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Assessment of Heavy Metals and Microbial Pollution of Lettuce (*Lactuca sativa*) Cultivated in Two Sites (Paspanga and Tanghin) of Ouagadougou, Burkina Faso

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Abstract

Environmental pollution can have detrimental effects on crop yield and its consumers. The current study was designed to investigate the potential human health risks associated with the consumption of lettuce crop contaminated with toxic heavy metals and microbiological status. Irrigated water, soil and lettuce were analyzed by AAS technics for heavy metals including Cd, Cr, Cu, Mn, Ni, Pb and Zn. Transfer factor (*TF*), daily intake of metals (*DIM*) and health risk index (*HRI*) were also calculated. Microbial analysis was carried out for the presence foodborne pathogens. The results showed that the heavy metals contents were higher in the soil than wastewater and the vegetables. Heavy metals ranged ($mg \cdot Kg^{-1}$) for Cd (1.27 to 2.93), Cr (7.28 to 7.38), Cu (0.91 to 1.70), Mn (0.29 to 6.60), Ni (1.74 to 2.16), Pb (1.32 to 1.69), Zn (3.08 to 3.79); and were higher than the WHO maximum limit permissible (ML) in vegetables. *HRI* < 1 indicates minimal risk. *TF* values designated an enhanced bio-contamination. Microbial numeration revealed the presence of spoiler and pathogenic microorganisms. The lettuce tested was not safe for human use, especially for direct consumption by human beings.

Keywords

Heavy Metal, Microorganisms, Pollution, *Lactuca sativa*, Risk Assessment, Burkina Faso

1. Introduction

Heavy metal contamination of vegetables cannot be underestimated as these foodstuffs are important components of human diet. Vegetables are rich sources of vitamins, minerals, and fibers, and also have beneficial antioxidative effects. However, intake of heavy metal-contaminated vegetables may pose a risk to the human health. Heavy metal contamination of the food items is one of the most important aspects of food quality assurance [1] [2] [3]. Rapid and unorganized urban and industrial developments have contributed to the elevated levels of heavy metals in the urban environment of developing countries such as Egypt [2] Iran [4], China [5] and India [1] [6].

In sub-Saharan Africa like other developing countries, the urban and out-of-town agriculture is an income source of many households. It contributes to the reduction of poverty, of unemployment and contributes to the food security. In Burkina, agriculture is the main source of income, and occupies 80% of population and contributes to 33% of gross national proceeds. The total market production in 2008 is valued to 747,488 tons [7].

However, agricultural activities are made around dams and restraints of water. The streaming water drained various organic and inorganic matters of urban environment in dams which could cause more pollution. In spite of it, this water serves for many uses (cleaning of vehicles, laundry, drink for animals, irrigation etc.) could be polluted [8]. The quantity of water used for irrigation is valued to 323 million m^3 per year and represents 84% demand according department environment of Burkina [8].

Linked with environmental pollution, water pollution is also a problem of worldwide concern and ground water is extremely polluted due to unplanned disposal of untreated domestic sewage and industrial effluents into watercourses [9].

Sanitary and environmental risks are also due to practice of agriculture in urban environment by using of agricultural inputs as pesticides, nitrogen, phosphor, and raw organic matter containing undesirable residues as traces elements [10]. Heavy metals get accumulated with time in soils and plants due to waste water irrigation and absorbed minerals settle in edible tissue of the vegetables [11]. Heavy metals are potentially toxic for plants: phytotoxicity results in chlorosis, weak plant growth, yield depression and may even be accompanied by reduced nutrient uptake, disorders in plant metabolism and in leguminous plants, and reduced ability to fix molecular nitrogen [12] [13].

Heavy metals uptake by crops growing in contaminated soils is a potential hazard to human health because of transmission in the food chain [14] [15]. Recent reports indicated that heavy metals take driver's seat among the chief contaminants of leafy vegetables.

