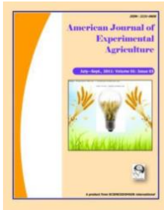

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Contamination of Lettuce Plants Irrigated with Waste Water in Yaounde, Cameroon

Valerie Njitat Tsama[1], Godwill Mih Chewachong[2], Ives Magloire Kengne Noumsi[1], Wilfried Arsene Letah Nzouebet[1], Nkeze Nyochembeng[1] and Zachée Ambang[2*]

[1]Department of Plant Biology (Waste Water Research Unit), Faculty of Science, University of Yaounde I, P.O.Box: 8250 Yaounde, Cameroon.
[2]Department of Plant Biology (Phytopathology Research Unit), Faculty of Science, University of Yaoundé I, P.O.Box 812 Yaoundé Cameroon.

Authors' contributions

This work was carried out in collaboration between all authors. Author VNT designed the study, wrote the protocol and wrote the first draft of the manuscript. Author GMC reviewed the experimental design and all draft of the manuscript; author IMKN reviewed the experimental design and all drafts of the manuscript. Authors WALN and NN managed the analyses of the study. Author ZA identified the plants, designed the study and protocol, and reviewed all drafts. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the effects of various treatment combinations of water contaminated with faecal sludge on the growth and microbiological characteristics of lettuce (*Lactuca sativa* L.) cultivated around the river Avo'o discharged zone.
Study Design: We used randomized plots in which young lettuce plants obtained from a nursery were planted in four sites (S0, S1, S2 and S3) situated 3000, 810, 100 and 350 m from the raw discharge area along river Avo'o.
Place and Duration: Field studies were carried out in Nomayos (Yaounde) during the 2009-2010 growing seasons while laboratory analysis were effected at the waste water research unit of the Department of Plant Biology at the University of Yaounde I.

**Corresponding author: E-mail: zachambang@yahoo.fr;*

Methodology: Faecal streptococci, faecal coliforms and parasitic characteristics were determined in raw discharged sludge from river Avo'o water samples collected at three different points (P1, P2, P3) and in lettuce using standard protocols. Growth parameters of the lettuce plants were also evaluated.
Results: Faecal sludge, water from river Avo'o and *L. sativa* showed high levels of bacteriological and parasitological pollutants. The results also revealed high concentrations of faecal coliforms and faecal streptococci (6092 and 3390 CFU/100 ml respectively), and helminth eggs (> 273 eggs / L) in water. Treatment 3 (S3) showed the largest number of leaves with an average of 34.63 leaves in the ninth week for site 1 and 40.33 leaves for site 2. Plant height increased gradually with time and treatments. The mean values of leaf surfaces obtained after nine weeks were 11.5 cm2 and 13.99 cm2 for treatment S3 in sites 1 and 2 respectively.
Conclusion: Though the best growth of lettuce was observed in plots irrigated with water from river Avo'o, the high levels of parasite concentrations on their leaves (43 eggs/100 g) highlight the necessity for a better management of faecal sludge in Yaounde city.

Keywords: River Avo'o; faecal sludge; irrigation; growth of lettuce; microbial pollutants; Cameroon.

