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Microbial contamination of Lettuce (*Lactuca sativa*) and soil profile in Kirkuk regions under controlled application of domestic wastewater

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Introduction

ABSTRACT

Background: In arid countries, the growing scarcity of potable water causes untreated wastewater to be used for crop production, but improper irrigation techniques could pose a serious risk of sewage water contamination of plants, soils, and groundwater, This study was prompted by the increasing risk of harmful bacterial contamination in vegetable crops and groundwater due to the application of wastewater on land. **Methods:** The purpose of the study was to investigate (1) how field-grown lettuce plants' microbial contamination is affected by irrigation with untreated wastewater; (2) Fecal coliform bacteria contamination of agricultural soil. From the wastewater flowing in the Al-Khasa River and from the waters of the Lower Zab River in the city of Dibs, Kirkuk Governorate, Iraq. Lettuce plants were grown in two different locations. **Results:** Bacterial isolates were extracted from wastewater and plants, *Alcaligenes faecalis, Burkholderia gladioli, Enterbacter kobei, Enterbacter hormaechei, Enterbacter ludwigii, Enterococcus columbae, Escherichia coli, Globicatella sulfidifaciens, Klebsiella oxytoca, Klebsiella pneumonia, Morganella morganii, Pseudomonas otitidis, Pantoea agglomerans, Sphingomonas poucimobilis, Staphylococcus lentus* and *Staphylococcus sciuri*. Total bacterial isolates 38 (63.16 % isolation from wastewater running in khasa river, 5.26 % isolation from lower zab river and 31.58% isolation from plant irrigated with wastewaters). Bacteria were detected per 100 g of dry matter and compared with the control. In order to detect fecal coliform, a sample of the soil profile was also taken at a precise distance of 3 cm from the soil surface. The findings showed that when soil is irrigated with water from the lower Zab River, the number of all bacterial species has significantly decreased. The higher the percentage of wastewater in the irrigation water, the higher the bacterial count/g of the plant root system. By measuring BOD, COD , pH, , chloride, ammonia, conductivity, suspended particles, total dissolved solids, lipids, phosphate, nitrite, and , nitrate. **Conclusion:** each step of pollution levels in the untreated wastewater of the Alkhasa River was examined, and comparisons with the lower Zab River were made and Human behavior and natural processes.

Introduction

Contaminated water sources are frequently recycled, especially in developing nations. An estimated 2 billion people depend on fecal microorganism-contaminated water for food and crop irrigation, among other uses [1]. Per 100 g of dry matter, bacteria were found and compared to the control. In order to detect fecal coliform, a sample of the soil profile was also taken at a precise distance of 3 cm from the soil surface. The findings showed that the