Trace metals may enter humans' through direct ingestion of soil, inhalation of dust and consumption of plants grown in metal-contaminated soil [16] [17]. Hazardous metal intake via food chain by humans has been reported in many countries [18]. Trace metal contamination of vegetables must be of great concern to scientist because of the health threat hazardous metals pose to humans and other living organisms. The concentrations of trace metals in most of these vegetables sold in the markets are unknown as much research had not been carried out.

The elements metallic traces are the third source of risk for the human and animal food after the mycotoxines and the micro-organisms [19]. The pollution of vegetables throughout trace elements is raised-up and risks on consumers health that ingest them through by food chain are enormous [20] [21].

Studies concern impact associated to illnesses diarrheic due to consumption of contaminated vegetables are more and more realized. Most studies have investigated the sanitary risk of vegetables associate to several sources of contamination [22].

Wastewater irrigation poses several threats to the environment through contamination by nutrients, heavy metals, salts, and nitrates [23]. It also poses a number of potential risks to human health via the consumption of or exposure to pathogenic microorganisms, heavy metals or harmful organic chemicals [24]. Effective wastewater treatment can reduce pathogen levels, but in most developing countries it is not an option for the municipal authorities due to the high costs involved [25].

Vegetables found in Ouagadougou are lettuce, cucumber, aubergine, carrot, tomato, onion, cabbage etc. are commonly grown in peri-urban areas and irrigated by water of dams.

Therefore, wastewater application on agricultural lands and their heavy metal accumulation by vegetables at Ouagadougou, is a cause of serious concern due to the potential public health impacts. It is often argued that heavy metals in sludge, when applied to soils, may enter in the food chain through plants or animals, contaminate surface and ground water, and thus cause health hazards [26] [27].

In Burkina Faso and particularly at Ouagadougou, lettuce is cultivated on a commercial scale using waste water for irrigation, but there is no information on the level of heavy metals and microbial contain in soils and vegetables produced.

This study was mainly investigated to evaluate the microbial load and the concentration of accumulated trace elements by lettuce irrigated with wastewater of dams.

2. Material and Methods

2.1. Study Area

Two main zones were selected on the basis of ground water and urban drains water irrigation. GPS Garmin eTrex Vista was used to registrate GPS coordinates of study sites at Ouagadougou. The site of Tanghin was located from north 12˚23.577' to East 001˚32.274' Latitude. The coordinates of Paspanga site were from north 12˚23.137' to East 001˚30.830'.

2.2. Collection of Samples

Soil sampling

Surface (0-30cm) complex soil samples were collected from two sites (Tanghin and Paspanga) for chemical soil analysis. The complex sample was collected from different locations at the same time 6.00 am (October, 2018). All the samples were completely mixed together to form a representative surface sample for analysis. The temperature of study site was ranged from 24.0˚C to 36.5˚C and 24.2˚C to 36.6˚C respectively for Tanghin and Paspanga.

Vegetable and wastewater sampling

Vegetable samples (roots and leaves of lettuce), and Low Groundwater (wastewater) were collected from two sites (Tanghin and Paspanga) of production. The samples were homogenized to obtain representative samples and were properly labelled and transported to the laboratory in clean polythene bags for analysis.

Processing of samples

The collected samples were cleaned to remove the dust particles. A part of soil and sliced vegetable samples were dried in an oven at 105˚C for 24 h until they were brittle and crisp [28], after drying the samples were grinded into a fine powder using a commercial blender and stored in polyethylene bags, until used for acid digestion [29]. The untreated parts of soil, vegetable and wastewaster samples were used directly for microbial analysis.

2.3. Acid Digestion and Metals Determination of Samples

Tri-acid mixture (15 ml, 70% high purity HNO_3, 65% $HClO_4$ and 70% H_2SO_4; 5:1:1) was added to the beaker containing 1g dry vegetable sample [29]. The mixture was then digested at 80˚C till the transparent solution was achieved.