1. INTRODUCTION

Excreta management especially around dumping sites in urban areas of most developing countries do not respect standard hygiene and health norms. Thousands of tons of sludge from onsite sanitation systems such as traditional latrines, public toilets, and septic tanks are disposed of without prior treatment in different parts of the world every day [1]. During the last decade, an increasing number of countries explored the possibility of municipal wastewater and sludge reuse [2,3,4]. Municipal sludge utilization and the use of wastewater for irrigation is becoming widely practiced not only in countries with water deficits, but also in countries with more temperate climates. The methods and extent of reuse however varies with respect to the local infrastructure and circumstances prevailing in each country. Faced with rapidly growing populations, cities in developing countries are subjected to food insecurity and high levels of unemployment. Urban agriculture, a booming business could be a viable approach to reducing unemployment and ensuring food security, at least, for those in the cities. In effect, many people have taken refuge in the production of vegetables, especially in lowlands and swamps which are floodplains with permanent moisture, rich in organic matter [5,6,7]. Several studies have shown the effect of wastewaters on the growth and productivity of plants in various countries around the world [5,8,9,10,11,12]. A study focused on the evaluation of the biomass of several species of *Eucalyptus camaldulensis, Acacia cyanophyllus* and *Popolus nigra* grown on farmlands irrigated with wastewater revealed that *Popolus nigra* plants had the best growth in height while *Acacia cyanophyllus* manifested the highest growth in diameter [13]. In addition, *Acacia cyanophyllus* produced the greatest biomass followed by *Arundo donax*, while biomass generation was low for *Eucalyptus sp.* and lowest in *Populus nigra*. Nawaz et al. [12] assessed the amounts of heavy metals in irrigation wastewater, soils and wheat plants irrigated with contaminated wastewater from municipal sources in Abbottaba in Pakistan and reported levels of metals that were generally above the threshold limits for irrigation water and food as per international regulations. In one other study, the feasibility of using wastewater and sludge from secondary sewage treatment plants to irrigate forest plant species of *Pinus brutia* (Greek origin) and *Pinus maritime* was tested [14]. The present research was conducted in Nomayos, a peri-urban area of Yaounde, Cameroon. The aim of the study was to evaluate the effects of various treatment combinations of water contaminated with faecal sludge on the growth and microbiological characteristics of lettuce (*Lactuca sativa* L.) cultivated around the river Avo'o discharged zone by using ordinary water and water contaminated with faecal sludge. The objectives were: (a) to characterize different kinds of water used for the irrigation of *L. sativa* in the study area, (b) to evaluate the growth parameters of the plant, and (c) to evaluate the sanitary risks associated with lettuce produced by farmers in this area.

2. MATERIALS AND METHODS

2.1 Site Description

The study site is located in Nomayos, a locality situated 20 km away from Yaounde, Cameroon. The zone serves as an indiscriminate dumping site for sludge collected by vacuum trucks from onsite sanitations in Yaounde (Fig. 1). Sludge is discharged daily into the few wells available (Fig. 1a) or directly on the soils surface (Fig. 1b). The sludge deposited then leaches into rivers near the area of discharge where farmers coincidentally collect water for irrigation of their crops (mostly vegetables).

The work was carried out along the borders of two tributaries of river Avo'o. The site has a surface area of about 300 m^2 and receives approximately 1350 m^3 of untreated faecal sludge per week.

2.2 Methodology

2.2.1 Microbiological tests

Samples of faecal sludge water from river Avo'o and lettuce irrigated with water from this river were collected for different microbiological and parasitological analysis. For this, four (04) points (S0: control site located at Mbankomo, 3 km away from the faecal sludge discharge area; S1: site 1 located 810 m before the faecal sludge discharge area; S2: site 2 located 100 m near the faecal sludge discharge area and S3: site 3 located 350 m after the faecal sludge discharge area) were mapped out for sampling. The microbiological analysis involved mostly the determination of the presence of faecal streptococci and coliforms using the membrane filtration protocol described by [15]. Parasitological analysis to determine the presence of helminth eggs were carried out using the protocol described by Bailenger and modified by [15].

2.2.2 Evaluation of lettuce growth parameters

The lettuce plants used for this study were obtained from a nearby nursery and transplanted at the different sites. Plants were watered twice daily (morning and evening) by sprinkling. Plant height, number of fresh leaves as well as leaf length and width of lettuce plants in the different sites mentioned above were evaluated. Plant height was measured using a double decameter; the number of fresh leaves and number of dead plants were determined by counting while the length of leaves was measured with a caliper.

2.2.3 Data analysis

The ANOVA test was conducted to determine the effects of different treatments applied on the bacteriological, parasitological and morphological parameters of *Lactuca sativa* L. Specifically, a two way ANOVA was used to determine any correlations and interactions between the different sites (S0, S1, S2 and S3) and the different growth parameters. On the other hand, a one way ANOVA was used to determine the means of the different microbiological and parasitological parameters.

3. RESULTS AND DISCUSSION

3.1 Microbiological Characteristics of Water Used for Irrigation

Analyses of sludge water from river Avo'o and lettuce plants from the study sites revealed the presence of indicators of faecal pollution: faecal coliforms and faecal streptococci (Table 1). Mean values for these bacterial indicators in faecal sludge were 22.86 x 10^5 CFU/100 ml and 10.68 x 10^5 CFU/100 ml respectively for faecal coliforms and faecal streptococci thus indicating a significant difference ($P< .05$).

