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Article

Treated Greywater Reuse for Hydroponic Lettuce Production in a Green Wall System – Quantitative Health Risk Assessment

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Abstract: The scarcity and pollution of freshwater are extremely crucial issues today and the expansion of water reuse have been considered as an option to reduce its impact. This study aims to assess the efficiency of an integrated greywater treatment system and hydroponic lettuce production as a part of a green wall structure and to evaluate the health risk associated with the production and consumption of lettuce through quantitative microbial risk assessment (QMRA) and chemical health risk assessment. The study was conducted based on the unique configuration of source separation system; on-site greywater treatment system; green wall structure as a polishing step; and hydroponic lettuce production in the green wall structure. The final effluent from the system was used to grow three lettuce varieties by adding urine as a nutrient solution. Both water samples and plant biomass were collected and tested for *E. coli* and heavy metals contamination. The system has gained a cumulative 5.1 $\log_{10}$ reduction of *E. coli* in the final effluent and no *E. coli* found in the plant biomass. QMRA results indicated that the system attained the health-based targets, 10^{-6} DALYs per person per year. Similarly, health risk index (HRI) and targeted hazard quotient (THQ) results did not exceed the permissible level, thus the chemical health risk concern was insignificant.

Keywords: Source separation system; Greywater treatment; Water reuse; Hydroponic system; Green wall; Heavy metals bioaccumulation; QMRA; Health risk assessment

1. Introduction

Water scarcity and water pollution are causing serious health and environmental challenges around the world for a large proportion of the world population, either proper infrastructure is absent or wastewater is discharged untreated to the recipients. Water, energy and nutrients in wastewater also represent valuable resources, which are needed to supply a growing population. Future wastewater infrastructure should therefore serve as combined resource recovery factories and wastewater treatment facilities, established as decentralized, semi-centralized, centralized or combined systems, depending on local needs and constraints. Some regions are facing water stress and groundwater depletion because of population growth, frequent drought occurrence (low rainfall), or in combination with over exploitation of local groundwater and wastewater transported far away from the point of water extraction/use. In those areas local groundwater recharge and reduced water consumption is crucial and this calls decentralized or semi-centralized treatment and recovery systems. Systems that are based on source separation have been suggested as an efficient strategy for nutrient recovery and water reuse [1,2].

Source separation of domestic wastewater is a system that provides an opportunity to collect the toilet waste separately, containing the majority of the nutrients and carbon but also waterborne pathogens, which may constitute a major risk factor unless handled properly. Simultaneously, the system collect greywater, with is much less concentration of pathogens than in combined domestic wastewater and constitutes most of the wastewater quantity in households' wastewater. The source of greywater is from kitchen and bathroom sinks, shower and laundry; whereas blackwater consists of urine, faecal material, toilet paper and flushing water from the toilet. In addition to the two broad

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classes, urine or urine with minimal flush water can be collected separately as yellow water [3-6]. Approximately, 90% of the total Nitrogen and 80-90 %of total Phosphorus in domestic wastewater originates from the urine fraction, which constitutes only 2 % of the wastewater volume. Greywater separation from blackwater offer chances to treat most of the wastewater easily using on-site treatment systems to a quality that can be discharged into the local water recipients or reused for non-potable purpose without negative effects on health and environment if it is treated properly.

Major concerns associated with water reuse is the quality of the wastewater in terms of microbial pathogens, heavy metals, organic pollutants, components in pharmaceutical residues and personal care products, which is threatening the public health when reused directly with insufficient treatment option. This potential threat can be reduced through proper wastewater treatment technologies as well as through efficient utilization systems. One of the most promising strategy to rise the coverage of domestic wastewater reuse as well as to reduce associated public health risk is the integration of source separation system with appropriate wastewater treatment technologies and growth systems that are effective, simple to operate, consuming less energy, environment friendly, and low cost (investment, operation and maintenance) [7]. The system becomes more effective and robust when the regular treatment system is further integrated with polishing steps like granular filtration. Moreover, selection and use of less risky irrigation methods for plant growth further reduce public health and environmental risks.

Treated greywater can be utilize for non-potable purposes such as agriculture, flushing toilets, landscaping, and aquifers recharge thereby address the issue of imbalance between water supply and demand in a given region [8]. Treated wastewater reuse for agriculture is widely applied in the arid and semi-arid areas around the world. Likewise, treated domestic wastewater reuse in urban areas is increasing, especially in large cities [9]. However, health risk is one of the limitations of treated greywater utilization for plant production. The health risk associated with treated wastewater reuse for vegetables production and non-potable consumption depends on factors like the quality of treated wastewater, the irrigation method used, the time interval between irrigation-harvest-consumption, and producer and consumer habits [10]. Treated domestic wastewater may contain limited amounts of essential nutrients for plant growth, however it might be inadequate for plant growth and we need to supplement additional nutrients. Crop production using treated wastewater has been challenged by inadequate supply of nutrients, particularly nitrogen [9]. This could potentially be supplied by use of source-separated urine as a nutrient solution. The application of an integrated system between treated greywater and source-separated urine for hydroponic crop production could increase the efficiency of the system in terms of utilizing nutrients from wastewater, maximizing water reuse potential, increasing control on quality of water and reducing the risk of pathogen contamination.

Green walls, also known as vertical gardens is a plant growth system attached to the walls of buildings that refer to all forms of vegetated wall surfaces. The advancement of green wall technologies provide a broad range of options for designers to realize multiple objectives and to bring freestanding design features on the interior and exterior of buildings [11]. One of the option is to integrate with a building infrastructure like a component of on-site greywater treatment and at the same time, green wall plants get water and nutrients from the system. The integration of such treatment system with a green wall technology provide many environmental and financial benefits. The green wall provides additional layer with dual effects as it act as an insulator, reducing the need for cooling energy during summer and heating energy during winter, respectively. It is also aesthetically appealing, and improve air quality by reducing CO_2 level and increasing oxygen. Moreover, a green wall designed for urban agriculture can bring various benefits such as providing the basis for healthier community interaction (community gardening) and improving access to fresh food [11-13].

Greywater, however, may contain various microbial pathogens and hazardous chemicals depending on the nature of raw greywater and the treatment efficiency. Irrigation of wastewater for vegetables and food crops may result in bioaccumulation of heavy metals and at the same time, it

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may cause contamination of plant products with microbial pathogens. Various health problems can occur and develop due to the consumption of contaminated vegetables and the consumption of heavy metals contaminated food and this may cause disruption of various biological processes in the body leading to a decreased immunological defence, growth retardation, disability associated with malnutrition, cardiovascular, neurological, kidney and bone diseases [14,15]. Quantitative microbial risk assessment (QMRA) models and chemical health risk assessment (CHRA) approaches will enable us to evaluate the adverse health effects of operational activities and the consumption of vegetables, and support the risk management decisions.

Quantitative microbial risk assessment (QMRA) models have been used to evaluate the health risk associated with treated wastewater irrigation of vegetables and food crops [10,16-21]. On the other hand, the health risk of heavy metals bioaccumulation in vegetables and food crops irrigated by untreated and treated wastewater evaluated in different studies [22-26]. This study was conducted in a unique configuration of an on-site greywater treatment system, granular filtration as part of green wall structure and hydroponic lettuce production system. The aim of this study is to assess the efficiency of an integrated system and to evaluate the health risk associated with the production and consumption of lettuce through quantitative microbial risk assessment (QMRA) and chemical health risk assessment (CHRA) approach.

