية الدقيقة أحد الاهتمامات الرئيسية للبيئة المائية. وتزيد نفايات الفضلات البشرية من المستشفيات والصناعة ومزارع الماشية من الحمل البكتيري في نظام المياه، ومن هنا فإن الهدف الرئيسي من هذه الدراسة هو التحقق من صلاحية ري المحاصيل المائية التي قد تكون ملوثة بالميكروبات البكتيرية وتمثل مصدر خطر ميكروبيولوجي على الصحة العامة إذا لم يتم علاجها. في هذه الدراسة تم جمع عينات المياه من الدائرتين الأولى والسادسة بمدينة أنجمينا وفقا للطرق المعيارية الموصى بها من قبل جمعية الصحة العامة الأمريكية (APHA) وتم تقييم النتائج باللوغاريثم كوحدات تشكل مستعمرة بكتيرية لكل مليجرام (CFUs/mg) وأيضًا تم اقتباسها عند مستويات ثقة P <0.05 باستخدام برنامج التحليل الإحصائي (R×643.2.5. Lnk) Rrecomander، ثم تمت مقارنتها بالقيم التي تم الحصول عليها من منظمة الصحة العالمية (WHO) في نيجيريا وغيرها من المؤسسات الصحية والبيئية. ونتيجة لذلك، وجد أن الحمل الميكروبي لجميع عينات مياه الري المجمعة يقدر بـ $10^2\times7.02$، $10^2\times9.85$ و$10^2\times3.89$ log cfu/mg للبكتيريا الهوائية الكلية الكلية (FAMT)، البكتيريا البرازية الكلية والمكورات العنقودية الذهبية على التوالى. كانت الأعداد الملحوظة في عينات مياه ري الخضروات أعلى من المعايير الموصى بها وهي: 1000 ≤ CFU/mg. تم إجراء هذه التجارب في معمل البكتيريا لمعهد ابحاث الثرة الحيوانية من اجل التنمية (IRED) التابع لوزارة الثروة الحيوانية والانتاج الحيواني.