When water samples collected at different points (S1, S2, S3) were compared to the control S0 (1112 and 264.20 CFU/100 ml), significant differences ($P< .05$) were observed between the amount of faecal coliforms and faecal streptococci (Table 1). Analysis of the amount of faecal coliforms and faecal streptococci on the leaves of *L. sativa* revealed significant differences between samples from S2 and S3 when compared to those of the control (S0) as exemplified in (Table 1). In addition, a very strong Pearson's linear correlation was observed between the levels of faecal pollution indicators in the sludge water from river Avo'o and leaves of *L. sativa* (Table 1).

The data obtained reveals very high levels of faecal pathogens in sludge suggesting high levels of pollutants in the raw sludge. Similar results were obtained by [16] working on factors affecting the watering constructed wetlands using faecal sludge in tropical regions. The bacteriological characteristics of water from river Avo'o contaminated with drainage from the Nomayos sludge discharge area revealed pollution levels that were above standard restrictive values recommended for irrigation waters used in the cultivation of edible crops (< 1000 CFU for faecal bacteria and 1 helminth ova/100 g of *Lactuca sativa*) [15]. These values reflect the impact of sludge on environmental pollution. Similar observations were made by [16] who noted that the uncontrolled discharge of

Table 1. Microbiological characteristics of samples (n= 33 for each sample)

Site	CF (CFU/100 ml)		SF(CFU/100 ml)	
	Water samples	***L. sativa***	**Water samples**	***L. sativa***
Faecal sludge*	$22.86x10^5±25.47x 10^2$		$10.68x10^5 ±16.46 x 10^2$	
S0	1112.00±1013.25	10.33±1.40	264.20 ±189.52	4.67±5.65
S1	1400.00±116044	13.03±8.7	370.00 ±132.06	4.63±3.01
S2	3200.00±2280.77	361.66±295	1480.00±1200.00	138.33±12.76
S3	4580.00±2902.06	583.33±45.75	1540.00 ±1420.56	250±23.5

S0: control, S1: before discharge point, S2: discharge point, S3: after discharge point
**The number of germs for lettuce is expressed in CFU/100 g of leaves*

Fig. 1. Partial view of two types of faecal sludge disposal systems at Nomayos, Yaounde. (a) discharge into a well, (b) direct discharge on the ground

excreta in the environment does not respect the principles of ecological and health care especially in developing countries. In addition, these authors described disease (amibiasis, ankylostomiasis, cholera, and typhoid fever) transmission routes: direct contact with faeces during swimming and the consumption of food, especially salads prepared with contaminated water. Waterborne diseases like typhoid fever and diarrhea reportedly affects 32 and 43% respectively of the Cameroonian population [20].

Wastewater contaminated with excreta can be used in agriculture if such water is treated to avoid contamination since municipal sludge and wastewaters reportedly contains pathogens like bacteria, protozoa, viruses, and helminth eggs [17]. The contamination of lettuce reported in this study is favoured by the irrigation method, mainly sprinkling. However, most farmers use wastewater to irrigate their crops by spraying with watering cans. Several authors have studied the health risk associated with consuming wastewater-irrigated food crops and vegetables [14,17,18,19]. Consumption of such wastewater-irrigated food without respect of proper hygiene can result in waterborne diseases.

3.2 Parasitological Characteristics

Mean numbers of helminth eggs in raw faecal sludge were estimated at 3601 eggs/L. In the control site (S0) river Avo'o and the leaves of *Lactuca sativa* irrigated with, the mean number of parasites were 285 eggs/L and 43 eggs/100 g, respectively (Table 2). These parasite concentrations show that faecal sludge is highly polluted and influences the parasitological characteristics of water from river Avo'o and consequently lettuce irrigated with such water. Based on the concentrations of helminth eggs we obtained, the sludge analyzed can be ranked as category a sludge type following the classification of [21]. The high levels of pathogens in irrigation water obtained in this study indicates a possible public health risk that should be prevented. The reuse of wastewater for irrigation in different agricultural systems is associated with different risk factors. Some risk factors are short term and vary in severity depending on the potential for human, animal or microbial pathogens to have contact with the environment [22,9]. It is worth mentioning that most common human microbial pathogens found in wastewater are of enteric origin. Uncontrolled use of wastewater in agriculture has significant health implications for farmers and their families, vendors and producer communities as well as consumers in wastewater irrigated areas [23].

