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Mercury in basil (*Ocimum basilicum*) grown simultaneously with shrimp (*Litopenaeus vannamei*) by aquaponics

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ABSTRACT

The accumulation, mass balances, and potential health risks associated with the presence of Hg were evaluated in basil cultivated simultaneously with shrimp by aquaponics (~1.7 g L^{-1}). Two aquaponic (well water (WW) and diluted seawater (DSW)) treatments and one hydroponic control (hydroponic solution (HS)) were examined. The trend of Hg accumulated in basil tissues was roots > leaves > stems. No significant differences ($p > 0.05$) were found between aquaponic treatments. Mercury concentrations in the edible basil tissues were below the maximum allowable levels (0.2 mg kg^{-1}) for vegetables established by international guidelines (WHO, 2007; CREU, 2018). The mass balances evidenced that most Hg (> 99%) entered the aquaponic system through the input water, while the main output route (> 90%) was through the zeolite used in plants. Mercury inorganic speciation in water was similar between WW and DSW treatments. The dominating speciation was $HgCl_2$ (50%), which is a bioavailable form that biota can use. In the HS treatment, the chemical speciation was different as it formed complexes mainly with ammonia. The health risk index for Hg was < 1.0, which indicates that the population exposed to the consumption of basil grown with shrimp would not be at risk.

1. Introduction

Aquaculture is one of the fastest-growing food production sectors, contributing to 49.2% of the world's fishing production in 2020 (FAO, 2022). However, its growth has been accompanied by an increase in diseases and diverse environmental impacts. Some are associated with the discharge of effluents that cause contamination of the receiving waters by the presence of organic matter, nutrients, and residues of pollutants such as food additives, fertilizers, disinfectants, and antibiotics (Páez-Osuna, 2001; Lyle-Fritch et al., 2006). As part of these residues, As, Cu, Zn, and Hg have been found at relatively high levels, which could pose a potential risk to human health (Lacerda et al., 2006; 2011; León-Cañedo et al., 2017; Dietrich and Ayers, 2021).

Traditional agriculture requires a huge expense of water and land space; thus, an increasing interest in the development of new agricultural methods for sustainable food production has emerged (Ramos-Sotelo et al., 2019). Current research has focused on the development of sustainable food production systems, which face one of the biggest

Abbreviations: WW, well water; DSW, diluted seawater; HS, hydroponic solution; DWC, deep water culture; HDPE, high density polyethylene; EC, Electrical conductivity; HRI, health risk index; EDI, estimated daily intake; RfD, reference oral dose; DO, dissolved oxygen.

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challenges of the 21st century: to guarantee food and nutritional security for ~10 billion people by 2050 (FAO, 2021). In this regard, aquaponics appears as an alternative to achieve the sustainability of aquaculture by implementing concepts of water recirculation and nutrient recycling. This reduces water consumption and effluent discharges, and it also increases productivity. Additionally, it contributes to water, food, and nutrition security, as well as to the minimization of environmental impacts on receiving water bodies (Rakocy et al., 2006).

In traditional aquaculture systems, only 20–30% of the food supplied is converted into biomass. The rest of the food remains accumulated at the bottom (sediments) of the shrimp farming ponds, deteriorating the water quality and representing a potential risk for the receiving water bodies when wastewater is discharged (Páez-Osuna et al., 1997; Páez-Osuna, 2001). In the aquaponic systems, the nutrient-enriched waters are used as fertilizer for plant growth (Tidwell, 2012; Fierro-Sañudo et al. 2020). Therefore, these nutrients are transformed into biomass and their accumulation is prevented by the improvement of the culture water quality (Alarcón-Silvas et al., 2021). In addition, the combined cultivation of plants and animals reduces the space needed for production, as less water is required and the discharge of pollutants into surrounding ecosystems is lowered (Fierro-Sañudo et al., 2020).

Aquaponics research has been developed mainly by the integration of fish and plants (vegetables, aromatic herbs, and halophytes) from different water sources such as freshwater (Rakocy et al., 2006), brackish water (Nozzi et al., 2016), and seawater (Thomas et al., 2019). However, studies regarding crustacean and plant integration are scarce. Limited studies have been carried out primarily on freshwater or low-salinity water that integrates shrimp or prawns with tomatoes (Mariscal-Lagarda et al., 2012, 2014; Mariscal-Lagarda and Páez-Osuna, 2014), lettuce (Farias-Lima et al., 2019), and basil (Ronzón-Ortega et al., 2012). However, most of them only report production and describe technical approaches, while studies focused on chemical issues such as the accumulation of heavy metals, mass balances, health risk assessment (León-Cañedo et al., 2019), or nutrient removal (Alarcón-Silvas et al., 2021) are scarce.

Basil (*Ocimum basilicum*) is one of the most cultivated plants in aquaponic systems (Love et al., 2015). It is a commercially important herb as both fresh and dried leaves are used for culinary and medicinal purposes (Chalchat and Ozcan, 2008). Basil plants grow well indoors and outdoors under sunny conditions, in which warmth, light, and moisture are the basic ecological requirements for its cultivation (Putievsky and Galambosi, 1999). This plant is produced commercially in many countries such as Egypt, India, Indonesia, Mexico, and the United States of America (Li and Chang, 2016). It is also suitable for soilless production and some studies (Roosta, 2014; Mangmang et al., 2016) suggest that basil has a better yield under soilless systems compared to conventional ones. Conversely, basil cultivation in aquaponics with catfish (*Clarias gariepinus*) has been developed with various hydroponic components (grow pipes, raft, and ebb-and-flood gravel substrate) without additional fertilizer during the plant grow-out phase (Knaus et al., 2020). Similarly, carp (*Cyprinus carpio*) and basil were cultured in an aquaponic recirculating system, where one kg of leaves was obtained in 60 days of growth; Filep et al. (2016) conclude that basil grows much better when using aquaponics than conventional cultivation.

In aquaponics, mineral nutrients have complex and crucial roles in plant growth and metabolism when they are available in adequate amounts. The content of micro- and macronutrients in plants is conditional for optimal development; this content can be affected by the characteristics of the soil (except in aquaponics), irrigation water composition, and the ability of plants to selectively accumulate some metals (Lozak et al., 2002). Metals such as Cu, Fe, Mn, and Zn have important positive and negative roles in human life. However, other elements such as Hg have toxic roles in the biochemical reactions of the human body (Baslar et al., 2005). On occasion, these elements can be toxic to humans even at considerably low concentrations (Sparks, 2005). In aquaponic systems, the main sources of these metals are originated from the addition of feeds (Hg as impurities) in the fish culture tanks followed by the input water, while organic sludge and output water are the main routes of removal (Lacerda et al., 2011; Leon-Cañedo et al., 2017). Moreover, some external sources of heavy metals could be associated with rainfall, atmospheric dust, fertilizers, etc. (Lacerda et al., 2006). Plants could then absorb them through the leaves and increase the concentrations in their tissues (Kovacheva et al., 2000). Considering that most herbaceous plants can easily accumulate heavy metals in their leaves (even more than cereal, vegetables, and fruit crops; Mapanda et al., 2005), it is important to analyze the accumulation in commercial aromatic herbs, such as basil, and their potential human health risk by consumption.