After cooling, the digested samples were filtered using Whatman No. 42 filter paper and the filtrate was diluted to 50 ml with deionised water. Determination of the heavy metals such as, Cd, Cr, Cu, Mn, Ni, Pb and Zn in the filtrate of vegetables and atmospheric deposits was achieved by atomic absorption spectrophotometer (Shimadzu Model 6800 with graphite furnace Model GFA 7000). Thus wastewater parameters were analyzed according to the calibration methods used by the American Public Health Association for water and wastewater Analysis [30].

2.4. Translocation Factor Calculation

Transfer factor is the ratio of the concentration of heavy metals in a plant to the concentration of heavy metals in soil. The transfer factors (*TF*) for each heavy metal was computed based on the method described [31] [32]. The *TF* value for the selected heavy metals was calculated according to the following equation:

$$TF = \left[P_s \left(\text{mg} \cdot \text{kg}^{-1} \text{ dry weight} \right) \right] / \left[S_t \left(\text{mg} \cdot \text{kg}^{-1} \text{ dry weight} \right) \right] \quad (1)$$

where:

P_s: plant-metal content originating from the soil, S_t: the total heavy metal contents in the soil.

2.5. Daily Intake of Metals (*DIM*)

Daily intake of vegetables in adult was calculated by data obtained during the survey though a questionnaire. *DIM* was calculated by the following equation [33]:

$$DIM = \left[C_{metal} \times C_{factor} \times D_{food\,intake} \right] / B_{average\ weight} \quad (2)$$

C_{metal}: Heavy metal concentrations in plants (mg·kg^{-1}).

C_{factor}: Conversion factor (CF) of 0.070 was used for the conversion of fresh vegetables to dry weight.

$D_{food\ intake}$: Daily intake of the food crops was 0.50 kg·person^{-1}·d^{-1}.

$B_{average\ weight}$: Body weight for the adult population was 55.0 kg.

These values were used for the calculation of *HRI* as well.

2.6. Health Risk Index (*HRI*)

The *HRI* refers to the ratio of the daily intake of metals in the food crops to the oral reference dose (*RfD*) [32] [34] and was calculated using the following equation:

$$HRI = DIM / RfD .$$

An *HRI* > 1 for any metal in food crops indicates that the consumer population faces a health risk.

2.7. Microbiological Analysis

The microbial analysis of samples was appreciate by the numeration of total mesophilic aerobic on Plate Count Agar at 37˚C and the count recorded after 24 hours according to standard method [35].

Yeasts and moulds were enumerated on Sabouraud agar after inoculated and incubated at 30˚C for up to 5 days according to method NF ISO21527-1 [36]. *Salmonella spp.* were numerated according to ISO6579 [37] at 37˚C/24 h on *Salmonella-Shigella* medium. The numeration of total coliform and fecal coliform 44˚C on Violet Red Bill Agar (VRBG) is according to standard method ISO4832 [38].

E. coli was numerated on Eosine Methylene Blue by ISO4832 [38]. Method of NF ISO21528-2 [39] was used to enumerate *Enterobacteriaceae*. *Staphylococcus aureus* was detected by using ISO 6888-1 [40]. The counting was done by evaluating the colonies found in media [41]. Cetrimide medium was used to detect

Pseudomonas spp. for 48 h at 30˚C [42].

2.8. Statistical Analysis

The recorded data were subjected to two-way analysis of variance (ANOVA) to assess the influence of different variables on the concentrations of heavy metals in the vegetables tested.

ANOVA for each vegetable was performed separately using variables such as sites. All the statistical analyses were computed with STAT5 software version 8.

3. Results and Discussion

3.1. Physico-Chemical Characteristics of Wastewater

Some of the physico-chemical properties of the wastewater are shown in **Table 1**. No significant difference ($p > 0.01$) was found on pH, Temperature and Dissolved oxygen. The pH of wastewater closed to FAO values and ranged from 6.58 to 6.73 respectively for Tanghin and Paspanga. Values of pH were lower than those of Oster and Shainberg [43] ranging from 7.29 to 7.45. They have reported that this range of irrigation water is not an acceptable criterion of water quality because it tends to be buffered by the soil, and most crops can tolerate a wide pH range. Temperature ranged from 25.10 to 24.90 and dissolved oxygen ranged from 3.11 to 4.67. Conductivity and turbidity presented significant difference ($p < 0.01$) respectively from 433.00 to 561.00 and from 4.35 to 50.03 between the two sites.