Ascaris lumbricoides, *Enterobius* sp., *Trichuris trichura*, *Tenia* sp., and *Schistosoma* sp. Were the different species of parasites identified? observed at different sampling points (Table 2). Heterogeneity was observed in the type and number of parasite species recorded at the sampling sites. For example, S0 sample site registered only 02 parasite species: *Ascaris lumbricoides (*28.33 eggs/L) and *Trichuris trichura* (3.33 eggs /L). *Ascaris lumbricoides* was the most represented species with a frequency of 84.16%. Sites S0 and samples of wastewater from river Avo'o revealed the presence of 05 species of parasites: *Ascaris lumbricoides*, *Enterobius* sp., *Hookworm* sp*., Trichuris trichura, and Schistosoma* sp. Average numbers of eggs per liter of samples were 540, 123.33, 141.66, 62.25 and 13.26 respectively for each parasite. The presence of parasites in the water samples collected from river Avo'o can be explained by its proximity to the indiscriminate sludge discharge site. This has a direct influence on the quality of water from the site.

3.3 Evolution of Growth Parameters

Plant growth is expressed in terms of number of fresh leaves, height of plant and increase in leaf area with time irrespective of the different treatments applied (Fig. 2). There was a significant difference (P=.001) with Student test in the number of fresh plant leaves, height of plant, length and width of leaves between treatments. After nine weeks of treatment, an increase in the growth parameters studied was recorded. Such increases were proportional to the treatment administered from S0 to S3. The use of wastewater resulted in greater number of plant leaves, plant height and leaf area. Treatment 3 (S3) located after the faecal sludge discharge site showed the largest number of leaves with an average of 34.63 leaves for site 1 and 40.33 leaves for site 2 in the ninth week. A gradual increase in plant height was also observed following an increase in time and the different treatments for all investigation sites. The mean values obtained in the ninth week were 11.5 cm^2 and 13.99 cm^2 for treatment S3 on sites 1 and 2 respectively. Site 2 yielded plants with the largest leaf surfaces. From the first to the fifth week, growth was similarly irrespective of site or treatment. The rapid increase in these parameters could be explained by the presence of excess nutrients found in faecal sludge, all of which are mineralized and rendered bio-available by bacteria present in the soil [8,24,25]. These results corroborate with those obtained by [26,27] which showed that there exist a relationship between an increase in nutrient concentration in wastewater and absorption by plants for growth. Similar observations were made by [14,28,29] while working on the assessment of sewage effluent quality from a sewage treatment plant. The death of plants observed in sites S2 and S3 were about the same, but significantly higher (P< .001) than those for S0 and S1 (Fig. 2d). These results could be explained by the fact that S0 is completely located out of the discharge zone while S1 is found upstream and receive very limited amounts of the pollutants poured into river Avo'o. On the other hand, S2 and S3 are found downstream. A previous study by [20] suggested that higher amounts of pollutants in waste water could accelerate the death of plants.

Table 2. Mean number of helminth eggs in different samples n= 33 for of each sample

Parasites	Control (eggs/L)	River Avo'o (eggs/L)	*Lactuca sativa* L. (eggs/100 g)
Ascaris L.	28.33±10.30	540±221	33±5.61
Enterobius sp.	0	123.33±75.5 9	3.7±1.07
Hookworm sp.	0	141.66±86	4.3±2.02
Trichuris trichura	3.33±11.5	62.25±25	2±0.75
Schistosoma sp.	0	13.26±420	0
Mean number	6.32±4.36	178.10±82.36	8.20±1.89