2. Materials and Methods

2.1. System configuration

Source separation for wastewater management system was established in 1997 at Norwegian University of Life Sciences (NMBU) student dormitory serving for 48 students at Kaya, Ås. The greywater system collect wastewater from washbasins, showers, kitchen sink, and laundry whereas the blackwater system, collect toilet waste separately. Both systems pumped into the laboratory (fløy 4) in a separate pipeline, for different experiments and the source separation system is described in detail in [27]. In this study, the greywater first treated with package greywater treatment plant (biofilter system), which encompasses a sequence of a primary settler, an unsaturated fixed-film biofilter and a secondary clarifier. Furthermore, the effluent from biofilter system polished by infiltration system. Three filtration column (2.5 meter height and 31.5 cm diameter), as a part of green wall were constructed in order to polish the effluent discharged from the greywater treatment plant (GWTP). The filtration column were constructed with three layers, thus are; 1-meter bottom layer is 0.8-1.6 mm diameter filtralite, 0.3-meter in the middle consisted granular activated carbon, and 1.1-meter on top of the activated carbon is 2-4 mm filtralite. The top 10 cm air space used to feed the water uniformly from the top of the column by using nozzles. The effluent from the filtration columns collected in the bucket at the bottom of the column and used to grown lettuce hydroponically by adding human urine, which stored for three months. The plantation pots mounted on the green wall shelves and irrigated with the treated greywater from the buckets by using small submersible pumps for circulation (Figure 1).

2.2. Lettuce pots alignment and hydroponic system

A flush and drain hydroponic system was designed with perlite as a growth medium. Perlite is a volcanic porous lightweight and inert material, which is commonly used for hydroponic plant growth. Three lettuce varieties namely, a) *Lactuca sativa 'Lobjoits Green Cos'*, b) *Lactuca sativa 'Red Salad Bowl'* and c) *Lactuca sativa 'Australische Gele'*, are used for this study. Each plantation container holds eight pots and each pots contain three lettuce plants. The three lettuce varieties were mounted on the top, middle and bottom shelves respectively. The first green wall column effluent mixed with urine with the proportion of 0.3% for the first three weeks and then increased to 0.6% until the harvesting time. The second column effluent also mixed with 0.15% of urine for the first three weeks and then increase into 0.3% until the harvesting time. The third column effluent directly irrigated from the

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treated greywater without urine. Each mix batch circulated every 30 minutes using small-submerged pumps controlled by PLC, and the mixed batch changed every three days.

2.3. Water sample collection and lab analysis

Water samples were collected every two weeks from raw greywater, biofilter system effluent, filtration column effluent (green wall), and circulated irrigation water. The sample were collected in 1-liter bottles and analysed within an hour. The water samples were analysed for total phosphorous (P) and total nitrogen (N) using spectrophotometric test kits (Hach-Lange); total coliforms (TC) and *E. coli* were quantified using the most probable number method (MPN) with Colilert-18 (IDEXX) and Quantitray 2000 (IDEXX) according to ISO 9308-2:2012. In addition, grab samples were collected from the same position to analyse heavy metals by using inductively coupled plasma mass spectrometry (ICP-MS).

2.4. Plant sample collection and lab analysis

Seven to ten replicates of lettuce plant, from each treatment plots, were collected for plant growth examination and heavy metal bioaccumulation analysis. Whereas for microbial assay, 25 g of composite lettuce sample from each treatment plots were collected and put into stomacher plastic bags containing 225 ml, sterile buffered peptone water (0.1%), homogenized by using a stomacher for 1 minute. *E. coli* was enumerated from the homogenised supernatant using the most probable number method (MPN) with Colilert-18 (IDEXX) and Quantitray 2000 (IDEXX) according to ISO 9308-2:2012.

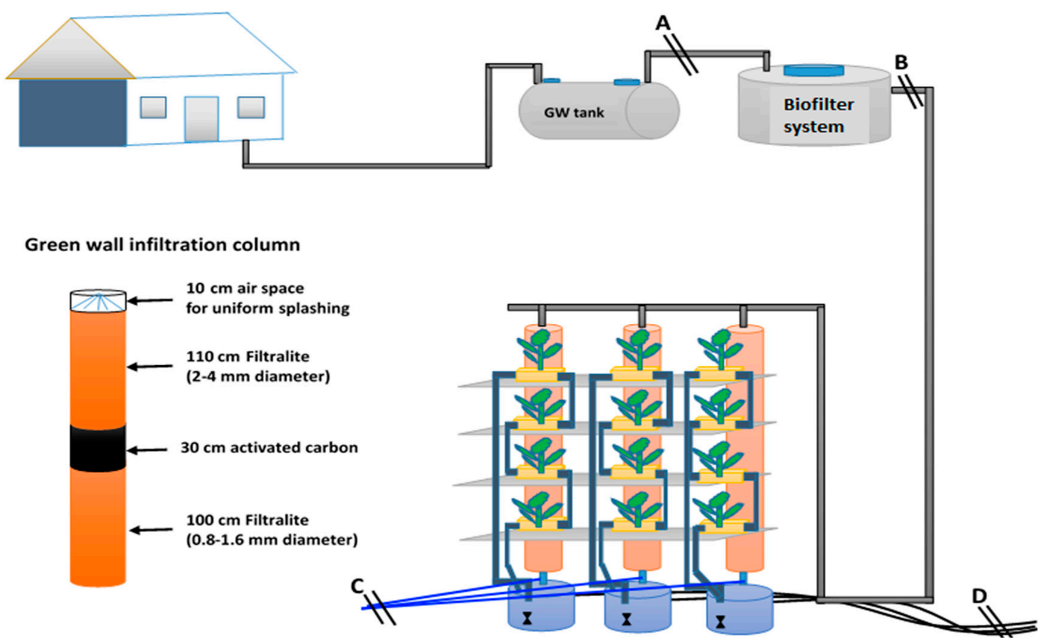

Figure 1. Greywater treatment steps, green wall and lettuce production configuration

2.6. Hydroponic nutrient uptake and lettuce growth analysis

Nitrogen (N), Phosphorus (P), and Potassium (K), are essential elements in plant nutrition and their presence in the plant tissue analyzed to understand the growth, development and health of the plant. Moreover, C-N ratio indicates the nutritional quality of the lettuce. Moreover, lettuce growth analysis were performed using plant growth index to describe the performance of the lettuce plant grown under this experimental set up. These plant growth indexes are:

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a) ***Specific leaf area (SLA)***: SLA is the surface area of a fresh leaf divided by its oven dry mass. It reflect an essential trade-off in plant functioning between a rapid production of biomass in the case of high SLA and an efficient conservation of nutrients low SLA. Moreover, species in permanently or temporarily resource-rich environments tend to have a higher SLA than do those in resource-poor environments [28,29].

b) ***Leaf weight ratio (LWR)***: LWR is the ratio of total leaf dry weight to total dry weight of the plant. It describe the leafiness of the plant on a dry weight base, and measure the distribution of dry materials between the leaves and the rest of the plant [30].

c) ***Leaf area ratio (LAR)***: Computed from the photosynthetic surface area per unit dry weight of a plant. It is a measure of the efficiency with which a plant deploys its photosynthetic and respiration per unit of its biomass [30].

d) ***Root-shoot ratio***: Root-shoot ratio is the ratio of the dry weights of the root system and aerial part of a plant. It is an index of plant response to their environment through growth balance between root and shoot of the plant. When nutrient availability increases, plants allocate relatively less to their roots, which means that less effort is required to acquire this resource. An alternative view is that relatively greater root growth in response to shortages of nutrients or water could maximize a plant's probability of capturing those resources [30,31].

2.5. Health risk assessment

Both quantitative microbial risk assessment (QMRA) and chemical health risk assessment (CHRA) approaches were applied to evaluate the health risk of microbial contamination and heavy metal bioaccumulation associated with reusing treated greywater for lettuce production (Figure 2).