3.2. Concentration of Heavy Metal Levels in Soils Irrigated with Wastewater

The concentration of heavy metals in the soil samples were presented in **Table 2**. The levels of Cd, Cr, Cu, Pb and Zn were significantly different ($p < 0.01$) in soil of the two sites. The mean values ranged of Cd from 3.66 to 6.60.01; Cr, from 196.39 to 218.63; Cu, from 1.01 to 6.20; Pb, from 20.65 to 40.26; Zn, from 44.56 to 53.91 $mg \cdot kg^{-1}$ soil, respectively for Tanghin and Paspanga. No significant ($p > 0.01$) was found concern Mg (62.96 to 65.70 $mg \cdot kg^{-1}$ soil) and Ni (15.86 to 16.18 $mg \cdot kg^{-1}$ soil). Excepted to Cd which were higher than standards limits values (1.5 $mg \cdot kg^{-1}$), the means values of heavy metals were lower than permissible limits

Table 1. Physico-chemical of irrigation waste water.

Parameters	Site of Tanghin	Site of Paspanga	FAO standards
pH	6.58^a	6.73^a	6 - 8.5
T˚C	25.10^a	24.90^a	-
Conductivity (µS/cm)	433.00^a	561.00^b	0 - 3 (mS/cm)
Turbidity (NTU)	4.35^a	50.03^b	NTU > 50
Dissolved oxygen (mg/L)	3.11^a	4.67^a	-

Values with the different letter in the same line are significantly different at $p < 0.01$.

for metals (mg·kg^{-1}) in soil [44]. Also, the results obtained in this study were lower than the values reported in the soils irrigated by Uwah *et al.* [28]. And yet values obtained ranges exceed the permissible limits for mineral soils in arid regions [45].

The sequence of heavy metals in the soil was found in the order of Cr > Mn > Zn > Pb > Ni > Cd > Cu. A comparison of the data with the criterion established by Kabata-Pendias and Pendias [46] for the approximate concentrations of heavy metals in plants revealed that Cr was in excessive or toxic concentrations. According to Doelman [47], N mineralization and nitrification of soil were inhibited at high concentrations of heavy metals in mg·kg^{-1} around 1000 for Zn, Cu and Ni, around 100 - 500 for Pb and Cr and around 10 - 100 for Cd. The rate of mineralization of soil organic matter appears to be sensitive to metal contamination in soil [48].

At high concentrations in soil, all heavy metals have also strong toxic effects on plants which results in weak growth, yield depression, disorders in plant metabolism and reduced nutrient uptake [12] [13]. Heavy metals not only inhibit root growth but also can hamper many physiological processes and, in particularly the uptake of nutrients [49]. The rate of absorption of elements by plants depends upon the cultivated plant and the soil properties, such as the pH, cation exchange capacity (CEC) and distribution of metals in different soil fractions [50]. At an acidic pH, high Mn concentrations in cultivated soils could pose a risk of toxicity to plant. Under an acidic pH, free Mn may be the predominant form in the soil solution, making it readily available for the plants. Renella *et al.* [51] have reported that in Mn and Zn-polluted soils, the solubility of Mn and Zn was significantly higher in the presence of organic acids, which are typically released by plant roots, thus suggesting that plants can mobilize trace elements via their root exudates.