In the first study, we examined the water quality, nutrient use efficiency, and growth of the whiteleg shrimp *Litopenaeus vannamei* in an aquaponic system coupled with basil *Ocimum basilicum* (Alarcón-Silvas et al., 2021). Results evidence that this system removes N and P from water, improves water quality, enables efficient use of water and nutrients, reduces effluent discharge to the environment, and increases production. Shrimp were selected as the animal component of the aquaponic system mainly for three reasons (Fierro-Sañudo et al., 2020; Alarcón-Silvas et al., 2021): (i) shrimp, particularly *L. vannamei*, is a species with a wide osmoregulation capacity that can be cultured in marine water, brackish water, low-salinity water, and freshwater; thus, it cultivates successfully in waters where plants of commercial interest grow; (ii) this species is the most cultured shrimp in the world (52.9% of all crustaceans cultivated (FAO, 2020)); and (iii) various studies have shown that this shrimp can be successfully integrated with various plants such as tomato, lettuce, and basil in low salinity waters.

In this study, we evaluated Hg accumulation in basil plants grown in a closed recirculating aquaponic system using two types of low-salinity waters frequently used in aquaculture and agriculture in Northwest Mexico. In addition, mass balances for Hg were developed to quantify the distribution of this element in the different reservoirs involved. Finally, the potential health risk associated with human consumption of basil edible tissues was estimated. The hypothesis is that the basil grown with low salinity shrimp effluents exhibits (in terms of Hg content) an enhanced quality compared to a commercial hydroponic solution.

2. Materials and methods

2.1. Description of the experiment

An aquaponic experimental system was carried out in the YK Experimental Module, located in Mazatlan, Sinaloa, Mexico.

2.1.1. Experimental design and experimental units

The aquaponics system (Fig. 1) consisted of one shrimp culture component and one hydroponic unit for basil crop coupled simultaneously through a constant flux recirculation system. The shrimp culture component comprised six high-density polyethylene (HDPE) tanks (2 m diameter × 1.2 m height) with a capacity of 3.14 m^3 per tank. One clarifier tank (180 L) and one biofilter (120 L) were connected to each shrimp tank. HDPE plastic beads and gravel were used as a substrate for the fixing of nitrifying bacteria. The common variety of *O. basilicum* known as large basil was used. This variety usually has silky-textured leaves that are wider and brighter. They have an intense green color and give off an unmistakable aroma with notes of camphor and cinnamon (Hiltunen and Holm, 1999). The unit for basil growth consisted of one deep water culture (DWC) hydroponic system (HS) for each tank. The DWC system consisted of beds constructed with cement blocks and covered with plastic (3.0 m long × 1.0 m wide × 0.2 m high) with a slope of 2%. A sump tank (50 L) was placed at the end of the DWC system to return the water to the shrimp tanks. Water was transferred by gravity from the shrimp culture tanks to the rest of the system using a ½" diameter HDPE hose. At the end of the DWC system, the water was

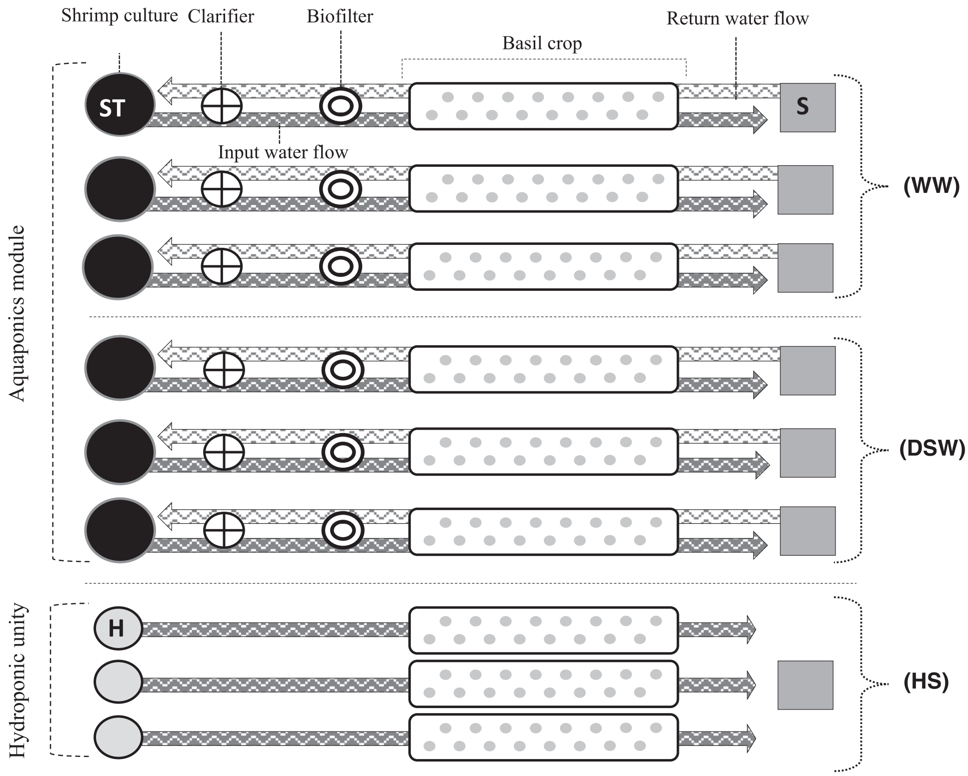

Fig. 1. Scheme of the experimental system used in this study. Treatments for aquaponics (WW, well water treatment at 2.7 dS m^{-1}, and DSW, diluted seawater treatment at 2.7 dS m^{-1}) and hydroponics (HS, nutritive solution at 2.5 dS m^{-1}) performed in triplicate. ST: shrimp tanks; S: sump tank; H: hydroponic nutritive solution.

received in a 50 L sump tank and later pumped back into the shrimp culture tanks by a ½ HP peripheral pump. Water recirculation through the aquaponics system had a constant flow of 2 L min^{-1}. The water lost through evaporation, evapotranspiration of the plants, and the draining of the clarifier tanks were replaced from a 450 L tank connected to the pump reservoirs where water was restored after reaching a predetermined level. The exchange of water was zero during the culture cycle.