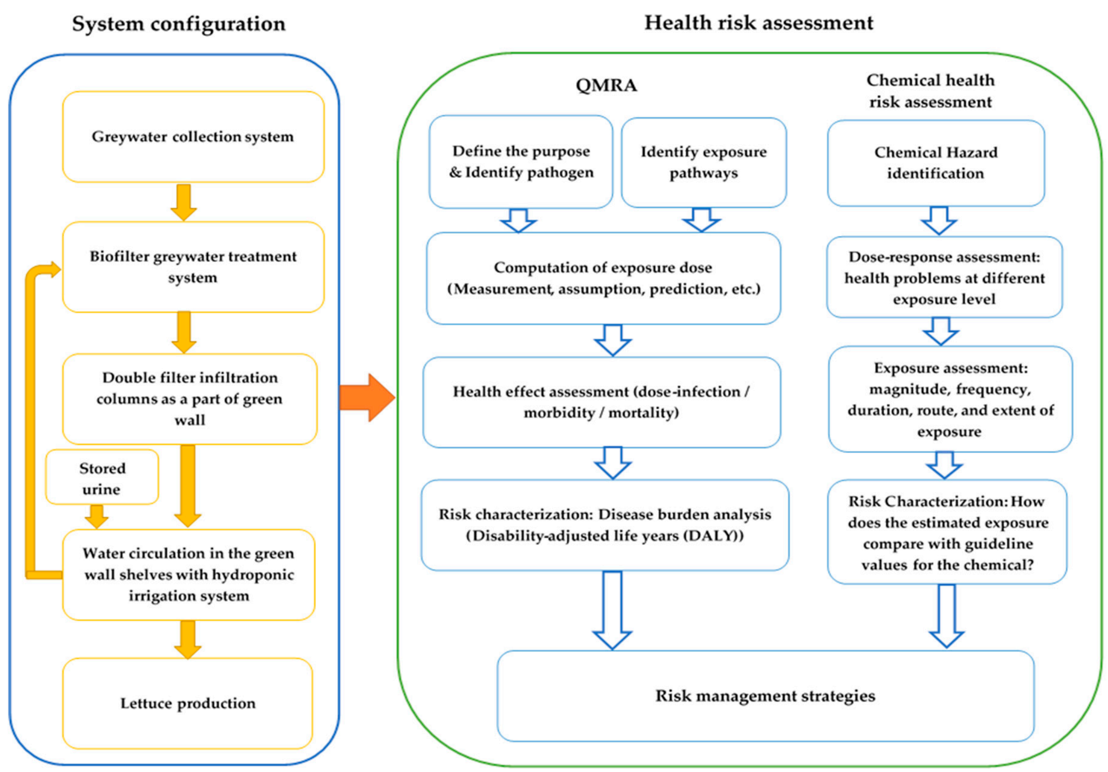

Figure 2. System configuration and health risk assessment procedures

2.5.1. Quantitative microbial risk assessment (QMRA)

Given the probability of having pathogen-infected person in the system, the study included single reference pathogen from each group of enteric bacteria, viruses and protozoa and follow water harmonized QMRA approach, which includes, problem formulation, exposure assessment, health effect assessment, and risk characterizations.

Problem formulation: The main purpose of this study is to evaluate the health risk associated with operational activates for lettuce production and consumption in terms of achieving health-based targets, 10^{-6} DALYs per person per year is not exceeded. The study intended to address enteric pathogens that may present in greywater and three reference pathogens were selected from each of the three-pathogen groups: Protozoa: *Cryptosporidium* was selected, as it has high infectivity, resistant to disinfection units and one of the most important waterborne human pathogens. Bacteria: *Campylobacter* selected, as it is the most common cause of bacterial gastroenteritis. Viruses: Norovirus, which is a very contagious virus that can infect anyone and found in abundance in sewage systems.

Exposure assessment: The main exposure pathway considered in this study, is operational activities in relation to lettuce production and raw lettuce consumption. The operational activities in relation to lettuce production that can potentially expose the operator to microbial pathogens are routine ingestion and accidental ingestion, assumed 0.0001 liter per event and 0.001 liter per event, respectively. The exposure dose, on the other hand, depends on the microbial quality of the circulated irrigation water. Exposure from lettuce consumption quantified using the equation:

$$Exposure \quad D = C * v * q \tag{1}$$

Where exposure D is the mean dose per event. C is the concentration of pathogens in the circulated water applied to the plant through hydroponic irrigation system (organism·L^{-1}), v is the volume of irrigation water in contact to the lettuce (L·g^{-1}) and the assumed value is based on observation during harvesting, and q is the quantity of lettuce consumed per event (Table 1).

Table 1. Values to compute exposure for lettuce consumption

Pathogens	C (pathogen*L^{-1})	v (L*g^{-1})	q (g)
Cryptosporidium	4.7E-04	1.0E-07	50
Norovirus	3.5E-05	1.0E-07	50
Campylobacter	8.2E-06	1.0E-07	50

Health effects assessment: The two important pathogen-specific factors for the risk assessment are the dose-response relationship and the illness per infection assuming that the health end-point this study is illness. Therefore, the dose-response models recommended for the reference pathogens to assess the probability of infection is shown in Table 2. In addition, since the probability of illness are often viewed as independent of dose given that infection has occurred, the estimated values of the probability of illness for a given infection of *Cryptosporidium*, *Campylobacter*, and norovirus are 0.39, 0.33, and 0.73 respectively [32-34].

Table 2. Dose-response relationships for reference organisms

Organism type	Distribution	Model	Parameters
Norovirus	Beta-Poisson	$P_{inf} = 1-(1+d/\beta)^{-\alpha}$	$\alpha = 0.04$ $\beta = 0.055$
Campylobacter	Beta-Poisson	$P_{inf} = 1-(1+d/\beta)^{-\alpha}$	$\alpha = 0.145$ $\beta = 7.58$
Cryptosporidium	Exponential	$P_{inf} = 1-\exp^{(-rd)}$	$r = 0.059$

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Risk characterization: The final step in this risk assessment approach is to determine the magnitude of risk by integrating information from problem formulation, exposure assessment, and health effect assessment. In this study, the computed health risk was based on the cumulative greywater treatment efficiency, the volume of routine and accidental ingestion of treated greywater during operational activities, and the volume of consumed lettuce. The probability of infection per exposure event were taken from dose-response relation and adjusted to reflect yearly risk of infection and illness by estimating the frequency exposure per year. The equations used for risk characterization in this study are listed Table S1.

2.5.2. Heavy metals health risk assessment

Metal pollution: Heavy metals bioaccumulation may differ in different crops depending up on the environment they are produced. In order to measure the combined effect of all expected heavy metals, Metal Pollution Index (MPI) is commonly applied. In addition, MPI was used to normalize and compare the total metal content between the different plant verities and treatment level as it is proposed by [35].

$$MPI = [M_1 \ x \ M_2 \ x \ M_3 \ldots . M_n]^{1/n} \qquad (2)$$

Where M_n is the mean concentration of metal n (mg/kg dry wt) in the examined crop.

Plant uptake rate of heavy metals: A number of factors can affect the plant uptake mechanism of heavy metals. These factors are the plant species, properties of plant growth medium, root growth, vegetative growth, the bioavailability of the metal in the water phase, which depends on the retention time of the metal, as well as the interaction with other elements and substances in the water [36]. The daily heavy metals uptake rate across lettuce verities for each treatment level were described by the equation:

$$DUR_i = \frac{C_m}{T \ x \ BM_p} \qquad (3)$$

Where, DUR_i is average daily uptake rate of heavy metals normalized by dry biomass of lettuce variety i (µg/day), C_m is the concentration of heavy metals in the lettuce tissue (µg/g), T is the total growth time (days), and BM_p dry plant biomass (g).

Daily intake rate (DIR) is one of the exposure pathway of heavy metals through ingestion of vegetables was determined by using daily intake rate (*DIR*) (mg/kg*day). *DIR* estimate the average daily loading of metal into the body system of a specified body weight of a consumer. The daily intake of metals depends on both the heavy metal concentration and the amount of vegetable consumed. Moreover, its effect depends on the body weight of the consumer. *DIR* computed using the equation:

$$DIR = \frac{C_{metal} \ x \ C_{factor} \ x \ IR}{BW} \qquad (4)$$

Where, C_{metal} is the heavy metal concentration in vegetables (mg/kg), C_{factor} is the conversion factor that convert fresh lettuce weight to dry weight and our conversion factor is 0.065; IR is the daily intake of vegetables (g/day) assumed 0.05 kg/day, and BW is the average body weight assumed 70 kg for this study.