3.3. Concentration of Heavy Metal Levels in Wastewater

Concentrations of Cd, Cr, Cu, Mn, Ni, Pb and Zn in different wastewater collected from Tanghin and Paspanga were given in **Table 2**. The sequence of heavy metals in waste water was found in the order of Zn > Cd > Mn > Pb > Cu > Ni > Cr. Excepted Cd (2.50 to 3.30 mg·L^{-1}) and Zn (3.60 to 3.66 mg·L^{-1}), the samples presented significant different ($p < 0.01$) of concentrations of heavy metals in the samples. The levels are varied quietly such as Cr (0.51 to 2.27 mg·L^{-1}), Cu (0.88 to 3.83 mg·L^{-1}), Mn (0.29 to 6.60 mg·L^{-1}), Ni (0.50 to 2.30 mg·L^{-1}), Pb (1.37 to 3.72 mg·L^{-1}). Excepted to Pb which was found to be within the acceptable safe limit, the results showed that all of the heavy metal concentrations exceeded the permissible limits in the wastewater samples set by FAO [52] as Cd (0.2 mg·L^{-1}), Cr (0.1 mg·L^{-1}), Cu (0.2 mg·L^{-1}), Mn (0.2 mg·L^{-1}), Ni (0.2 mg·L^{-1}), Pb (5 mg·L^{-1}) and Zn (2 mg·L^{-1}). Exceeding the safe limit or having a shortage of these elements may cause diverse problems for irrigated crops according to Balkhair and Ashraf [32]. The toxic and micronutrient elements may have an inhibitory effect on plant growth when their concentrations exceed the safe limit. Using wastewater in irrigation for a long period may lead to an accumulation of heavy metals in agricultural soils and plants. Therefore, the use of wastewater in irrigation leads to contamination of the food chain [53].

3.4. Concentration of Heavy Metal Levels in Lettuce Irrigated with Wastewater

Levels of the heavy metals obtained in the vegetable samples are shown in Table 2. There is no significant difference in concentration of heavy metals between samples of Tanghin and Paspanga with the exception of Mn (0.29 to 6.60 mg·kg^{-1}), ($p < 0.01$). Heavy metals were ranging as the concentration of Cd (1.27 to 2.93 mg·kg^{-1}), Cr (7.28 to 7.38 mg·kg^{-1}), Cu (0.91 to 1.70 mg·kg^{-1}), Ni (1.74 to 2.16 mg·kg^{-1}), Pb (1.32 to 1.69 mg·kg^{-1}), Zn (3.08 to 3.79 mg·kg^{-1}). These values were agreement with previous study of Uwah *et al.* [28].

The heavy metal levels in this study were also higher than the WHO maximum limit permissible (ML) in vegetables. The WHO maximum limit (mg·kg^{-1}) of some metals in vegetables are: Cd (0.02), Cr (0.1 - 0.2), Cu (0.1), Mn (0.3), Ni (0.1), Pb (0.1) and Zn (0.1) [54]. In the similar study, Shagal *et al.* [55] noted that *Lactuca sativa* grown in the same soil contained Cd, Cu, Pb, Mn and Zn in variables concentrations.

The level of heavy metals in plants depends mainly on the levels of soil contamination and plant species [28]. The results from the present study demonstrated that plants grown on wastewater-irrigated soils are contaminated with heavy metals and pose a health concern.

In heavy metals polluted soils some plant species are able to accumulate fairly large amounts of heavy metals without showing stress, which represents a potential risk for animals and human health because of transmission in the food chain [14] [56]. Many people could be at risk of adverse health effects from consuming common vegetables grown in contaminated soils [57].

The results simply explained that the heavy metals accumulated in the soils

Table 2. Concentrations of heavy metals in the soil, wastewater and the lettuce plant.