2.1.2. System management and treatments

Two types of low-salinity water were used for the shrimp culture: (1) well water (WW) with a salinity of 1.7 g L^{-1} (WW treatment; ~2.7 dS m^{-1} of electrical conductivity, EC) and (2) filtered seawater (34 g L^{-1} of salinity) diluted with freshwater (0.2 g L^{-1}) at 1.7 g L^{-1} (DSW treatment; EC ~2.7 dS m^{-1}). The shrimp tanks were coupled to a filtration system (one clarifier and biofilter per tank) and then to a DWC system for basil growth. Additionally, (3) a control treatment consisting of a nutritive hydroponic solution (HS control treatment; EC~2.5 dS m^{-1}) was used to irrigate plants in a DWC system (without a clarifier and biofilter) (Fig. 1). The chemical components of the nutritive hydroponic solution were based on Samperio Ruiz (2000) (Table 1). Aeration for the system was supplied by a 1 HP regenerative air blower and aeration tubing (Aerotube) in each tank.

2.1.3. Shrimp stocking and farming

Litopenaeus vannamei post-larvae (PL) (0.73 ± 0.10 g) used for the shrimp culture were provided by Proveedora de Larvas S.A. de C.V. Post-larvae were acclimatized at 1.7 g L^{-1} of salinity according to McGraw and Scarpa (2004) and grown at 75 PL m^{-3} per culture cycle. Shrimp were fed with "Flake" food (52% protein; Brine Shrimp Co., Utah, USA) during the first week, and later with "Camaronina" food (35% protein; Nestlé Purina PetCare Company, St. Louis, Missouri, USA) until the harvest. The shrimp culture cycle lasted 48 days.

2.1.4. Basil sowing and density

The basil seedlings were transplanted into their respective treatments two weeks after the commencement of the shrimp cycle. Seedlings were placed to float in their corresponding DWC systems (WW, DSW, and HS) on polystyrene sheets (3 m long × 1 m wide × 1″ thickness); 48 seedlings (16 plants m^{-2}) were placed on each bed (25 cm of separation each) using a substrate of zeolite during a growth cycle of 65 days. Plants in each pot were placed in a 15 cm zeolite base (~200 g). The management of the aquaponic system and the production variables of the shrimp culture and basil crop are detailed in Alarcón-Silvas et al. (2021).

2.2. Speciation and physicochemical analyses of water sources

The water sources used from both WW and DSW aquaponic treatments were analyzed for water characterization (at the start and finish of each aquaponic culture cycle) according to the following four groups of variables: (1) physicochemical parameters, (2) major components, (3) nutrients, and (4) metals. Temperature, pH, EC, and dissolved oxygen (DO) were measured using a dissolved oxygen meter (model DO200, YSI, Ohio, USA), while pH and EC were measured using a Combo pH-EC tester (model HI 98129, Hanna Instruments, Texas, USA). The precision, estimated as the coefficient of variation, was 5.5%, 2.2%, and 4.1% for the DO, pH, and EC, respectively. The calibration of the pH and EC meter was performed using buffers of pH 4.01 (Orion 910104, Thermo Scientific) and 7.01 (Orion 910110, Thermo Scientific), as well as a Hanna EC (1413 μS cm^{-1} at 25 °C) solution. Samples used for major ions and nutrient analyses were collected (one sample per tank) at the initial and final stage of the crops directly from the tanks (25 cm below the surface water) and filtered using Whatman GF/F (0.7 μm pore; 47 mm diameter) filters. The water samples were stored in clean plastic bottles (120 mL) and transported to the laboratory at a low temperature (4 °C). Water samples for heavy metal analyses were collected (one sample per tank at the initial and final stage) in acid-washed (2 M HNO_3)

Table 1
Chemical characterization of the water sources (mean ± SD from triplicate) used during the initial and final stages in the aquaponic systems and the hydroponic nutrient solution (control).

		WW		DSW		HS
Macronutrients	n	Initial	Final	Initial	Final	(mg L^{-1})
TAN-N (μg L^{-1})	3	4.1 ± $1.3^{a,1}$	5.9 ± $1.1^{a,1}$	5.9 ± $2.6^{a,1}$	8.0 ± $4.7^{a,1}$	41.2
NO_2-N (μg L^{-1})	3	7.2 ± $6.3^{a,1}$	4.3 ± $0.8^{a,1}$	6.3 ± $2.4^{a,1}$	6.1 ± $2.8^{a,1}$	0.002
NO_3^--N (mg L^{-1})	3	1.2 ± $0.4^{a,1}$	0.3 ± $0.0^{b,1}$	1.5 ± $0.6^{a,1}$	0.4 ± $0.1^{b,1}$	141
TN (mg L^{-1})	3	3.4 ± $1.8^{a,1}$	2.2 ± $1.7^{a,1}$	3.2 ± $1.2^{a,1}$	1.2 ± $0.1^{a,1}$	190
PO_4^--P (μg L^{-1})	3	53.7 ± $12.6^{a,1}$	276 ± $200^{a,1}$	41.6 ± $8.3^{a,1}$	337 ± $141^{a,1}$	45.6
TP (μg L^{-1})	3	65.7 ± $2.2^{a,1}$	348 ± $189^{a,1}$	53.6 ± $3.9^{a,1}$	372 ± $164^{a,1}$	45.6
K^+ (mg L^{-1})	3	38.2 ± $9.5^{a,1}$	36.4 ± $22.8^{a,1}$	35.2 ± $12.3^{a,1}$	57.3 ± $7.3^{a,1}$	212
Mg^{2+} (mg L^{-1})	3	60.2 ± $14.8^{a,1}$	73.6 ± $8.6^{a,1}$	32.2 ± $9.5^{b,2}$	61.3 ± $1.5^{a,1}$	49.6
Ca^{2+} (mg L^{-1})	3	57.1 ± $8.7^{a,1}$	62.9 ± $7.5^{a,1}$	39.0 ± $8.6^{a,1}$	51.7 ± $0.7^{a,1}$	131
Micronutrients						(μg L^{-1})
Cu (μg L^{-1})	3	13.3 ± $2.5^{a,1}$	12.2 ± $0.4^{a,1}$	11.1 ± $2.0^{a,1}$	16.5 ± $4.8^{a,1}$	100
Mn (μg L^{-1})	3	3.3 ± $0.5^{a,1}$	1.8 ± $0.3^{a,1}$	2.0 ± $1.6^{a,1}$	1.9 ± $0.4^{a,1}$	400
Zn (μg L^{-1})	3	56.5 ± $17.6^{a,1}$	67.7 ± $19.2^{a,1}$	53.5 ± $17.1^{a,1}$	67.5 ± $21.6^{a,1}$	100
Mo (μg L^{-1})	3	2.9 ± $0.1^{a,2}$	3.2 ± $0.1^{a,1}$	3.4 ± $0.0^{a,1}$	2.6 ± $0.2^{b,2}$	–
Others						
Na^+ (mg L^{-1})	3	172 ± $9^{b,1}$	257 ± $20^{a,2}$	184 ± $57^{b,1}$	374 ± $1^{a,1}$	41.6
Cl^- (mg L^{-1})	3	831 ± $17^{a,1}$	616 ± $25^{b,2}$	770 ± $41^{a,1}$	748 ± $34^{a,1}$	13.6
Hg (μg L^{-1})	3	6.7 ± 0.6	0.7 ± 0.1	6.7 ± 1.3	0.7 ± 0.3	< 0.1