Health risk index (HRI) is the ratio between daily intake rate and the reference dose $(R_f D)$ (mg/(kg*day) that express the health risk of non-carcinogenic effects [37], and described by the equation:

$$HRI = \frac{DIR}{RfD} \qquad (5)$$

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Where *DIR* is the daily intake rate, and R_fD is reference dose expressed as an oral dose per kilogram of body weight, which is an estimate of the lowest daily human exposure that is likely to occur without appreciable risk of toxicity for non-cancerous effects during the life time [38]. $HRI < 1$ indicates that the exposed population is safe of health risk that comes from heavy metal consumption.

Targeted hazard quotient (THQ) is a ratio between heavy metal concentration and the oral reference dose, weighted by the duration and frequency of exposure, intake rate and body weight. [37]. A *THQ* vale less than one indicates that the exposed population to the heavy metals through lettuce consumption is unlikely to experience visible adverse health effects. *THQ* computed by the formula:

$$THQ = \frac{EF\ x\ ED\ x\ IR\ x\ C\ x\ 10^{-3}}{RfD\ x\ BW\ x\ TA} \quad (6)$$

Where *EF* is exposure frequency (days per year), *ED* is the exposure duration (years, equivalent to the average lifetime), *IR* is the vegetable ingestion rate (g per person per day), *C* is the metal concentration in lettuce (mg Kg^{-1}), *BW* is the average body weight (kg), and *TA* is the averaging exposure time for non-carcinogens (days per year * exposure duration). The average body weight of human are different from region to region and for this study, the average body weight of an adult is assumed 70 kg based on literature [39], and also the daily lettuce consumption assumed 50 g. In addition, exposure frequency and duration were assumed 104 days per year, and 70 years life expectancy respectively.

3. Results

3.1. Irrigation Water quality and greywater treatment efficiency

Raw and treated greywater quality were monitored every other weeks from the first day of planting of lettuce until harvesting and the results shown in Figure 3. Based on microbial water quality monitoring, greywater treatment efficiency of the barriers structures in the system varied. The reduction of *E. coli* in Log_{10} MPN/100 ml were 1.6, 1.9, and 1.6 for biofilter system, green wall filtration column, and circulated irrigation water, respectively. Therefore, the system has gained a cumulative reduction of *E. coli* about 5.1 log_{10} MPN/100 ml in the final effluent. In addition, the reduction of total coliform bacteria were 1.4, 2.1, and 0.2 Log_{10} MPN/100 ml for biofilter system, green wall filtration column, and circulated irrigation water respectively, resulting a 3.7 log_{10} reduction in the final effluent.

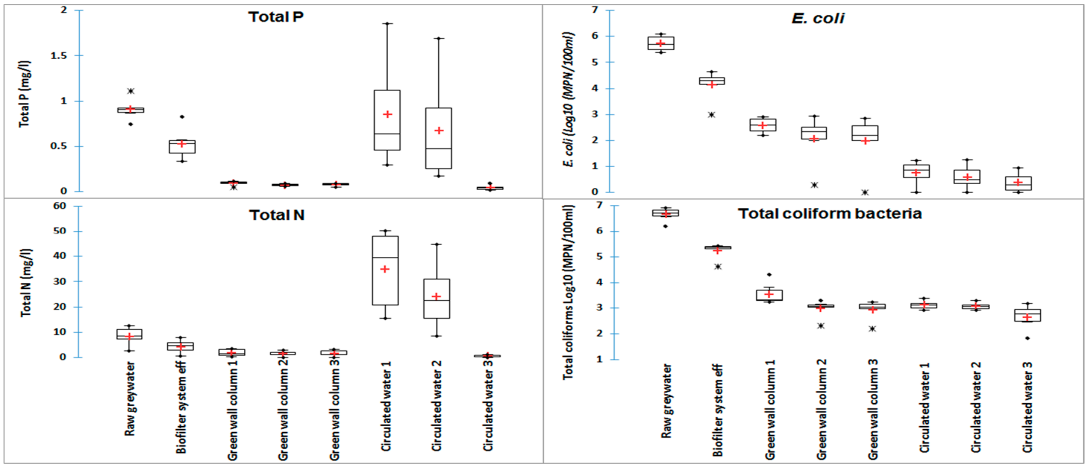

Figure 3. Water quality at different treatment steps of the system

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An average total phosphorus and total nitrogen in raw greywater during this experimental period were 0.91 mg/l and 8.52 mg/l respectively. It reduced to 0.53 mg/l and 4.37 mg/l by biofilter system and 0.08 mg/l and 1.73 mg/l by green wall filtration column respectively. When 3% - 6% urine added to the effluent of infiltration column 1, and 1.5% - 3% urine added to the effluent of infiltration column 2, the average total phosphorus and total nitrogen concentration rises to 0.85 mg/l and 34.97 mg/l in the first circulated water and 0.68 mg/l and 24.27 mg/l in the second circulated water respectively. On the other hand, heavy metal analysis result based on grab sample shows that the concentration of Zn and Cr were 87.3 µg/l and 20 µg/l respectively and which is relatively higher in the raw greywater as compared to the concentration of other heavy metals. Heavy metals removal efficiency of greywater treatment steps were different for each heavy metal elements, and it was up to 82 % for Cr in the case of biofilter system. The concentration of some of heavy metal elements increase in the treated gray water like Mn after biofilter system effluent; Cu, As, Cd and Pb in the filtration column effluent (Table 3).

Table 3. Heavy metal concentration (µg/L) in greywater and different treatment steps

Sampling points	Cr	Mn	Ni	Cu	Zn	As	Cd	Hg	Pb
Raw greywater	20.0	12.7	17.0	10.7	87.3	<0.26	<0.01	<0.02	0.6
Biofilter system effluent	3.6	15.7	9.6	5.6	34.7	<0.26	<0.01	<0.02	0.2
Filtration column effluent	<2.5	3.4	3.6	8.0	<21	0.67	0.036	<0.02	0.25
Human Urine	8	17	3.9	14	600	30	0.2	<0.02	0.4

3.2. The level of microbial contamination and heavy metal bioaccumulation in the lettuce

The lettuce biomass were subjected to *E. coli* test and the result shows that there was no positive sample in the case of all plots. On the other hand, heavy metal analysis result shows that the bioaccumulation of Zn, Mn, and Cu in the plant tissue relatively high as compared with the other elements (Table 4).

Table 4. Heavy metal concentration in three varieties of lettuce for different treatment

Urine in irrigation water (%)	Lettuce type	Heavy metals concentration (mg/kg)								
		As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
0.3-0.6	a	0.13	0.02	1.43	11.00	27.00	3.30	0.38	51.25	0.02
	b	0.40	0.02	2.50	13.00	32.00	3.50	0.96	59.00	0.02
	c	0.11	0.02	2.50	12.33	30.33	3.73	0.66	55.00	0.01
0.15-0.3	a	0.09	0.02	1.00	12.00	36.00	4.10	0.26	78.00	0.02
	b	0.11	0.03	1.20	10.00	43.00	2.70	0.30	75.00	0.02
	c	0.13	0.04	0.86	12.00	49.00	3.50	0.39	74.00	0.02
0	a	0.22	0.02	0.76	9.90	13.00	2.20	0.42	38.00	0.01
	b	0.22	0.03	1.20	43.00	27.00	3.10	0.63	55.00	0.01
	c	0.27	0.02	1.80	20.00	18.00	4.00	0.73	63.00	0.01

a - Lactuca sativa 'Lobjoits Green Cos' b - Lactuca sativa 'Red Salad Bowl' c - Lactuca sativa 'Australische Gele'

3.3. Hydroponic nutrient uptake and lettuce growth analysis

Growth analysis of lettuce was evaluated using four plant growth indexes and presented in Figure 4. As we can see from the figure, the value of specific leaf area (SLA) for the three lettuce species and associated treatment level were different. SAL value reduced with the reduction of urine in the irrigation water and it was clearly observed in the case of *Lactuca sativa 'Red Salad Bowl'* that SAL value of 0.014 m^2g^{-1} in the case of 0.3%–0.6% urine as compared to 0.003 m^2g^{-1} in the absence of urine. The average SLA value of all tree lettuce variety with the application of 0.3%-0.6% urine and in the absence of urine as a treatment were 0.012 m^2g^{-1} and 0.005 m^2g^{-1} respectively. The computed leaf weight ratio (LWR) and leaf area ratio (LAR) for the three species are 11.57 and 0.13 in the application of 0.3% - 0.6% urine with the treated greywater irrigation and 3.93 and 0.02 in the absence of urine respectively. The average root-shoot ratio of lettuce irrigated 0.3% - 0.6% urine mix is 0.35 and even get lower up to 0.22 in the case of *Lactuca sativa 'Lobjoits Green Cos'*, while for lettuce irrigated without urine, was 0.96 and it was higher up to 1.15 in the case of *Lactuca sativa 'Australische Gele'*.