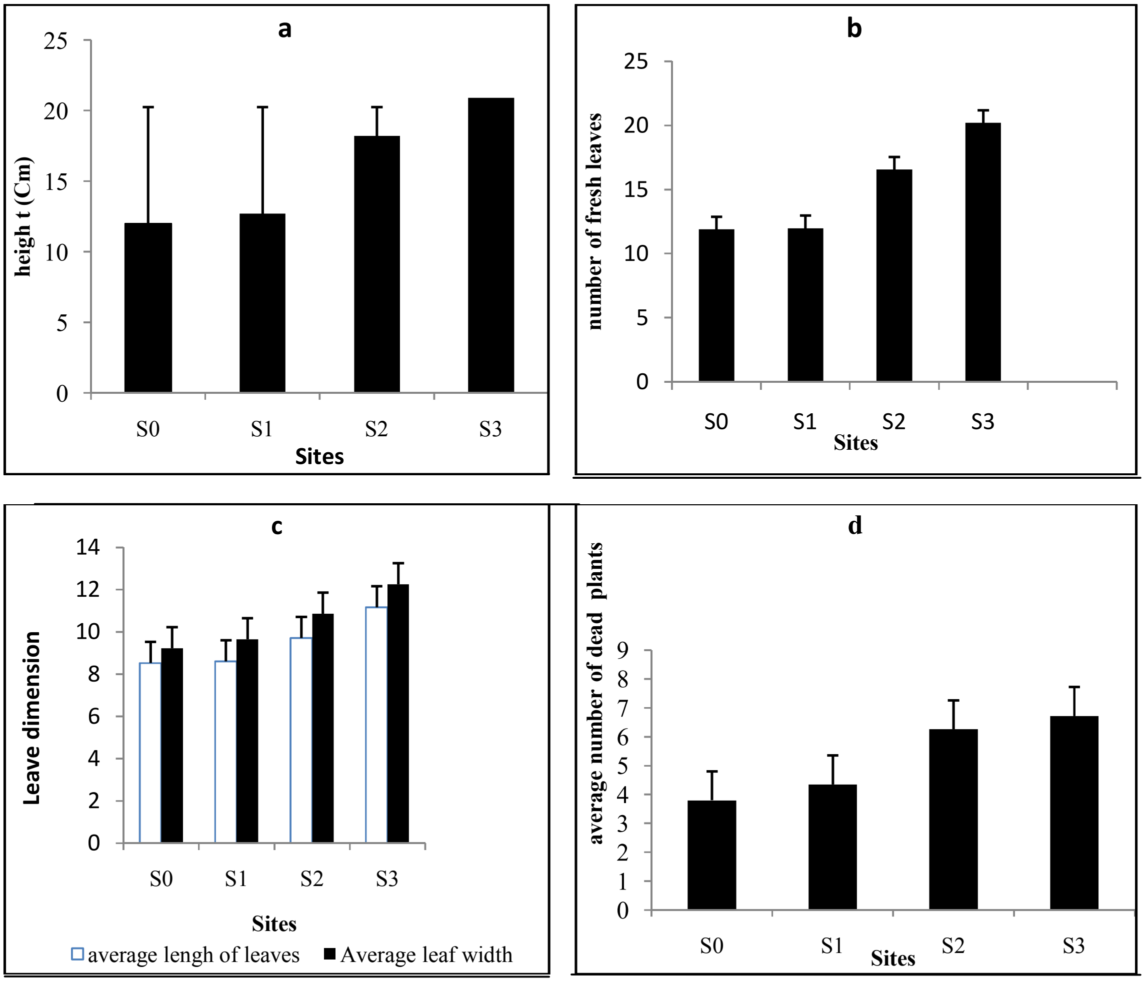

Fig. 2. Evolution of growth parameters of *Lactuca sativa* L. (a: height of plant; b: number of fresh leaves; c: average length and average width of leaves, d: average number of dead plants)

4. CONCLUSION

Most pathogens that affect humans are derived from faeces and transmitted by direct contact during swimming for example or orally through the food we eat and the water we drink. The reuse of wastewater contaminated with faecal sludge for irrigation of crops led to higher crop (*Lactuca sativa* L.) growth rates but was unfortunately associated with high concentrations of faecal coliforms, faecal streptococci and parasites on their leaves. Such high levels of pathogenic micro-organisms highlight an urgent need to better manage faecal sludge in this city. This is important in reducing the associated environmental and health hazards. Based on our results, it is recommended that the government reinforces already existing laws on the treatment of faecal sludge before discharge.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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