Means with different superscript letters between initial and final water for a same treatment indicate significant differences ($P < 0.05$); means with different superscript numbers between WW and DSW for a same type of water (initial or final) indicate significant differences ($P < 0.05$). Statistical tests were performed by two-way ANOVA.

polyethylene bottles (60 mL) and immediately filtered through 0.45-μm acid-washed (0.5 M HCl) filters (Type HA, Millipore). The filtrates were acidified (HNO_3 trace metal analysis, J.T. Baker) and used for the dissolved fraction analysis. Analyses of heavy metals and major ions were performed by an atomic absorption spectrometer (AAS-SpectraAA 220, Varian VGA-110) with flame (Ca, K, Mg, and Na), graphite (Cu, Zn, Mo, and Mn), and cold vapor (Hg) methods, respectively. Nutrient concentrations were measured using the procedures outlined by Grasshoff et al. (1990). The precision (expressed as the coefficient of variation at%) of the analyses varied depending on the metal and nutrient determined: from 3.5 (for K) to 8.2% (for Ca) for major components, from 3.5 (for Hg) to 7.8% (for Mn) for heavy metals; and from 5.0% for nitrates to 9.6% for ammonia.

It is important to indicate that the WW water selected for this study is commonly used by shrimp farmers from rural areas localized in El Rosario, Sinaloa, Mexico. This region is characterized by an extensive agricultural area, and these WW waters frequently receive the contribution of wastes from this economic activity, as well as from untreated municipal effluents. Inorganic speciation of Hg was calculated to investigate the distribution of its different chemical species in both WW and DSW treatments, as well as in the HS solution. This speciation model was developed using the Visual MINTEQ 3.1 v software with data on physicochemical parameters, and major and minor ions and nutrients (Table 1).

2.3. *Mercury accumulation in basil tissues*

During harvest, 16 whole plants were randomly selected from each bed and then separated into leaves, stems, and roots to determine Hg levels. Plants were firstly washed with purified Milli-Q water to remove impurities. After cleaning, these were separated by different tissues and grounded to obtain a portion by tissue (three pools of 100 g for bed) that were lyophilized afterwards (−49 °C and 132 × 10^{-3} mbar for 72 h). A total of 48 basil plants were analyzed per treatment; therefore, a total of 144 plants were examined. An aliquot of the dried homogenized tissue samples (0.25 g dry weight) was digested using 5 mL of HNO_3 concentrated in capped Teflon (Savillex) vials (León-Cañedo et al., 2019). Posteriorly, vials were heated at 140 °C for 3 h. When room temperature was reached, the digested samples were diluted with Milli-Q water to a final volume of 25 mL. Blank samples and the standard reference spinach material SRM-1570a (NIST, 2001) were digested (one per every 25 samples) using the same procedure to determine the accuracy and precision. Mercury analyses were performed by duplicate using the cold vapor method by atomic absorption spectrometry (SpectraAA 220, Varian VGA-110). The recovery value from the analyses of the reference material SRM-1570a for Hg was 90.5 ± 1.6%.

2.4. *Health risk assessment*

The health risk index (HRI) was calculated to evaluate the health threats of Hg associated with the consumption of basil grown in an aquaponic system with shrimp cultivated in low-salinity waters. The estimated daily intake (EDI) of Hg was needed to calculate the HRI using the reference oral dose (R_fD) for non-carcinogenic effects, which is an oral dose per kg of body weight. The R_fD for Hg was obtained from the USEPA (2018). The health protection standard of lifetime risks for the HRI is 1.0; therefore, values above 1.0 are considered a potential health risk (USEPA, 2006). The daily intake of metals was estimated using the equation (Arora et al., 2008):

$$EDI = \frac{C_m x DI_v x\ F}{bw}$$

Where C_m is the concentration of metals (mg kg^{-1} dry weight (dw) basis) in basil edible tissue, DI_v represents the average daily intake of basil, F is the conversion factor (0.085 + 0.002 in this case) used to convert fresh vegetable weight to dry weight, and bw is the body weight. The average body weight was considered to be 67.9 kg (for Latin-American and Caribbean populations; Walpole et al., 2012), while the average daily intake of basil for this study was assumed to be 0.02 kg (wet mass) $person^{-1}$ day^{-1}.

The HRI or the ratio between the EDI and the RfD that expresses the health risk of non-carcinogenic effects was calculated considering different body weights (10–120 kg of body weight) and diverse basil daily intake rates. The equation used was (Jan et al., 2010):

$$HRI = \frac{EDI}{R_fD}$$

where EDI represents the estimated daily intake of Hg and R_fD represents the reference oral dose. The R_fD value for Hg is 0.0003 mg kg^{-1} bw d^{-1}. R_fD values were obtained from USEPA (2018). An HRI < 1.0 indicates that the population is safe; while an HRI > 1.0 indicates that the population is not safe from the health risk associated with heavy metal consumption.

2.5. *Statistical analysis*

Normality (Kolmogorov-Smirnov test, Lilliefors Probabilities) and homoscedasticity (Levene test) tests were used to determine data distribution and equal variances. Statistical methods were performed to see if there were significant (at the 0.05 (or 5%) level) differences between the water quality variables of the two aquaponic water sources (WW and DSW) and the Hg levels in basil tissues between treatments. Statistical analyzes were grouped as follows: (1) comparisons from water quality variables between the aquaponic treatments (WW and DSW) (T-student test) and; (2) comparisons of Hg concentrations in different basil tissues between the two aquaponic and control treatments (WW, DSW, and HS) (One-way ANOVA). A post-hoc test (Tukey HSD test) was carried out to identify significant differences between the means of the treatments. The software employed for all statistical analysis was STATISTICA 7 (Statsoft Inc., Tulsa, USA).