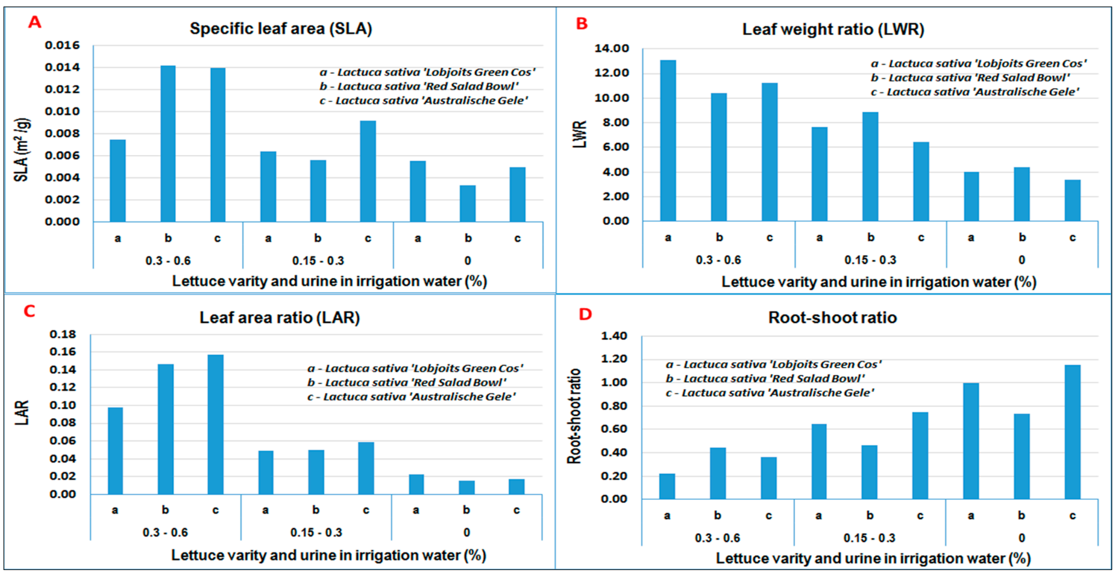

Figure 4. Growth indexes result for different lettuce verities and treatment level

3.4. Quantitative microbial risk assessment

3.4.1. Estimation of reference pathogens in irrigation water and lettuce biomass

The estimation of reference pathogens at different greywater treatment steps and in a produced lettuce were based on *E. coli* concentration as an indication of microbial contamination. As shown in Table 5, *E .coli* concentration decreased because of different greywater treatment steps. The base for the estimate of reference pathogen concentration was the combination of information about *E. coli* in each treatment steps with the concentration of reference pathogens in the sewage system, which was published in different studies. Moreover, 1% of the sewage assumed to be mixed with the greywater system (Table S2), from the previous studies, the average concentration of *Cryptosporidium, Campylobacter, and* norovirus in the sewage system estimated 678.1 oocysts/100ml, 118 MPN/100ml, and 5.1×10^4 gene copies/100ml respectively [40-42]. The concentration of reference pathogens in the irrigation water were dependent on the efficiency of greywater treatment steps of the system, which was based on *E. coli* removal efficiency. The final concentration of *Cryptosporidium, Campylobacter, and* norovirus in circulated irrigation water were 4.7E-04 oocysts/100ml, 8.2E-06 MPN/100ml, and 3.5E-08 gene copies/100ml respectively (Table 5). The microbial contamination of lettuce was assumed as unintentional contact with irrigation water during harvesting time.

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3.4.2. Health risk assessment computation

Routine ingestion and accidental ingestions are the two rout of exposure in the operation of lettuce production during irrigation and harvesting practices. Based on practical observation, routine ingestion was assumed to be 0.0001 liter per event and occurred more frequent in the irrigation practices whereas accidental ingestions was estimated about 0.001 liter per event and occurred less frequent for both operational activities. The exposure dose during operational activities of lettuce production was estimated 4.7E-07 to 4.7E-08 for *Cryptosporidium*, 8.2E-09 to 8.2E-10 for *Campylobacter* and 3.5E-11 to 3.5E-12 for norovirus. The exposure dose due to lettuce consumption is depend on the volume of irrigation water accidentally contaminated the lettuce and the volume of lettuce consumption. The estimated exposure dose due to contaminated lettuce consumption was 2.35E-09, 1.75E-10, and 4.1E-11 in the case of *Cryptosporidium, Campylobacter* and norovirus respectively (Table 6).

Table 5. Estimated reference pathogens concentration at different treatment steps

Pathogens	Variables	An average value
	Concentration (C) in raw greywater (L^{-1})	6.8E+01
	Biofilter system log_{10} reduction	1.59
	Concentration in biofilter system effluent (L^{-1})	1.7E+00
Cryptosporidium	Infiltration column log_{10} reduction	1.93
	Concentration in infiltration column effluent (L^{-1})	2.1E-02
	Circulated irrigation water log_{10} reduction	1.64
	Concentration in circulated irrigation water (L^{-1})	4.7E-04
	Concentration (C) in raw greywater (L^{-1})	1.2E+00
	Biofilter system log_{10} reduction	1.59
	Concentration in biofilter system effluent (L^{-1})	3.0E-02
Campylobacter	Infiltration column log_{10} reduction	1.93
	Concentration in infiltration column effluent (L^{-1})	3.6E-04
	Circulated irrigation water log_{10} reduction	1.64
	Concentration in circulated irrigation water (L^{-1})	8.2E-06
	Concentration (C) in raw greywater (L^{-1})	5.1E+00
	Biofilter system log_{10} reduction	1.59
	Concentration in biofilter system effluent (L^{-1})	1.3E-01
Norovirus	Infiltration column log_{10} reduction	1.93
	Concentration in infiltration column effluent (L^{-1})	1.5E-03
	Circulated irrigation water log_{10} reduction	1.64
	Concentration in circulated irrigation water (L^{-1})	3.5E-05

Treatment efficiency based on *E. coli* Log_{10} reduction in the system

The computed health risk that accounts lettuce production (irrigation and harvesting) and consumption expressed in terms of probability of infection for single exposure that ranges from 2.8E-08 in the case of accidental ingestion of *Cryptosporidium* to 2.5E-12 in the case of routine ingestion of norovirus during lettuce production process. On the other hand, the probability of infection due to lettuce consumption per single exposure was estimated 1.4E-10, 7.8E-13, and 1.3E-10 in the case of *Cryptosporidium*, *Campylobacter*, and norovirus respectively (Table 7).

3.5. Heavy metals health risk assessment

The relative daily uptake rate of heavy metals between lettuce varieties were different for different heavy metals elements (Figure 5). For example, the daily uptake rate of arsenic by *Lactuca sativa 'Red Salad Bowl'* was highest as compared to the other two varieties, whereas the daily uptake rate of nickel was relatively lowest. On the other hand, the relative daily uptake rate of heavy metals is also varied depending on the volume of urine mix in the irrigation water. For example, the relative daily uptake rate of chromium increases as the urine mix in the irrigation water increases and is the same for all other cases.