Site	Tanghin			Paspanga		
Parameters (mg·kg^{-1} or L^{-1})	Soil	Wastewater	Lettuce	Soil	Wastewater	Lettuce
Cd	3.66 ± 0.01^a	3.30 ± 0.03^a	2.93 ± 0.03^a	6.60 ± 0.13^b	2.50 ± 0.03^a	1.27 ± 0.06^a
Cr	196.39 ± 9.13^a	0.51 ± 0.03^a	7.28 ± 0.03^a	218.63 ± 10.2^b	2.27 ± 0.03^b	7.38 ± 0.03^a
Cu	1.01 ± 0.01^a	0.88 ± 0.03^a	0.91 ± 0.03^a	6.20 ± 0.35^b	3.83 ± 0.03^b	1.70 ± 0.03^a
Mn	62.96 ± 0.11^a	0.29 ± 0.03^a	0.26 ± 0.03^a	65.70 ± 1.48^a	6.60 ± 0.03^b	3.96 ± 0.03^b
Ni	15.86 ± 0.12^a	0.50 ± 0.03^a	1.74 ± 0.03^a	16.18 ± 0.03^a	2,30 ± 0.03^b	2.16 ± 0.03^a
Pb	20.65 ± 0.14^a	1.37 ± 0.03^a	1.32 ± 0.03^a	40.26 ± 0.03^b	3.72 ± 0.03^b	1.69 ± 0.05^a
Zn	44.56 ± 0.22^a	3.66 ± 0.03^a	3.79 ± 0.03^a	53.91 ± 0.03^b	3.60 ± 0.03^a	3.08 ± 0.03^a

Values with the different letter in the same line are significantly different at $p < 0.01$.

are transported to the vegetables through their roots by the process of absorption. The absorption and accumulation of heavy metals in plant tissues depend upon many factors according to Rupa *et al.* [58].

A variation in the metal concentration may be due to the variable factors like heavy metal concentration in soil wastewater used for irrigation and atmospheric deposition along with the plant's capability to uptake and accumulate the heavy metals [58].

3.5. Transfer Factor of Heavy Metals from Soil to Vegetables

The results of transfer factor (*TF*) of the heavy metals migrated from soil to lettuce of Tanghin and Paspanga sites were presented in **Table 3**. The *TF* values obtained were ranging for Cd (0.19 to 0.89), Cr (0.030 to 0.037), Cu (0.27 to 0.91), Mn (0.004 to 0.06), Ni (0.11 to 0.13), Pb (0.04 to 0.064), Zn (0.057 to 0.085) respectively in the samples of Tanghin and Paspanga. Statistical test of significance using ANOVA and t-test revealed no significant difference ($p > 0.01$) between the *TF* of heavy metals migrate from soil to lettuce samples of the two sites. The results of this study revealed that the mean values of Cd (0.63) and Cu (0.72) were closed to those found (Cd: 0.64, Cu: 0.67) by Ibrahim *et al.* [57]. But the mean values of *TF* of Cr (0.048), Mn (0.034), Ni (0.12), Pb (0.052), Zn (0.071) were lower than those reported by the same authors (Cr: 0.43, Cu: 0.67, Mn (0.68), Ni (0.36) Pb: 0.42, Zn: 0.71). The values of transfer factor of all the heavy metals in the lettuce were less than 1. According to Uwah *et al.* [59], it is easy for plants species with $TF > 1$ to translocate metals from roots to shoots than those which restrict metals in their roots (those with *TF* less than1). Khan *et al.* [60] on the other hand, explained that if the transfer coefficient of a metal is greater than 0.50, the plant will have a greater chance of the metal contamination by anthropogenic activities. This indicates that the levels of heavy metals in the investigated samples are low but, there is a chance for the samples of Tanghin to be contaminated with Cd ($TF = 0.80$) and Cu ($TF = 0.91$) by further anthropogenic activities. So the variations in transfer factors among lettuce samples in the

Table 3. Transfer factors (*TF*) of the heavy metals from soil to lettuce.

Heavy metals ($mg \cdot kg^{-1}$)	Site	
	Tanghin	Paspanga
Cd	0.80 ± 0.01^a	0.19 ± 0.02^a
Cr	0.037 ± 0.001^a	0.03 ± 0.001^a
Cu	0.91 ± 0.02^a	0.27 ± 0.02^a
Mn	0.004 ± 0.00^a	0.06 ± 0.001^a
Ni	0.11 ± 0.01^a	0.13 ± 0.01^a
Pb	0.064 ± 0.002^a	0.04 ± 0.002^a
Zn	0.085 ± 0.003^a	0.057 ± 0.001^a

Values with the different letter in the same line are significantly different at $p < 0.01$.

two studied sites may be attributed to differences in the concentration of metals in soil and differences in element uptake by different vegetables [61] [62].