3. Results and discussion

3.1. Quality of water sources

The chemical components (macronutrients, micronutrients, and sodium) present in the water sources (WW, DSW, and HS) at the initial and final stages are summarized in Table 1. The average concentrations of total nitrogen (TN), K^+, and Ca^{2+} found in WW and DSW during the culture cycle were low in the initial stage compared with those found in the commercial hydroponic nutritive solution (HS). Mg^{2+} in both treatments (WW and DSW) had similar concentrations to the HS treatment. No significant ($p > 0.05$) differences were found in most nutrient concentrations between WW and DSW. Only Mg^{2+} had significant differences ($p < 0.05$) at the beginning of the culture cycle. The initial and final water in both treatments were similar, except for TAN-N in the WW treatment, in which concentrations were higher in the final water compared to the initial water. Micronutrient concentrations (Cu, Mn, Zn, and Mo) were similar between WW and DSW for the initial and final water. Remarkably, Cu recorded a higher concentration in the final stage for both treatments, which could be associated with the remineralization of this element from the sludge fraction.

Physicochemical parameters (pH, temperature, EC, and DO) were similar between treatments during the shrimp culture cycle (48 days). Temperature average values at the start of the culture cycle were 31.8 and 32.1 °C and 28.9 and 29.1 °C at the end for WW and DSW, respectively. The pH values were maintained between 7.0 and 8.0 for both WW and DSW treatments during the cycle. Additionally, DO ranged from 6.5 to 6.7 mg L^{-1} in the beginning and from 6.0 to 6.1 mg L^{-1} in the end. The EC was kept close to 2.7 dS m^{-1}, a value that is within the restriction range classified as light to moderate (0.7–3.0 dS m^{-1}) considering the guidelines of water quality for irrigation (Ayers and Westcot, 1994). The physicochemical variables recorded in both treatments during the culture cycle were within the optimal and recommended ranges (Ferreira et al., 2011) for shrimp growth. The water from agricultural irrigation usually contains concentration ranges of 0–920, 0–60, 0–2, 0–1065, and 0–400 mg L^{-1} for Na^+, Mg^{2+}, K^+, Cl^-, and Ca^{2+}, respectively (Ayers and Westcot, 1985). In this study, chloride concentrations in both WW and DSW sources were higher than the restriction limits (< 355 mg L^{-1}) specified by Ayers and Westcot (1985) for plant irrigation. In excess, chloride is an element that could limit plant growth. It is emphasized that the concentrations of both Cl^- and Na^+ in the effluents from the shrimp culture grown with WW are even higher than those reported as habitual concentrations for this type of water (Millero, 2006). This could be due to the proximity of the marine wedge at the site where the WW was collected.

3.2. Mercury in waters: concentration and speciation

Mercury concentrations in the two water sources (WW and DSW) were higher in the input water (6.7 ± 0.6 μg L^{-1}) than in the output (0.7 ± 0.3 μg L^{-1}) water of the system. This could be associated with an accumulation of Hg in the organic sludge or/and in the tissues of the basil plant, or/and even by the retention of the zeolite substrate. Levels of Hg in rainwater are in the range of 0.01–0.1 μg L^{-1} (WHO, 1996). Some local mineral deposits can produce higher levels in groundwater; however, natural levels of Hg in groundwater and surface waters are less than 0.5 μg L^{-1}. The WHO (1996) guideline value for inorganic mercury in drinking water is 6 μg L^{-1}. However, the limit value in many countries is much lower, e.g., 2 μg L^{-1} in the USA and the European Union (UNEP, 2008).

Most of the elements contained in the selected waters, such as major ions (Cl^-, Na^+, Ca^{2+}, K^+, and Mg^{2+}) and micronutrients (Cu, Mn, Mo, and Zn), are present in shrimp pond waters due to their presence as natural components in water sources and commercial feeds (mainly fish meals). Furthermore, the presence of significant levels of Hg in these waters or even in the shrimp ponds could be associated with impurities from fertilizers, feeds, and chemical additives (Lyle-Fritch et al., 2006; Lacerda et al., 2011). The availability of major ions and trace metals in the water sources used for basil crop nutrition will depend on several factors, such as growth media and pH. This will directly influence crop bioavailability and affect speciation, transport, and solubility. In addition, the absence or excessive amounts of one essential element (except Hg) may induce deficiencies or toxicity (Salisbury and Ross, 1994).

Inorganic speciation of Hg presented the same chemical species and similar percentages of their distribution without significant differences during the initial and final stages of the culture in both WW and DSW treatments (Fig. 2). Mercury as free ion Hg^{2+}, which is considered the most bioavailable species (Ramírez-Rochin et al., 2021), was found in low proportion (<0.01%). The dominant species in both water treatments were $HgCl_{2\ (aq)}$ (53.6–56.1%) and $HgClOH_{(aq)}$ (27.9–32.5%). However, Laporte et al. (2002) suggests that Hg bioavailability is related to neutral chemical species, such as $HgCl_2$ and HgClOH in this case, through a passive Hg uptake. Therefore, the main species found in both WW and DSW treatments are bioavailable forms for shrimp and plants. The hydroponic solution exhibited a different distribution of the chemical species of Hg, as well as additional species compared to the treatments due to the chemical composition of this solution. The main Hg species were $Hg(NH_3)_2^{2+}$ (65.2%) and $Hg(OH)_2$ (29.0%) (Fig. 2), in which Hg forms complexes mainly with hydroxides, as well as N as ammonia due to the high concentration of this nutrient in the hydroponic solution.

3.3. Basil crop production

The total biomass produced as edible tissue (leaves and stems) was recorded for each treatment after the conclusion of the basil crop cycle (65 days) (Table 2). The results in the basil crop variables showed a production trend of WW > HS > DSW for total biomass and production per plant. However, the trend for leaves/stem ratio was DSW > WW > HS. Differences ($p < 0.05$) were observed between the DSW and HS leaves/stem ratio variable, while no significant differences ($p > 0.05$) were found between WW, DSW, and HS treatments for the remaining variables. Further details regarding shrimp and basil production are described and discussed in Alarcón-Silvas et al. (2021).

3.4. Mercury accumulation in basil

Mercury found in the basil tissues of this study showed a trend of roots > leaves > stems (Table 3), with concentrations of 11–21 μg kg^{-1} (dw) in both leaves and stems, and 32–36 μg kg^{-1} in roots for both WW and DSW. The Hg concentrations analyzed in all basil tissues for the control treatment (HS) were < 9 μg kg^{-1} (dw). No significant differences were found in Hg concentrations between both WW and DSW treatments. In this sense, the sources of Hg and the propensity for plants to accumulate and translocate them to edible and harvested parts depends extensively on soil and climate factors, plant genotype, agronomic management, and the composition (concentration and speciation) of irrigation sources (McLaughlin et al., 1999). For this reason, the substrate (zeolite) used for the cultivation of basil and the conditions of the system could have affected the translocation of Hg, and consequently a variable Hg distribution between the different tissues.