Table 6. Estimation of exposure dose (*D*) and exposure frequency for reference pathogens during lettuce production and consumption

Pathogens	Activities	Rout of exposure	Concentration (*C*)	Volume (*L*) per event	Exposure dose (*D*) per event	Frequency/ person/ year
Cryptosporidium	Hydroponic irrigation	Routine ingestion	4.7E-04	1.0E-04	4.7E-08	365
		Accidental ingestion	4.7E-04	1.0E-03	4.7E-07	10
	Lettuce harvest	Routine ingestion	4.7E-04	1.0E-04	4.7E-08	30
		Accidental ingestion	4.7E-04	1.0E-03	4.7E-07	5
	Lettuce consumption	Deliberate ingestion	4.7E-04	5.0E-06	2.35E-9	104
Campylobacter	Hydroponic irrigation	Routine ingestion	8.2E-06	1.0E-04	8.2E-10	365
		Accidental ingestion	8.2E-06	1.0E-03	8.2E-09	10
	Lettuce harvest	Routine ingestion	8.2E-06	1.0E-04	8.2E-10	30
		Accidental ingestion	8.2E-06	1.0E-03	8.2E-09	5
	Lettuce consumption	Deliberate ingestion	8.2E-06	5.0E-06	4.1E-11	104
Norovirus	Hydroponic irrigation	Routine ingestion	3.5E-08	1.0E-04	3.5E-12	365
		Accidental ingestion	3.5E-08	1.0E-03	3.5E-11	10
	Lettuce harvest	Routine ingestion	3.5E-08	1.0E-04	3.5E-12	30
		Accidental ingestion	3.5E-08	1.0E-03	3.5E-11	5
	Lettuce consumption	Deliberate ingestion	3.5E-08	5.0E-06	1.8E-10	104

Metal pollution index (*MPI*) value for three lettuce varieties and associated treatments of urine mix with irrigation water shown in Table 9. *MPI* value of *Lactuca sativa 'Lobjoits Green Cos'* was lowest in all treatment case as compared to the other varieties and it was 0.91, 0.83, and 0.67 for 0.3% - 0.6%, 0.15% - 0.3% urine mix and without urine respectively. While *MPI* vale of *Lactuca sativa 'Red Salad Bowl'* was highest and it was 1.26 in the case of 0.3% - 0.6% urine mix treatment level.

The health risks of heavy metal through lettuce consumption were evaluated based on the health risk index (HRI) and the target hazard quotient (THQ). For the computation of health risk indexes, daily intake rate is very crucial to estimate the level of exposure through food chain (lettuce consumption). The daily intake rate of heavy metals from different varieties of lettuce along with the treatment level presented in Table 10. The daily intake of Zn was found to be greater than the other heavy metals and it ranges from 3.5E-03 to 7.2E-03 μg/kg*day. In addition, the daily intake of Mn and Cu were also higher and it ranges from 1.2E-03 to 4.6E-03 μg/kg*day and 1.1E-03 to 9.3E-04 μg/kg*day respectively (Table 10).

Health risk index and the target hazard quotient are an indication of health risk level of lettuce consumption that produced in the system and computed using reference dose $(R_f D)$. The estimated

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values of reference dose $(R_f D)$ of heavy metals, which is an oral dose per kilogram of body weight were taken from EPA web page and shown in Table 8. The computational result of HRI shows that the highest value was obtained in the case of AS and it was 0.062 in the case of *Lactuca sativa 'Red Salad Bowl'* lettuce variety grown with 0.3% - 0.6% urine mix in the irrigation water. However, the value of HRI for all lettuce and treatment level were below one and it shows that the population is not at risk and the public health risk concern due to lettuce consumption is very less for the given assumptions (Table 11). The THQ value for As and Cr, are relatively higher but < 1 in all cases (Table 12) and it implies that the concern level of health risk is very low for a given assumption. The overall evaluation result shows that the lowest vale of THQ obtained in the case of *Lactuca sativa 'Lobjoits Green Cos'* and the highest value obtained in the case of *Lactuca sativa 'Red Salad Bowl'*

Table 7. The health risk of lettuce production and consumption

Pathogens	Rout of exposure	Activities	$P_{inf/event}$	$P_{inff/year}$	$P_{ill/expo}$	$P_{ill/year}$
Cryptosporidium	Hydroponic irrigation	Routine ingestion	2.8E-09	1.0E-06	1.1E-09	3.9E-07
		Accidental ingestion	2.8E-08	2.8E-07	1.1E-08	1.1E-07
	Lettuce harvest	Routine ingestion	2.8E-09	8.3E-08	1.1E-09	3.2E-08
		Accidental ingestion	2.8E-08	1.4E-07	1.1E-08	5.4E-08
	Lettuce consumption	Deliberate ingestion	1.4E-10	1.4E-08	5.4E-11	5.6E-09
Campylobacter	Hydroponic irrigation	Routine ingestion	1.6E-11	5.7E-09	5.2E-12	1.9E-09
		Accidental ingestion	1.6E-10	1.6E-09	5.2E-11	5.2E-10
	Lettuce harvest	Routine ingestion	1.6E-11	4.7E-10	5.2E-12	1.6E-10
		Accidental ingestion	1.6E-10	7.8E-10	5.2E-11	2.6E-10
	Lettuce consumption	Deliberate ingestion	7.8E-13	8.2E-11	2.6E-13	2.7E-11
Norovirus	Hydroponic irrigation	Routine ingestion	2.5E-12	9.3E-10	1.9E-12	6.8E-10
		Accidental ingestion	2.5E-11	2.5E-10	1.9E-11	1.9E-10
	Lettuce harvest	Routine ingestion	2.5E-12	7.6E-11	1.9E-12	5.6E-11
		Accidental ingestion	2.5E-11	1.3E-10	1.9E-11	9.3E-11
	Lettuce consumption	Deliberate ingestion	1.3E-10	1.3E-08	9.3E-11	9.7E-09

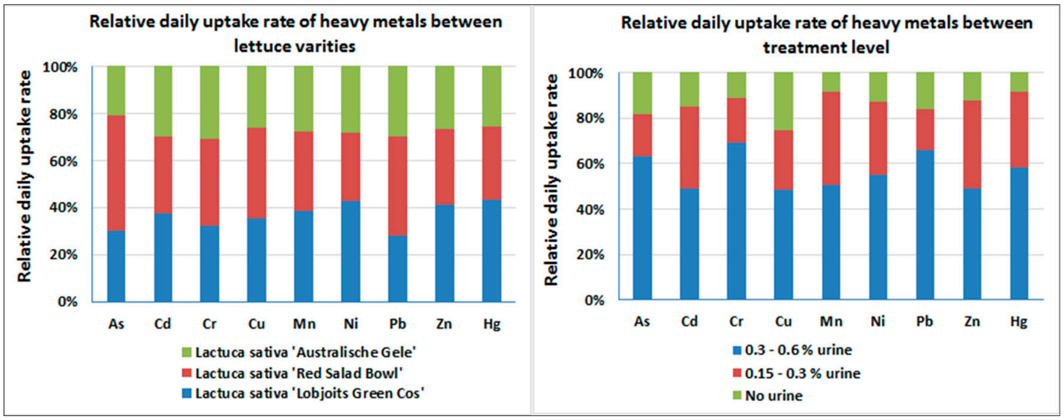

Figure 5. Relative daily uptake rate of heavy metals lettuce plant

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Table 8. Reference dose (RfD) of heavy metals (mg/kg/day)

Parameter	Heavy metals								
	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
Reference dose (RfD)	0.0003	0.001	0.003	0.04	0.14	0.02	0.0035	0.3	0.0003

Table 9. Metal pollution index for lettuce varieties

Urine in irrigation water (%)	Lettuce type	Metal Pollution Index (MPI)
0.3 - 0.6	*a - Lactuca sativa 'Lobjoits Green Cos'*	0.91
	b - Lactuca sativa 'Red Salad Bowl'	1.26
	c - Lactuca sativa 'Australische Gele'	0.99
0.15 - 0.3	*a - Lactuca sativa 'Lobjoits Green Cos'*	0.83
	b - Lactuca sativa 'Red Salad Bowl'	0.87
	c - Lactuca sativa 'Australische Gele'	1.02
0	*a - Lactuca sativa 'Lobjoits Green Cos'*	0.67
	b - Lactuca sativa 'Red Salad Bowl'	1.13
	c - Lactuca sativa 'Australische Gele'	1.10