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number of all bacterial species in the soil irrigation using water from the lower Zab River had significantly decreased. The more wastewater there was in the irrigation water, the higher the bacterial count/g of the plant root system. Ammonia, pH, BOD, COD, chloride, conductivity, and suspended particles can all be measured, total dissolved solids, Transmissions of intestinal nematode, pathogenic bacteria, those causing diarrhea, dysentery, typhoid, and cholera to farmers operating in the wastewater-irrigated fields and vegetable consumers were the principal dangers of the usage of untreated water [2]. Determination of diverse microbial pathogenic species in untreated water samples is crucial. The potential negative impact of using treated wastewater to irrigate edible crops is a constant source of concern and caution. As a result, establishing guidelines for the reuse of both treated and untreated wastewater in agriculture worries both local and international organizations. Corn, potato, and lettuce plants are irrigated using treated effluent were higher than plants that were irrigated using natural water resources. This could be because the treated wastewater contained plant nutrients, primarily nitrogen and phosphate, but the risk posed by some diseases is still being taken into account. Numerous interactions of plants, soil, and bacteria have a significant impact on plant productivity, water quality, and soil health [3]. Fecal coliform bacteria are used as indicators of water pollution and have been used for many years to assess the safety and quality of water for irrigation and human consumption. which are found in both human and animal feces, are generally being to the human intestines. The most prevalent species of these bacteria *Escherichia coli* [4]. In actuality, the objective is to have fecal coliform-free water that may be utilized to irrigate crops that are still raw. Effectively treated wastewaters that satisfy microbiological quality standards should be utilized for crop irrigation in order to stop the spread of numerous diseases; fecal water contamination is a major public health concern [5]. Although their absence does not always imply the absence of pathogens, the presence of these bacteria in treated wastewater suggests a breakdown in the disinfection process. Assessing the risks of water river contamination by land-applied untreated wastewater requires knowledge of the fate and movement of bacteria in soils. Contaminant transport studies can be used to both objectively assess microbial transfer in soils to plants and to explain the essential elements and mechanisms regulating microbial transport. Therefore, as bacteria are essential in revealing their ability to biodegrade in order to support environmental restoration, bioremediation, if used for this purpose, could be a potential solution to the growing problems caused by environmental contamination [6]. Soil contamination is arguably the world's most dangerous issue; these new pollutants need to be tackled to protect our living environment. Human activities and rapid industrialization have harmed the environment and negatively impacted the health of people and animals. Wastewaters from agro-industry must be adequately, economically, and completely treated because they endanger the environment [7]. Several studies have looked into how microorganisms move across soil following wastewater application. Polluted environments can be improved and sustainably cleaned thanks to their capacity to control nutrient release and establish stable growth inside plants. current studies, demonstrating noteworthy outcomes. Contamination of water poses a severe threat to human health [8]. An experiment comprising two site cultivation campaigns examined the impact of wastewater irrigation on the composition and diversity of bacterial communities in soil mesocosms containing lettuces. The goal of these investigations was to allay growing worries about fecal pollution in the environment and the possibility of human exposure to enteric pathogens through other environmental pathways, especially foodborne and recreational exposure routes [9]. Without any laws or rules, many local farmers in Kirkuk City grow vegetable crops using untreated wastewater. The major goal of this study was to investigate and determine the microbiological risks related to the use of untreated household wastewater for field irrigation of vegetable crops and the contamination of the soil profile by wastewater application that contains fecal coliform bacteria. To attempt accomplish this goal, the effluent of the wastewater flowing in the Khasa River was examined for certain pathogenic bacteria as well as chemical and physical examination. which was used to irrigate the lettuce plant and transport the experiment of wastewater and compare it with the lettuce plant irrigated with water from the Alzaab River. To measure the spread of fecal coliform, a soil column was also examined [10].

Material and Methods

Site description

The first site collected from rhizospheric lettuce plant samples were examined in the wastewater discharge flowing in the Kirkuk Governorate's Khasa River; about 264 km north of Baghdad city in Iraq, area of Interest' in **(Fig. 1)**. The second site for collecting samples is in the aldibs city, a lettuce plant irrigated with water from the lower zab river during the period from December 2023 and January 2024, The maturity date for lettuce in northern Iraq in two site.

Water collection and analysis

Samples of water used for irrigation of soil and plant crops, were collected from the farmland of selected areas of Kirkuk city. The first site to collect untreated wastewater samples (one-liter volume), from investigated in the effluent of the wastewater running in khasa river and The second site is in the aldibs city, water samples irrigating lettuce plants from the lower zab river, were collected, in sterilized, wide-mouthed glass bottles. The samples were promptly moved to the lab in an ice tank. The wastewater from the experimental initial locations was categorized as having a pH of 7 and an EC of 720 ms/cm, according to the physical and chemical properties provided. The second site for collecting samples is pH 8.3, EC 420 ms/cm.