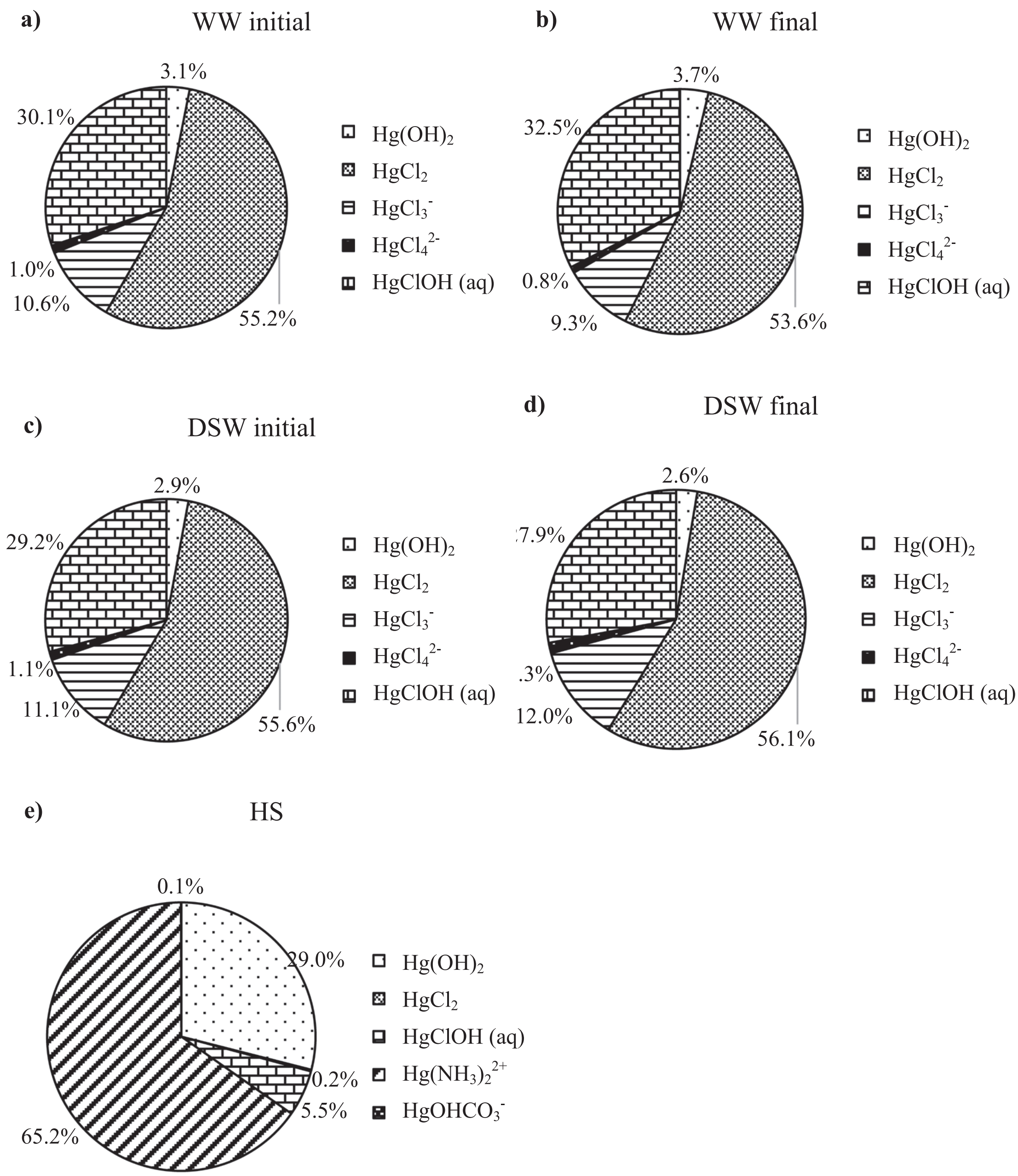

Fig. 2. Mercury inorganic speciation in the water of aquaponic systems: WW treatment at the (a) initial and (b) final stage; DSW treatment at the (c) initial and (b) final stage; and (e) the hydroponic solution HS (control).

Table 2
Production of basil (mean ± SD) grown with low-salinity waters (WW and DSW at 1.7 g L^{-1} of salinity) in aquaponic and hydroponic systems (control).

	n	WW	DSW	HS (control)
Production per plant (g)	48	70.4 ± 43.3^{a}	42.9 ± 22.1^{a}	64.6 ± 37.4^{a}
Total edible biomass (kg)	48	10.1 ± 3.8^{a}	6.2 ± 1.5^{a}	9.3 ± 3.2^{a}
Leaves/stem ratio	48	$2.1 \pm 0.3^{a,b}$	2.5 ± 0.3^{a}	1.8 ± 0.2^{b}

n = number of plants used for the production variables; Means with different superscript letters between treatments indicate significant differences ($P < 0.05$); Statistical tests were performed by two-way ANOVA.

Table 4 shows the Hg concentrations found in the basil tissues in this study, and those reported in several studies around the world and for different growth conditions (soil or soilless). Three studies (Majkowska-Gadomska et al., 2018; Shim et al., 2019; Marinescu et al., 2020)

Table 3
Mercury accumulation (mean ± SD dry weight) in basil tissues grown in aquaponic and hydroponic systems using different water sources.

Treatment		Hg (μg kg^{-1})		
	n	Leaves	Stems	Roots
WW	3	18 ± 6^{a2}	11 ± 3^{a2}	36 ± 6^{a1}
DSW	3	21 ± 5^{a2}	12 ± 6^{a2}	32 ± 5^{a1}
HS (control)	3	< 9	< 9	< 9

Means with different superscript letters between treatments indicate significant differences ($P < 0.05$); Means with different superscript numbers between tissues by the same treatment indicate significant differences; Statistical tests were performed by one-way ANOVA.

Table 4
Mercury accumulation (μg kg^{-1} in fresh weight) in basil edible tissue grown from different regions of the world.

Region	Study type and irrigation	Basil species	Hg	Reference
Various regions of Iran	[1]Greenhouse experiment	*O. basilicum*	25	Shariatpanahi et al. (1986)
Shantikunj, Haridwae, India	Market samples	O. sanctum	17	Kumar et al. (2005)
NE Poland	[1]Greenhouse experiment	Various	1	Majkowska-Gadomska et al. (2018)
Busan, Korean Republic	Local supermarkets	*O. basilicum*	1	Shim et al. (2019)
Galati County Romania	Spontaneous flora	*O. basilicum*	3	Marinescu et al. (2020)
NW Mexico	[1]Shrimp diluted seawater effluents	*O. basilicum*	2.3 ± 0.9	This study
NW Mexico	[1]Shrimp well effluents	*O. basilicum*	2.6 ± 0.9	This study

[1] Soilless cultivated plants.

reported the lowest levels of Hg, which are comparable to those found in our work. It should be noted that there is limited information regarding Hg accumulation in basil irrigated with wastewater or aquaculture effluents.