Table 10. Daily Intake rate (DIR) (mg/Kg*day) of heavy metals in different varieties of lettuce

Urine in irrigation water (%)	Lettuce type	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
0.3-0.6	a	1.2E-05	1.8E-06	1.3E-04	1.0E-03	2.5E-03	3.1E-04	3.6E-05	4.8E-03	1.8E-06
	b	3.7E-05	1.9E-06	2.3E-04	1.2E-03	3.0E-03	3.3E-04	8.9E-05	5.5E-03	1.7E-06
	c	1.0E-05	2.0E-06	2.3E-04	1.1E-03	2.8E-03	3.5E-04	6.2E-05	5.1E-03	1.4E-06
0.15-0.3	a	8.4E-06	1.9E-06	9.3E-05	1.1E-03	3.3E-03	3.8E-04	2.4E-05	7.2E-03	1.5E-06
	b	1.0E-05	2.4E-06	1.1E-04	9.3E-04	4.0E-03	2.5E-04	2.8E-05	7.0E-03	1.6E-06
	c	1.2E-05	3.3E-06	8.0E-05	1.1E-03	4.6E-03	3.3E-04	3.6E-05	6.9E-03	2.0E-06
0	a	2.0E-05	1.7E-06	7.1E-05	9.2E-04	1.2E-03	2.0E-04	3.9E-05	3.5E-03	7.2E-07
	b	2.0E-05	2.8E-06	1.1E-04	4.0E-03	2.5E-03	2.9E-04	5.9E-05	5.1E-03	1.0E-06
	c	2.5E-05	2.0E-06	1.7E-04	1.9E-03	1.7E-03	3.7E-04	6.8E-05	5.9E-03	1.1E-06

a - Lactuca sativa 'Lobjoits Green Cos' b - Lactuca sativa 'Red Salad Bowl' c - Lactuca sativa 'Australische Gele'

Table 11. Health risk index (HRI) of heavy metals caused by the consumption of lettuce

Urine in irrigation water (%)	Lettuce type	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
0.3-0.6	a	0.021	0.001	0.022	0.013	0.009	0.008	0.005	0.008	0.003
	b	0.062	0.001	0.039	0.015	0.011	0.008	0.013	0.009	0.003
	c	0.017	0.001	0.039	0.014	0.010	0.009	0.009	0.009	0.002
0.15-0.3	a	0.014	0.001	0.015	0.014	0.012	0.010	0.003	0.012	0.002
	b	0.017	0.001	0.019	0.012	0.014	0.006	0.004	0.012	0.003
	c	0.020	0.002	0.013	0.014	0.016	0.008	0.005	0.011	0.003
0	a	0.034	0.001	0.012	0.011	0.004	0.005	0.006	0.006	0.001
	b	0.034	0.001	0.019	0.050	0.009	0.007	0.008	0.009	0.002
	c	0.042	0.001	0.028	0.023	0.006	0.009	0.010	0.010	0.002

a - Lactuca sativa 'Lobjoits Green Cos' b - Lactuca sativa 'Red Salad Bowl' c - Lactuca sativa 'Australische Gele'

Table 12. Calculated target hazard quotient (THQ) for heavy metals in in different varieties of lettuce

Urine in irrigation water (%)	Lettuce type	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
0.3-0.6	a	0.32	0.01	0.34	0.20	0.14	0.12	0.08	0.12	0.05
	b	0.95	0.01	0.60	0.23	0.16	0.13	0.20	0.14	0.04
	c	0.26	0.02	0.60	0.22	0.15	0.13	0.14	0.13	0.03
0.15-0.3	a	0.21	0.01	0.24	0.21	0.18	0.15	0.05	0.19	0.04
	b	0.26	0.02	0.29	0.18	0.22	0.10	0.06	0.18	0.04
	c	0.31	0.03	0.20	0.21	0.25	0.13	0.08	0.18	0.05
0	a	0.52	0.01	0.18	0.18	0.07	0.08	0.09	0.09	0.02
	b	0.52	0.02	0.29	0.77	0.14	0.11	0.13	0.13	0.03
	c	0.64	0.02	0.43	0.36	0.09	0.14	0.15	0.15	0.03

a - Lactuca sativa 'Lobjoits Green Cos' b - Lactuca sativa 'Red Salad Bowl' c - Lactuca sativa 'Australische Gele'

The variation of THQ value due to the change in the input variable of lettuce intake rate per body weight was referred as the sensitivity of THQ for this particular input variable. The effect of the input on THQ were investigated by varying only this variable across its range of plausible values, while keeping all other inputs at their nominal values as it is described in the assumption for the other computations. The results were presented graphically to visualize how an output was sensitive for the change in inputs (Figure 6) with respect to its critical value. As we can see from the graph, THQ vale was above the critical value, when the value of lettuce intake rate per body weight of As, Cr, and Cu were above 1.65 g/kg, 2.05 g/kg, and 2.55 g/kg respectively.

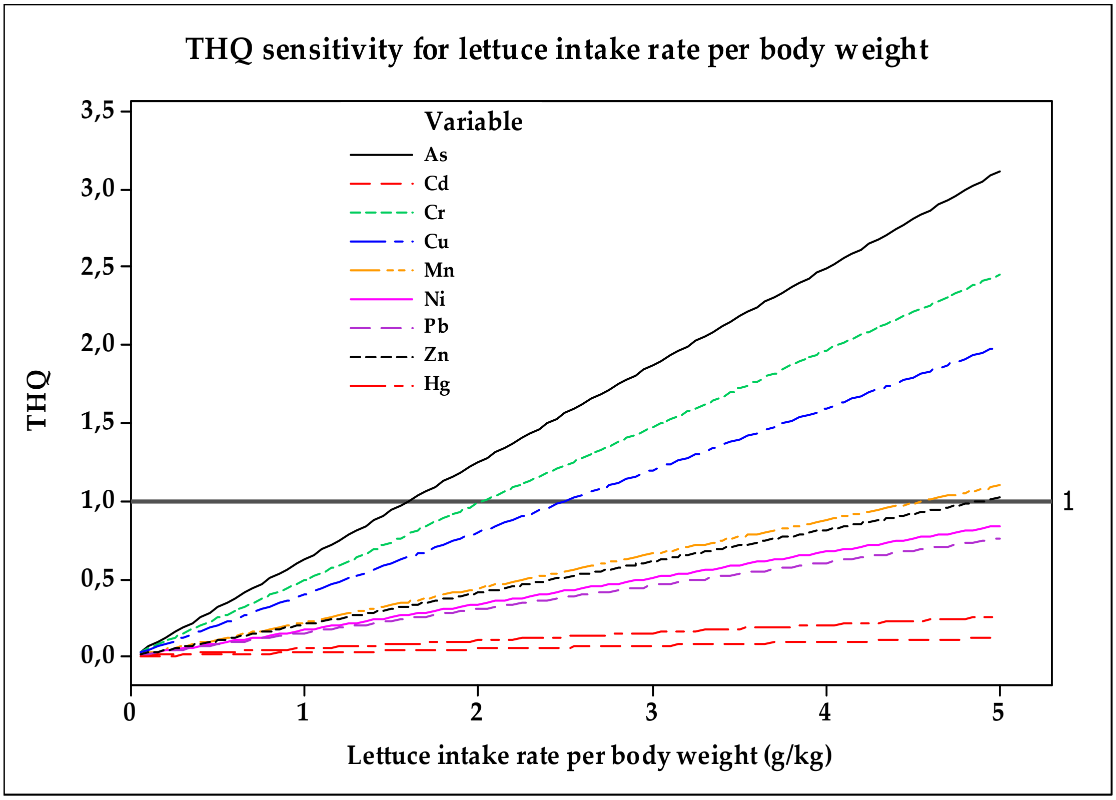

Figure 6. The sensitivity of calculated THQ for lettuce intake rate for a given body weight

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4. Discussion

Lettuce growth analysis showed that the performance of urine treated lettuce (0.3% to 0.6%) were better than lettuce plant produced with little urine (0.15% to 0.3%) and without urine in terms of appearance, height, and number of leaves (Figure S1). The overall better performance of urine treated lettuce attributed to adequate provision of essential nutrient for plant growth and it confirms the potential of urine for fertilizing in the hydroponic system. Different plants may require different proportion of urine in the irrigation water depending on the plant type and its growth stage. It has been observed that the application of 0.15% to 0.3% urine mix in the irrigation water was not optimal as lettuce is leafy plant that require higher nitrogen concentration for its vegetative growth, however, 0.3% to 0.6% give the impression as optimal level but further assessment required.