Transfer factor is one of the key components of human exposure to heavy metals through the food chain. Transfer factors were computed for the heavy metals to quantify the relative differences in bioavailability of metals to vegetables or to identify the efficiency of a vegetable species to accumulate a given heavy metal [59]. Others authors as Lokeshwari and Chandrappa [11] then Awode *et al.* [63] demonstrated that *TF* was based on the root uptake of the heavy metals and not the foliar absorption of atmospheric metal deposits.

3.6. Daily Intake of Metals and Human Health Risk Assessment

The data for the evaluation of the *DIM* and *HRI* from the heavy metal-contaminated lettuce in two sites of production were presented in **Table 4**. The *DIM* values were estimated according to the average vegetable consumption for adults and compared with the recommended daily intakes [64] [65].

The results showed that the *DIM* and *HRI* values were low in the lettuce crop. The *DIM* ($mg \cdot kg^{-1} \cdot person^{-1} \cdot d^{-1}$) of the lettuce were ranging for Cd (0.0019 to 0.0037), Cr (0.0046 to 0.0047), Cu (0.00058 to 0.0011), Mn (0.00016 to 0.0025), Ni (0.0011 to 0.0014), Pb (0.00084 to 0.0011), Zn (0.00196 to 0.0024) respectively in the samples of the two sites. These results obtained were lower compared to *DIM* values found by Umah *et al.* [28] respectively for Cr (0.012 to 0.049), Ni (0.0027 to 0.0052), Mn (0.0012 to 0.016), Pb (0.018 to 0.033) and Cd (0.001 to 0.0031). The *HRI* values were lower than 1 and ranged respectively for Cd (0.0019 to 0.0037), Cr (0.0046 to 0.0047), Cu (0.00058 to 0.0011), Mn (0.00016 to 0.0025), Ni (0.0011 to 0.0014), Pb (0.00084 to 0.0011), Zn (0.00196 to 0.0024). These values were lower comparing to those of Balkhair and Ashraf [32]. *HRI* < 1, but it could not exclude a possible future human health risk via the intake of lettuce coming from sites of Tanghin and Paspanga.

Table 4. Daily intakes of metals (*DIM*) ($mg \cdot kg^{-1} \cdot person^{-1} \cdot d^{-1}$) and the Health Risk Index (*HRI*) for individual heavy metals in lettuce irrigated with wastewater.

Heavy metals	*RfD* ($mg \cdot kg^{-1} day^{-1}$)	Risk assessment index			
		Tanghin		Paspanga	
		DIM	*HRI*	*DIM*	*HRI*
Cd	0.5	0.0019^a	0.0037^a	0.00080^a	0.0016^a
Cr	0.3	0.0046^a	0.015^a	0.0047^a	0.016^a
Cu	0.2	0.00058^a	0.0029^a	0.0011^a	0.0054^a
Mn	0.14	0.00016^a	0.0012^a	0.0025^a	0.018^a
Ni	0.4	0.0011^a	0.0028^a	0.0014^a	0.0034^a
Pb	0.6	0.00084^a	0.0014^a	0.0011^a	0.0017^a
Zn	0.3	0.0024^a	0.0080^a	0.00196^a	0.0065^a

RfD: Oral reference dose (*RfD*); Values with the different letter in the same line are significantly different at $p < 0.01$.

3.7. Microbial Quality of Fresh Lettuce

The present study was intended to provide some assessment on the microbiological quality of lettuce and the results were assigned in Table 5. The counts of the different microorganisms enumerated were higher than the limits values of FAO/WHO [54]. The total aerobic mesophilic count (AM) ranged with significant difference ($p < 0.01$) from 8.25 to 9.96 log cfu·g^{-1} in lettuce samples respectively from Paspanga and Tanghin. This result was higher than values of reported by Soriano *et al.* [66] with a range of 3.0 - 7.8 log cfu·g^{-1}. According to Pianetti *et al.* [67], aerobic mesophilic count is useful for indicating the overall microbial quality of food product: generally it does not relate to food safety hazards but acts as an indicator for food quality and shelf-life duration.