Mercury accumulation in the edible tissues of basil grown with shrimp effluents from both WW and DSW treatments were below the maximum allowable levels for food, vegetables, and products established in the guidelines for diverse world regions (mg kg^{-1} in fresh weight basis): 0.5 Malaysia and Singapore (WHO, 2007); 0.2 Canada (WHO, 2007); 0.05 Slovak (RGS, 2009); 0.01 China (MHPRC, 2012); 0.03 European Parliament (CREU, 2018); and 0.03 in general (FAO/WHO, 2001). This suggests that the reuse of shrimp low-salinity effluents (WW and DSW at 1.7 g L^{-1}) in an integrated aquaponic system for shrimp-basil culture does not represent a risk for the consumption of basil in terms of Hg accumulation. However, certain factors must be considered for a more accurate perspective, such as the intake periodicity and age and body weight, as this could have negative effects on human health. Therefore, health risk was examined to show any type of potential threat by the consumption of basil grown with this type of water in an aquaponics system.

3.5. Mercury mass balances

A mass balance (Fig. 3) of Hg was developed simulating typical conditions of an inland shrimp farm according to Páez-Osuna et al. (1997) and Leon-Cañedo et al. (2017) under specific conditions. (1) The

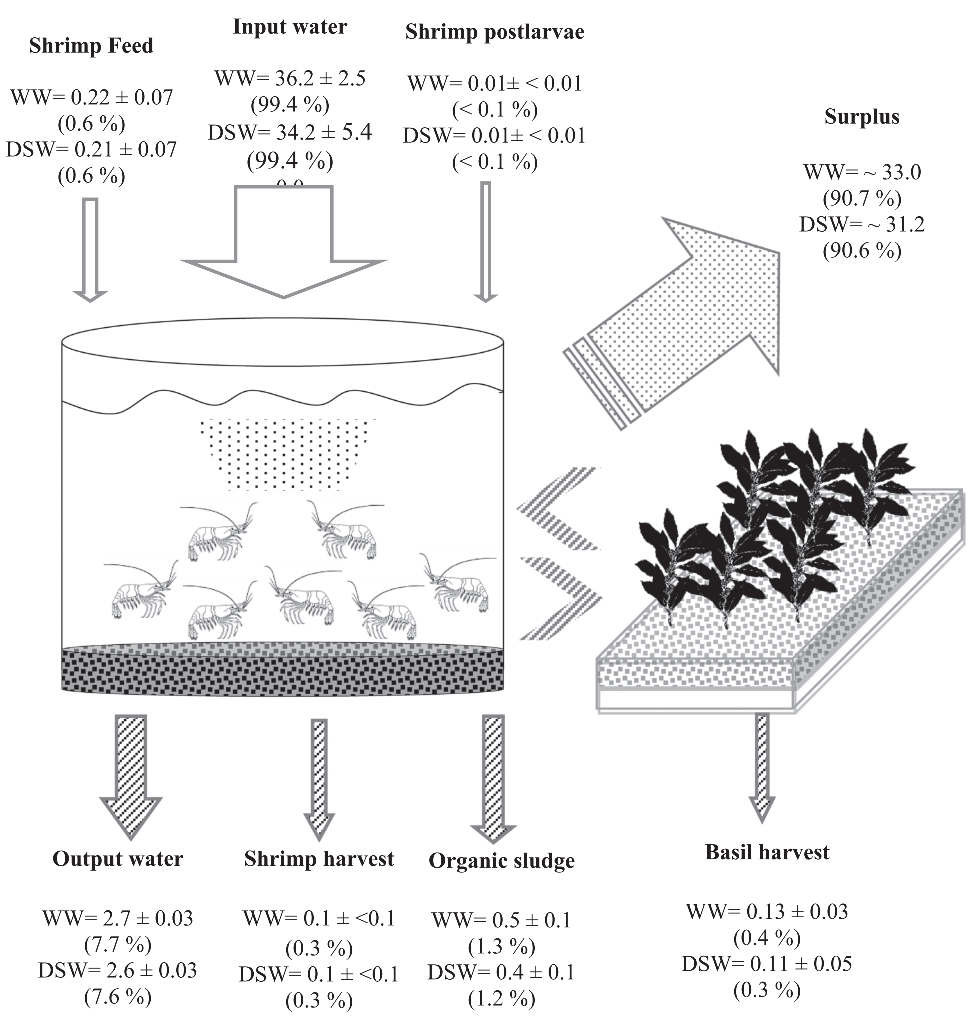

Fig. 3. Mercury mass balances in the aquaponic system with WW and DSW treatments. Values represent the mean ± standard deviation in mg $tank^{-1}$ $cycle^{-1}$; percentages are % of total mercury input into the tank. Mercury concentrations were calculated on the humidity of shrimp (75%) and basil (85%).

period of the culture cycle in commercial farms varies according to market demands and the threat of disease, which was assumed to be 78 days (cycle time for the aquaponic system) in this study. (2) The daily water exchange varies from 1% to 10%; a value of 1% was used in the present study. (3) Most farmers use food that contains 35% protein. The food used in this study was Camaronina (35% protein; Nestlé Purina PetCare Company); 3.98 and 3.89 kg cycle^{-1} (on a dry weight basis) were used for WW and DSW, respectively, and the Hg content was 0.055 ± 0.003 μg g^{-1} dw. (4) The stocking density of postlarvae was 75 PL m^{-2} (0.73 g fresh weight, with 75% moisture), in which the Hg found was 0.21 ± 0.02 μg g^{-1} dw. (5) The Hg concentration in the harvested shrimp (2.0 and 1.7 kg of fresh biomass for WW and DSW, respectively) was 0.23 ± 0.07 μg g^{-1} dw. (6) 5.2 m^3 of water was used in the culture cycle, in which the Hg content of the input water (filling and replacement waters) was 6.7 μg L^{-1}, while the output water (effluent, ~3.7 m^3) had 0.7 μg L^{-1} of Hg. (7) The whole harvested basil (16.8 and 13.1 kg of fresh biomass for WW and DSW) showed a Hg concentration of 0.05 μg g^{-1} dw. (8) Finally, we consider that the surplus from the sum of the inputs and outputs corresponds mainly to the zeolite used as substrate in the pots, the film/sludge of the clarifier and filter system, and the emission of Hg to the atmosphere.

Chemical analysis revealed that the shrimp feed used in this study contained 55 μg of Hg for each kg of feed supplied (León-Cañedo, 2019). Considering the rations of the feed applied during the culture cycle, a total average of 219 μg for WW and 214 μg for DSW of Hg entered each shrimp system pond through the shrimp feed.