Lettuce growth analysis within the natural development cycle may enable us to evaluate the adaptive feature of the plant. In order to evaluate lettuce growth in a quantitative terms, specific leaf area (SLA), Leaf weight ratio (LWR), Leaf area ratio (LAR), and root-shoot ratio were assessed. SLA is a measure of thickness of leaves relative to area, which is associated with the availability of sufficient plant nutrients.The high specific leaf area (SLA) associated with the application of 0.3% to 0.6% urine in treated greywater irrigation, and low SLA in the absence of urine, demonstrate the challenge of reuse of treated greywater alone for plant biomass production. The insufficient plant nutrient in the case of irrigation without urine significantly constrained the plant growth in all of the three varieties. LWR is a measure of biomass allocation to leaves, and the highest LWR in the case of 0.3% to 0.6% urine mix in the irrigation water associated with highest leaf biomass production whereas the lowest LWR in the absence of urine linked with the less leaf biomass production. Leaf area ratio is a measure of leafiness or photosynthetic area relative to respiratory mass of the plant that characterize the plant-atmosphere interaction where most of the energy fluxes exchange through photosynthesis and respiration. The highest LAR (0.16) in urine treated irrigation compared to the lowest LAR (0.01) in the absence of urine was reflected the value of urine as a nutrient solution for the growth performance of the lettuce in this system. The average root-shoot ratio varied widely between lettuce varieties of the same treatment. Relatively high root-shoot ratio was found in the case of lettuce grown without urine as a response to the shortage of nutrients.

In order to utilize treated greywater for irrigation purpose, it is important to select greywater treatment steps that reduce microbial pathogens and at the same time retain the nutrients. However, it is often difficult to find a treatment processes that perform the two demands simultaneously. Therefore, stored human urine (stored for three months and free from *E. coli*) were mixed with the final effluent of greywater treatment system as a nutrient solution for this system. Significant reduction of *E. coli* were observed in the final effluent of the greywater treatment steps of the system (Table 5) and also no *E. coli* were observed in any of the plant samples collected from each treatment plots in this preliminary study of water reuse potential assessment. The results of this study point out that the greywater treatment systems were efficiently remove *E. coli* and the integrated hydroponic irrigation system can minimize the risk of contamination of the edible part of the lettuce. Therefore, this study confirmed that the type of irrigation plays an important role in terms of reducing the risk of contamination.

Quantitative microbiological risk assessment (QMRA) is the process of estimating the risk from exposure to microorganisms and with the intention to determine whether the production and consumption of treated greywater irrigated lettuce has a health risk, a quantitative microbial risk assessment (QMRA) model was developed. The *E. coli* concentration in the final effluent of greywater treatment system has been considered as a base for the level of microbial contamination of irrigation water. The average concentration of reference pathogens (*Cryptosporidium*, *Campylobacter*, and norovirus) in our system was derived from the pathogen load of the municipal sewer system. The volume of water retained by the lettuce leaves and ingested with the lettuce is assumed very little (1.0E-07 l/g) because of the limited accidental contact of irrigation water during harvesting. On the other hand, the volume of water ingestion either accidentally or routinely in the production activities

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that means during irrigation and harvesting were determined from practical observation and experts opinion, assuming that it is very little in a closed hydroponic system.

The two major activities for lettuce production that expose the operator to microbial contamination were irrigation and harvesting. Most of these activities often carried out manually and the probability of hand contamination is high. Pathogen transmission through consumption of food with contaminated hands and accidental splashing of irrigation water into the mouth most likely considered as a route of exposure for this study. Considering the routes of exposure and other assumptions, the computed QMRA result showed that the infection risk of *Cryptosporidium*, *Campylobacter*, and norovirus due to lettuce consumption event were very low, 1.4E-10, 7.8E-13, and 1.3E-10, respectively. The health risk of both lettuce consumption and production activities based on the corresponding assumptions and scenarios were below WHO health-based targets, 10^{-6} DALYs per person per year. However, the conversion of *E. coli* concentration into reference pathogens based on the pathogen load of the sewer system could give us a clue about the average pathogen load of our system but it will undermine the peak risk when the incidence of pathogens are elevated at household level and at the same time in the greywater system.

The chemical health risk due to lettuce consumption was expressed in terms of health risk index (HRI) and targeted hazard quotient (HQ), which is commonly used to evaluate non-carcinogenic health effect. The exposed population will experience at risk if the value of both indexes are greater than one, which means if the exposed dose is greater than reference dose. The major risk contributor element due to lettuce consumption were As and Cr whereas the lowest risk contributor element in the system was Cd. However, the value of both HRI and THQ indexes were less than one for all lettuce verities and treatment level, for the given assumptions. This result shows that heavy metal health risk from the consumption of lettuce produced in this system was not significant. On the other hand, heavy metals bioaccumulation potential varied substantially among the different lettuce varieties. Thus, *Lactuca sativa 'Australische Gele'* have relatively less THQ value as compared with the other lettuce varieties and this indicates the opportunity to reduce the health risk caused by heavy metals through proper selection of plant varieties.

5. Conclusions

The typical configuration of greywater treatment systems and hydroponic lettuce production as a part of a green wall structure was the unique feature of this study. Considering its distinctive arrangement, this study provides key information about the health risk associated with treated greywater reuse for lettuce production. The integration of greywater treatment system with a green wall technology provide additional environmental benefits through aesthetically appealing, and improved air quality by increasing the oxygen level. Moreover, a green wall may also act as an urban and semi-urban agriculture that can bring various economic and social benefits, including generate additional household income, provide good opportunity for healthier community interaction, and improving access to fresh food.

Performing both microbial and heavy metal health risks assessment on the same subject enable us to observe the most critical risks to prioritize mitigation measures, and at the same time to perceive the health risk in different directions. The results of QMRA demonstrate the importance of microbial removal efficiency of integrated greywater treatment system and hydroponic irrigation scheme to minimize health risk below the health-based targets, 10–6 DALYs per person per year. Different studies have linked wastewater reuse with excessive bioaccumulation of heavy metals in the produced crops that could potentially pose both short and long-term health risks. Contrary to these studies, heavy metals risk assessment based on HRI and THQ indexes were not exceeded the permissible level (one), and as a result, the health risk concern of consuming lettuce was insignificant. Heavy metal uptake rate of plant varieties are different, this study reveals that heavy metal uptake rate of Lactuca sativa 'Australische Gele' was relatively less, and the result highlights the importance

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of having selected plant varieties that uptake a minimum amount of heavy metals in order to reduce health risk. Therefore, the selection of potential varieties should be considered in future studies.

By considering all the benefits that may arise from this scheme, this study points out some vital health risk minimizing strategies that may potentially further reduce health risks and these include: 1) Improving microbial and heavy metal removal efficiency of greywater treatment systems through appropriate research approaches. 2) Selecting plants that can be served cooked may potentially further reduce microbial health risk. 3) Growing plant varieties that have the potential of reduced heavy metal bioaccumulation and 4) Taking regulatory measures on consumable goods that can potentially release heavy metals at household level.

Supplementary Materials: The following are available online, Figure S1: The appearance of lettuce at different growth stage, A. after three weeks of planting, B. after six weeks of planting, and C. after eight weeks of planting, the left column is treated greywater with (0.3% - 0.6% urine), the middle column is with (0.15% - 0.3% urine), and the right column is without urine for each growth stage, Table S1: Health risk characterizing computational equations, Table S2. The average concentration of pathogens in the sewage system assuming 1% of sewage present in the greywater considering E. coli as a surrogate for faecal contamination, Table S3. The proportion of essential plant nutrient uptake by lettuce variety

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