The mean counts of total and fecal coliforms were ranged with no significant difference ($p > 0.01$) from 5.27 to 5.77 log cfu·g^{-1} and 4.85 to 4.99 log cfu·g^{-1} from lettuce, respectively. Total coliforms were higher than those (3.3 log cfu·g^{-1}) reported by Agbossou *et al.* [68]. But concentration of fecal coliforms was lower comparing to 5.35 log cfu·g^{-1} numerated by Thiaw [69]. *E. coli* were detected with no significant difference ($p > 0.01$) and ranged from 4.01 to 4.74 log cfu·g^{-1}. *Salmonella spp.* was reported with significant difference ($p < 0.01$) in the analyzed samples (25 g of food) from to 2.07 to 3.12 log cfu·g^{-1} respectively for Paspanga and Tanghin.

The microflora of vegetables is diverse but consists predominantly of gram negative bacteria such as *Enterobacteria*. The range for this group in both samples was 3.92 to 5.48 log cfu·g^{-1} and presented significant difference ($p < 0.01$). Agbossou *et al.* [69] found lower value (2 log cfu·g^{-1}).

The level of *Enterobacteria* found in this study is common in raw vegetables and are not necessarily associated with fecal contamination, because the majority of the genera are part of the endogenous microflora of the product.

Staphylococcus spp. occurred with no significant difference ($p > 0.01$) respectively

Table 5. Microbial characteristics of lettuce from Tanghin and Paspanga.

Microorganismes (log cfu·g^{-1})	Vegetable (Lettuce)	
	Tanghin	Paspanga
Total mesophilic aerobic	9.96^{a}	8.25^{b}
Total coliform	5.77^{a}	5.27^{a}
Fecal coliform	4.85^{a}	4.99^{a}
E. coli	4.01^{a}	4.74^{a}
Salmonella spp.	3.12^{a}	2.07^{b}
Enterobacteria	5.48^{a}	3.92^{b}
Staphylococcus spp.	3.10^{a}	2.35^{a}
Pseudomonas spp.	0.30	00
Yeast and moulds	6.97^{a}	5.88^{b}

Values with the different letter in the same line are significantly different at $p < 0.01$.

2.35 to 3.10 log cfu·g^{-1}. Cesar *et al.* [70] assumed that *Staphylococcus* contamination could cause production of toxins in lettuce when count over 5 log cfu·g^{-1}.

Pseudomonas spp. mean counted only on lettuce samples of Tanghin (0.30 log cfu·g^{-1}) and was lower those of Pianetti *et al.* [67] in ready-to-eat vegetables salads (7 log cfu·g^{-1}). This germ is undesirable because it is often responsible for spoilage of fresh vegetables due to the production of pectinolytic enzymes which cause breakdown of the peptic polymers in plant cells [71].

Yeasts and moulds (YM) mean counts were 5.88 to 6.97 log cfu·g^{-1}from Paspanga and Tanghin lettuce, respectively. These means were higher those of Abadias *et al.* [72]. Tournas and Katsoudas [73] referred the possible health problems associated with the presence of moulds in vegetables, as some may produce mycotoxins and others are known to cause allergies when they are able to produce large numbers of conidia.

4. Conclusions

This study reveals that the untreated wastewater is the primary source of soil pollution, and irrigation with contaminated water containing high amounts of heavy metals and microorganisms lead to increase the concentration of metals and foodborne pathogens on the *Lactuca sativa*. Long-term use of wastewater as irrigation purpose may lead to severe risk to consumer's health as, this study has already shown a severe risk to human health.

The continuous monitoring of the soil, plant and water quality along with preventing metals from entering the vegetables are prerequisites for the prevention of potential health hazards to human beings.

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Conflicts of Interest

Authors declare that we have no conflict of interest.

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