Fig. 3 shows that the main contribution of Hg in the aquaponic system during the growth cycle in both treatments (WW and DSW) was through the filling and replacement waters (99.4%), while the feed had a reduced contribution (0.6%). This contrasts with Cu and Zn, which resulted mainly from feeding (91.8–97.0%) in intensive inland shrimp tanks (León-Cañedo et al., 2017). Nevertheless, it agrees with Lacerda et al. (2011) who reported that the main entry routes for Hg in commercial shrimp farming are through the input water, followed by the commercial feed. In this study, the surplus or the organic sludge retained in the biofilter and clarifier, volatilization, and the zeolite substrate of plants was the main Hg output route for WW and DSW, with 33.0 and 31.2 mg cycle^{-1} (~90.7%), respectively; while the biomass of the harvested shrimp (0.1 mg cycle^{-1}) and basil (0.1 mg cycle^{-1}) represent ~0.7%. From these results, it is clear that the organic sludge, and in this case zeolite, are the main routes of removal of Hg, similar to the observed in commercial farms (Lacerda et al., 2011) and intensive shrimp tanks for Cu and Zn (León-Cañedo et al., 2017).

3.6. Health risk assessment

The EDIs of Hg for the consumption of basil grown with two types of low-salinity waters from a shrimp-basil aquaponic system were 4.5×10^{-7} and 5.3×10^{-7} for WW and DSW treatments, respectively. These EDIs were calculated considering a body weight of 67.9 kg and a daily intake of 20 g for basil as a reference. These EDIs are below the guidelines for the provisional tolerable daily intake (PTDI = 6.0×10^{-4}, JECFA, 2018) and reference oral dose (RfD = 3×10^{-4}, USEPA, 2017) for both WW and DSW treatments.

Diverse scenarios with different body weights (10–120 kg) and daily intakes of basil (from 0.01 to 35 kg) were considered to estimate the HRI, which evaluates the potential risk according to the variation of a person's body weight and the quantity of basil consumed (Fig. 4). The risk to human health by the consumption of Hg in basil occurs from a daily intake of 5 kg for infants and 10 kg for adults, which is far removed from reality. This could be related to a greater accumulation of mercury in the roots compared to the edible tissues of the basil plant. Considering a basil daily intake value of 0.20 kg per day and a body weight of 67.9 kg (Walpole et al., 2012), the HRI calculations for Hg do not exceed the threshold value (HRI = 1) of health risk for the consumption of basil grown with shrimp effluents under aquaponic conditions and two different ionic water compositions.

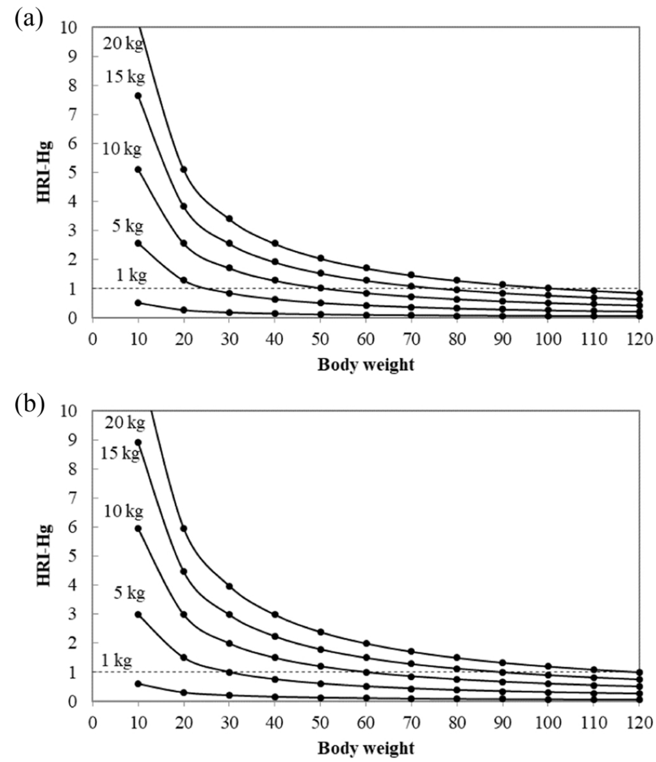

Fig. 4. Health risk index of Hg by the consumption of basil grown with different waters. (a) WW and (b) DSW. Continuous lines represent the HRI for different body weights and daily basil intake. The discontinuous line is the threshold of HRI= 1.0; values above 1.0 represent a potential human health risk.

4. Conclusions

In general, the integration of whiteleg shrimp (*L. vannamei*) and basil (*O. basilicum*) culture through a low-salinity aquaponic system is feasible in terms of production for both WW and DSW treatments. Mercury levels found in both WW and DSW treatments were lower than those typically registered in municipal and industrial effluents. Mercury accumulation in basil showed a general trend of root > leaves > stems, without significant differences between the concentrations of both WW and DSW treatments. The Hg levels in edible basil tissues for this study are comparable and even similar to those recorded in other studies under experimental conditions or from markets. In this study, Hg concentrations in the edible tissues of basil were below the maximum allowable limits established by the guidelines from different countries. The calculations of the EDI and HRI in this study indicate that the accumulated levels of Hg in edible basil tissue do not represent a risk for human health considering an average consumption of 20 g per day per person.

The mass balances evidence that the main route of incorporation of Hg in the aquaponic system comes from the input waters (99.4%), and the main Hg output route is through the sludge and zeolite of the pots (90.7%). However, we do not know if Hg emissions to the atmosphere are important since they were not quantified. It is significant to denote that the Hg associated with the harvest of shrimp and basil was very low (<0.5%). A final perspective from this study is that this aquaponic system could be developed commercially in Mexico since shrimp farming in this country (using low-salinity waters, 0.5–2 g L^{-1}) has been established in the states of Colima, Baja California, Jalisco, Hidalgo, and Sinaloa. Thus, future research should evaluate the economic and financial costs involved in commercial aquaponics shrimp-basil systems, followed by the overall quality of harvested products assessing bioactive molecules, and nutritional and nutraceutical properties, including secondary metabolites (Braglia et al. 2021).

CRediT authorship contribution statement

Jesús A. León-Cañedo: Conceptualization, Investigation, Methodology, Field work, Writing-original draft. **Suammy G. Alarcón-Silvas**: Investigation, field work, Writing-Review & Editing. **Juan F. Fierro-Sañudo**: Investigation, field work, Writing-Review & Editing. **Gustavo A. Rodríguez-Montes de Oca**: Conceptualization, Methodology, Funding Acquisition, Writing-Review & Editing. **Marcela G. Fregoso-López**: Methodology, Writing-Review & Editing. **Federico Páez-Osuna**: Conceptualization, Visualization, Funding Acquisition, Supervision, Project Administration, and Writing-Review & Editing.

Data availability

No data was used for the research described in the article.

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