Microbiological examination

Bacteria were isolated using the standard plate count method. Serial dilution and gram staining was done. For serial dilution, 9 ml of diluent or distilled water was pipetted into six test tubes for making three-fold dilutions. Next, a micropipette must be used to collect 1 milliliter of the material. Each dilution's aliquots were spread-plated after being moved to the plates. For 24 to 48 hours, these plates were incubated at 37 oC. The plates showed adequate development the next day, and colony forming units (CFU) were counted [11]. Isolation of bacteria had been accomplished were identified using biochemical tests and staining in microbiology laboratory/ college of education for women / University of Kirkuk. The species of these isolates during the period from December 2023 and January 2024 were identified by VITEK 2 system. The bacterial families identified are (*Enterobacteriaceae, Staphylococcaceae, Enterococcaceae, Burkholderiaceae, Aerococcaceae, Sphingomonadaceae* and *Erwiniaceae)*. Following the description of physical and chemical characteristics, 100 ml of each water sample from every site were filtered using Microfil S filtration devices (0.22-lm pore size; Millipore, Billerica, MA) to determine the total viable bacterial counts in the water sample (CFU/100 ml). The filters were incubated at 35°C after being put on heterotrophic tryptic soy agar plates [12]. From lettuce roots, river water, and sewage water, 38 distinct bacterial species were identified. The number obtained from (**Fig. 2**) study sites and percentage of bacteria isolated from the first site, the Khasa River, was 24 (63.16%) from the sewage water, and from the roots of the lettuce plant watered with untreated sewage water for the same site, 12 (31.58%). The bacteria isolated from the second site were 2 (5.26%) from the roots of the lettuce plant irrigated with the water of Lower Zab River. No bacterial species was isolated from water of the Lower Zab River.

Detection and enumeration of *E. coli* were determined using membrane filter procedure and mTEC Agar (Difco 0334) as described by EPA (2002). Colonies confirmation was determined using Urea Substrate Medium (100 ml of distilled water, 2.0 g of urea, and 0.01 g of phenol red). Multiple tube fermentation (MTF) techniques were used to count the total number of coliforms in the wastewater [13].

Results and Discussion

Many nations throughout the world are concerned about the quality of their water due to the limited availability and rising demand brought on by population growth. Therefore, it is crucial to promote the use of treated wastewater for irrigation in arid and semi-arid regions. Water used for irrigation or supplied to consumers should at the very least be free of harmful compounds and pathogenic organisms, as well as meet the strict standards of contemporary hygiene, sewage for agricultural irrigation remains a source of argument among government authorities and policymakers [14]. Research into new or more effective antibacterial chemicals is crucial because of the rise of multidrug-resistant pathogens. The World Health Organization released a list of priority multidrug-resistant bacteria that will need new antibiotics to eradicate. Among these, the "critical" (most urgent) group includes *Pseudomonas aeruginosa*, *Enterobacteriaceae*, *Burkholderiaceae, Aerococcaceae, Sphingomonadaceae* and *Acinetobacter baumannii*. As a result, significant research is being conducted all over the world to

find novel antibacterial substances [15]. This has led to global initiatives to track and assess treated wastewater's microbiological quality, Further knowledge of the molecular relationships between plant pathogens and their human and animal hosts is necessary to comprehend the magnitude of disease incidence and pinpoint disease prevention strategies. Until recently, it was believed that plant pathogenic bacteria and fungi had little significance in causing diseases in both human and animal systems. However, new research indicates that infections in humans and animals brought on by bacteria, viruses, and plant pathogenic fungi may have serious effects on both human and animal health [16]. The current study was conducted to assess the water quality, physico-chemical characteristics, and microbial flora of the effluent of the Alkhasa and Lower Zab rivers in addition to looking into the effects of wastewater quality use. This is because before water is widely used in agriculture, a strong monitoring program is necessary to offer reliable information on its current quality. By measuring biological oxygen demand (BOD), chemical oxygen demand (COD), chloride, ammonia, pH, conductivity, suspended particles, total dissolved solids, nitrate, nitrite, lipids, and phosphate, the pollution levels of the untreated wastewater from the Alkhasa River were evaluated and compared to those of the lower Zab River. Surface waters are harmed, and their usefulness for drinking, industry, and agriculture is limited by both natural processes and human activities (development, industry, and agriculture; growing demand for water resources) [17]. **(Table 1)** displays the chemical and physical analysis of the alkhasa river and lower zab river. The average water pH were 7.65, respectively, while the electrical conductivity of the alkhasa river sample showed a higher value to lower zab river samples was 720 ms/cm and 420 ms/cm, respectively, was positively correlated with both total bacterial numbers and anion concentrations, high temperatures have been shown to promote the growth and development of human infections.

Research revealed that fecal coliform, and total coliform were positively correlated with water temperature[18]. Both processes are strongly impacted at high pH values. The normal range established by the World Health Organization is between 7.0 and 8.5, which is the usual range of measured pH levels. For the lower zab river and untreated wastewater of alkhasa river, the COD values were 11 and 218 mg/L, respectively, identified a comparable circumstance in Jordan, where two monitoring wastewater treatment stations were examined for water quality both before and after station upgrades. The suspended solids (SS) and biochemical oxygen demand (BOD) of alkhasa river untreated wastewater, on the other hand, were 70 and 5 mg/L, respectively. According to APHA (1992), the presence of microorganisms had the biggest impact on BOD. (17, 3), (0.015, 0.01) and (1.3, 0.1) mg/L were the concentrations of NH_3^+, NO_2 and NO_3, respectively. The presence of

The presence of numerous nutrients and ions in wastewater, particularly nitrate (NO_3), was recognized to promote plant development. However, irrigating wastewater in excess of the soil-plant system's capacity to absorb it can result in the release of easily leachable contaminants or nutrients [19]. The O&G concentration in the untreated wastewater of alkhasa river and lower zab river 56 and 0 mg/L as well.. Industrial processes have adverse effects on ecosystems and contribute to environmental contamination, especially through unregulated wastewater discharges. Because they are insoluble, vegetable oils worsen this pollution by creating impermeable layers and interfering with oxygen transfer, which causes major habitat disruption [20]. Many biological agents present significant health risks in addition to dangerous heavy metals, including the potential to spread bacterial infections and intestinal worms, particularly to consumers and agricultural workers. Gastrointestinal disturbances, including nausea, vomiting, and diarrhea, are the most frequent signs of waterborne sickness. In vulnerable groups, including young children, the elderly, and people with weakened immune systems, the symptoms can be more severe, long-lasting, or even lethal, highlights some of the main foodborne microbial organisms that public health professionals and doctors must deal with it [21]. The amount of heterotrophic, total coliform (TC), fecal coliform (FC), and total streptococci per 100 milliliters of alkhasa river untreated wastewater (ARUWW) and lower zab river water (LZRW) samples was measured. **(Table 2)** compares the results to the FAO-recommended benchmark. ARUWW showed somewhat lower TC and FC counts than WHO's 1989 recommendations but had higher TC, FC, TS, *E.coli*, concentrations than LZRW. These bacteria are a sign of water untreatment, However, the presence of E. coli is a far more reliable indicator of

fecal contamination. A positive TC test result should be followed by FC, *E.coli*, or enterococci tests to confirm contamination, though, as TC bacteria may not always be fecal in origin [22]. The most accurate contamination indicators were found to be TC and FC tests, according to data from a source with known contamination. With a few exceptions, such as sterilized surfaces, bacteria are present everywhere in the world. The majority of normal, nonpathogenic flora and the few pathogenic species are made up of them. Thus, microbial activity and human activity are closely related. Though they are a great component of the human diet, fresh fruits and vegetables are tainted with a variety of dangerous microbes. Vegetables must be grown as rapidly as possible to meet the growing demand, which increases the risk that they may become contaminated with dangerous germs and raises questions over consumer safety. Examining the sources of contamination and the types of dangerous etiological agents present in fresh produce [23]. Depending on where they are found in the human body, many Bacteroides species can have both beneficial and damaging functions. They are frequently beneficial in the gut but opportunistic pathogens elsewhere, where they can lead to bacteremia and the formation of abscesses [24], [25]. Because there was a risk of bacterial infection to agricultural workers and surrounding communities, it has a direct impact on the health of workers in the fields, there was no fecal coliform guideline for restricted irrigation. The addition of a fecal coliform recommendation was proposed by evidence of enteric infections in farming households who were in close proximity to sprinkler-irrigated fields and in populations who had direct contact with partially treated effluent when the water quality exceeded 10^6 FC/100 ml [26].

The ability of microorganisms to remove toxic pollutants makes bioremediation an innovation that can be used in a variety of soil and water conditions. Various methods have been tried to remove the contaminants from the water and maintain water quality, and microbial bioremediation is a promising strategy to reduce these contaminants from the contaminated water [27]. New waste treatment procedures are necessary as a result of the environment's harsh waste discharge limits being put into place and enforced [28]. Finding effective substances to inhibit the activity of multidrug-resistant (MDR) bacteria is one way to address the urgent problem that needs immediate attention, as many antibiotics are no longer effective due to the fact that pathogenic bacteria in hospitals and various contaminated environmental communities are becoming more resistant to multidrug [29]. Finding novel antibiotics or altering already-existing ones is the main strategy used to fight bacteria that are resistant to antibiotics. The employment of bacteriophages is a viable substitute, nevertheless. Hospital sewage and wastewater have yielded more potent bacteriophages than other sources [30]. As expected, wastewater had an impact on the amount of bacteria; the higher the wastewater fraction, the higher the bacterial count. Every category of bacteria showed the effects of different wastewater properties, with the exception of the total quantity of bacteria. In other words, the significance of every distinct effluent quality is always clear. Because of this, it is not a single noteworthy similarity between two distinct water qualities. The results obtained from (**Fig. 2**) study sites and percentage Al-Khasa River untreated wastewater (ARUWW) were confirmed by isolating the following species (63.16 %) : *Alcaligenes faecalis, Burkholderia gladioli, Enterbacter kobei, Enterbacter hormaechei, Enterbacter ludwigii, Enterococcus columbae, Escherichia coli, Globicatella sulfidifaciens, Klebsiella oxytoca, Klebsiella pneumonia, Morganella morganii, Pseudomonas otitidis, Pantoea agglomerans, Sphingomonas poucimobilis, Staphylococcus lentus* and *Staphylococcus sciuri*. From samples of the Lower Zab River water (LZRW) (5.26 %), *Alcaligenes faecalis, Escherichia coli*, were isolated, which is the best evidence of microbial contamination. Twelve bacterial species (31.58 %) emerged from a lettuce plant that was irrigated with steam water, while no bacteria were found in a lettuce plant that was watered from the Lower Zab River in Dibs. This indicates that while the Zab River was either very clean or had a low rate of pollution, private water was contaminated at very high levels. The situation is significantly different in terms of the overall number of germs, which enumerates some of the main foodborne microbiological agents that public health professionals and doctors must deal with [31]. However, bioremediation methods, which employ plants both alone and in conjunction with other beneficial bacteria, are innovative methods for restoring contaminated soils. The bioremediation of soils contaminated in a variety of novel methods is covered [32]

Table 1. The irrigation characteristics alkhasa river untreated wastewater (ARUWW) and lower zab river water (LZRW) .

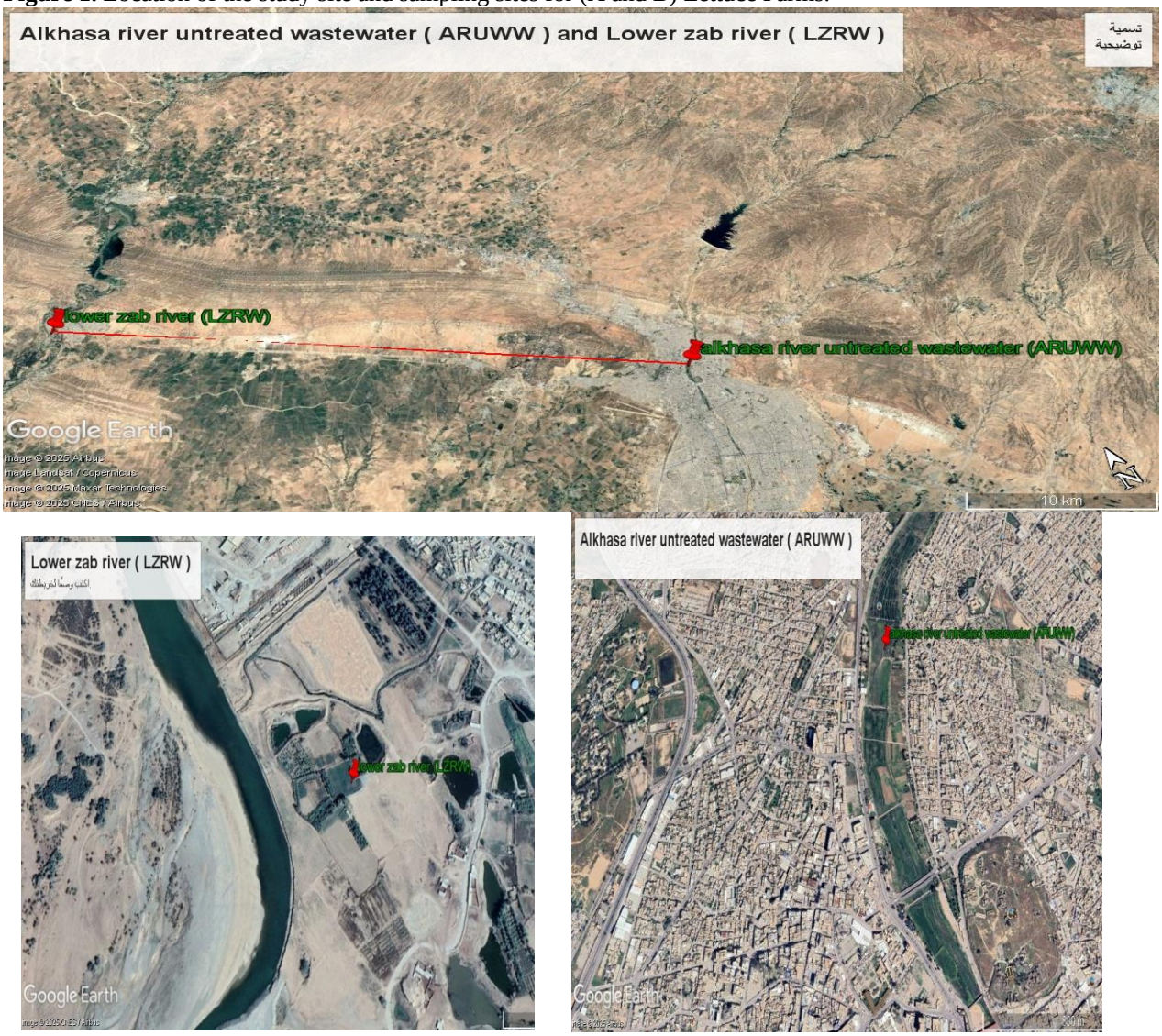
Parameter	ARUWW	LZRW	Effluent Discharge Maximum Standard
PH	7	8.3	6.0 -9.5
EC ms/cm	720	420	1000
TDS (mg/L)	574	216	--
TSS (mg/L)	200	38	60
BOD (mg/L)	70	5	40
COD (mg/L)	229	9	100
NO_2 (µg/l)	0.015	0.01	Nil
NO_3 (µg/l)	1.3	0.1	50
CL (mg/L)	75	11	600
NH_3 (mg/L)	17	3	10
PO_4 (µg/l)	0.371	0.012	3.0
O&G mg/L	56	0	10

EC- electrical conductivity (ds/m), COD – chemical oxygen demand (mg/l), BOD- biological oxygen demand (mg/l), TDS- total dissolved solids, TSS- Total suspended solids, SAR- sodium adsorption ratio.

Table 2. Frequency and percentage of bacterial contamination in lettuce plant and water collected from Kirkuk Governorate, Iraq during December 2023 to January 2024.

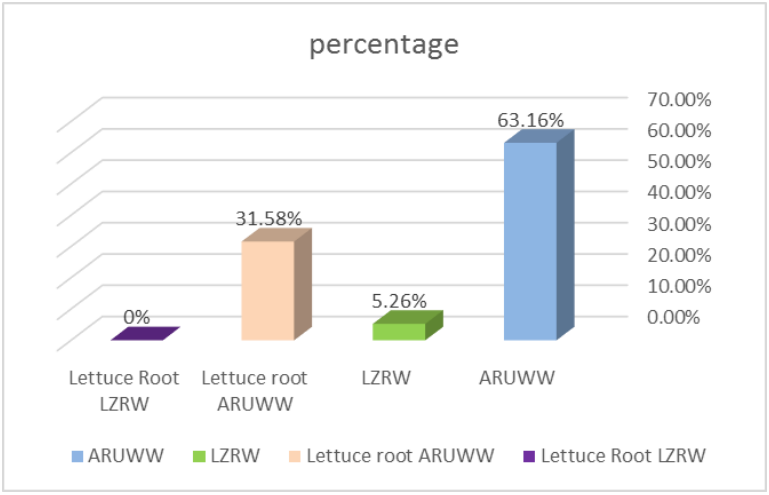
Organism	Bacteria Isolated from Water ARUWW N (%)	Bacteria Isolated from Water LZRW N (%)	Bacteria Isolated from Lettuce root ARUWW N (%)	Bacteria Isolated from Lettuce root LZRW N (%)	Total N (%)
Alcaligenes faecalis	3 (12.5)	1 (50)	1 (8.3)	0	5 (13.16)
Burkholderia gladioli	1 (4.17)	0	2 (16.7)	0	3 (7.9)
Enterbacter kobei	2 (8.33)	0	0	0	2 (5.3)
Enterbacter hormaechei	1 (4.17)	0	1 (8.3)	0	2 (5.3)
Enterbacter ludwigii	1 (4.17)	0	0	0	1 (2.7)
Enterococcus columbae	2 (8.33)	0	1 (8.3)	0	3 (7.9)
Escherichia. coli	3 (12.5)	1 (50)	0	0	4 (10.5)
Globicatella sulfidifaciens	1 (4.17)	0	2 (16.7)	0	3 (7.9)
Klebsiella oxytoca	1 (4.17)	0	1 (8.3)	0	2 (5.3)
Klebsiella pneumoniae	2 (8.33)	0	0	0	2 (5.3)
Morganella morganii	2 (8.33))	0	1 (8.3)	0	3 (7.9)
Pseudomonas otitidis	1 (4.17)	0	1 (8.3)	0	2 (5.3)
Pantoea agglomerans	2 (8.33)	0	1 (8.3)	0	3 (7.9)
Sphingomonas poucimobilis	1 (4.17)	0	0	0	1 (2.7)
Staphylococcus lentus	0	0	1 (8.3)	0	1 (2.7)
Staphylococcus sciuri	1 (4.17)	0	0	0	1 (2.7)
Total	24 (63.16 %)	2 (5.26 %)	12 (31.58%)	0 (0 %)	38 (100%)

Figure 1. Location of the study site and sampling sites for (A and B) Lettuce Farms.



A: Lower zab river (LZRW) and Lettuce Farms. B: Alkhasa river untreated wastewater (ARUWW) and Lettuce Farms.

Figure 2. study sites and percentage for Al-Khasa River untreated wastewater (ARUWW), Lower Zab River water (LZRW), Lettuce root (ARUWW) and Lettuce root (LZRW).



Conclusion

The study most likely observed that the use of household wastewater had an impact on the soil's microbial content as well. An increase in the soil microbial load, particularly pathogenic microorganisms, which may survive or build in the soil over time, may have been found and emphasized the hazards to public health, particularly for areas where agriculture depends on such irrigation techniques. To lessen contamination in crops and soil, suggestions for better wastewater treatment or other irrigation techniques may have been made. This study also confirms that the number of bacteria in the developing plant rises in proportion to the amount of wastewater present in the irrigation water. Additionally, it suggested the need for additional research on crop contamination brought on by other causes, such as climatic circumstances, and partially supported the reuse of wastewater without restrictions. Use of wastewater with restrictions. To ensure that developing bacterial pathogens are reduced to undetectable levels or to levels associated with no risks to human health, wastewater treatment plants must be used before discharging it into Khas River.

Recommendations for Further Research

More research on effective wastewater treatment systems, especially during dry seasons, and their effects on soil, human health, and crop output may have been required by the study's findings. The importance of regulations or laws regarding the use of domestic untreated water in agriculture following the disposal of harmful organisms is emphasized.

Conflict of interesr

None declared

Financial disclosure

None declared

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