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Han-Yong Jeon *Editor*

# Sustainable Development of Water and Environment

Proceedings of the ICSDWE2020

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Han-Yong Jeon  
Editor

# Sustainable Development of Water and Environment

Proceedings of the ICSDWE2020

 Springer

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# Preface

Dear Distinguished Authors and Guests,

It was a great pleasure to welcome all participants in the 2020 the 3rd International Conference on *Sustainable Development of Water and Environment* (ICSDWE2020) held in Inha University, South Korea, during January 13–14, 2020. ICSDWE2020 is sponsored by Inha University.

The aim of ICSDWE2020 is to present the latest research and results of scientists (professors, students, Ph.D. students, engineers, and postdoc scientists) related to sustainable development of water and environment. The key goal of the conference provides opportunities for academic scientists, engineers, and industry researchers to exchange and share their expertise, experience, new ideas, or research result and discuss the challenges and future in their expertise. ICSDWE2020 also provides a platform for the students, researchers, and engineers to interact with experts and specialists on the technical matters and future direction of their research area.

The papers were selected after the peer review process by conference committee members and international reviewers. The submitted papers were selected on the basis of originality, significance, and clarity for the purpose of the conference. The papers should provide the reader an overview of many recent advances in the field related to *sustainable development of water and environment*. The conference program is extremely rich, featuring high-impact presentation. We hope that the conference results constituted a significant contribution to the knowledge in these up-to-date scientific fields.

On behalf of the Organizing Committee, we would like to especially thank all technology committee members, reviewers, conference chairs, keynote speakers, sponsors, and conference participants for their support and contributions to ICSDWE2020.

We look forward to your participation in the 4th ICSDWE2021.

With our warmest regards

Incheon, Korea (Republic of)

Han-Yong Jeon

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# Environmental and Microbiological Assessment of Small-Scale Vegetable Farming Systems Irrigated With Wastewater from Upper Balili River, La Trinidad Benguet, Philippines



Rosemary M. Gutierrez and Venus Montalla

**Abstract** In developing countries like the Philippines, it is a common practice to use river wastewater for the irrigation of agricultural lands. The determination of coliform bacteria in the Balili River wastewater, agricultural soils irrigated by it, and vegetable, particularly lettuce, *Lactuca sativa*, grown in these areas were carried out to serve as indicators of their microbiological quality and potential risks. Bacteriological counts of the water, soil, and vegetable samples were enumerated via membrane filter technique and multiple tube fermentation technique. Primers of the *wecA* gene, which encode for the protein responsible for the enterobacterial common antigen (ECA), were used for the detection of *E. coli* by the Polymerase Chain Reaction Method. The results revealed that the total coliform and fecal coliform of the samples for the four sampling periods all exceeded the acceptable standards (>6000–13,000 MPN/100 mL). The low Water Quality Index values ranging from 21 to 28, let alone the presence of coliform bacteria such as *Enterobacter*, *Pantoea*, *Escherichia*, and *Klebsiella* in the samples, confirm the reports about the worsening quality of the river and stress the danger of directly introducing the wastewater to these agricultural fields. Furthermore, lettuce is a very high-risk crop for coliform contamination and as fecal coliforms were isolated from this vegetable, it can be inferred that the continued use of Balili River wastewater for crop irrigation is unsuitable and an unhealthy practice to consumers.

**Keywords** Balili River · Benguet · Philippines · *Lactuca sativa* · *Escherichia* · Enterobacteriaceae

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## 1 Introduction

Water from rivers, soil and agricultural products such as vegetables can easily be contaminated because of agricultural practices such as the use of untreated river water for irrigation. The use of this untreated river wastewater for irrigation of vegetable farmlands is a common practice in developing countries like the Philippines. However, this practice can be of danger to human health because of the spread of disease-causing microorganisms contaminating vegetables especially those that can be eaten raw in the form of fresh salads made of vegetables like lettuce, tomatoes, green pepper, cabbage, spinach, watercress and others.

It has been recommended by the World Health Organization (WHO) that crops and vegetables, in order to be eaten raw, should be irrigated only with biologically treated effluent which has undergone a disinfection process in order to achieve a safe coliform level of not greater than 100 most probable number (MPN) of coliforms per 100 mL in 80% of the samples (WHO 1976).

A major river found in La Trinidad, Benguet in Northern Philippines is the Balili River, which stretches from Baguio City to La Trinidad, Benguet. It spans approximately 50 km from Baguio City from Benguet province to La Union province. Water coming from the said river aids in the irrigation of many vegetable farmlands and flower gardens in the municipality (Bengao et al. 2015). Under the standards of the Philippine Water Act, the Balili River was classified before as Class A body of water. The standard of Class A water is that its water quality should be potable, i.e., it should only contain 1.1 MPN fecal coliform per 100 milliliters (mL) of sample. However, it was reported by the Environment Management Bureau, Cordillera Administrative Region (EMB-CAR) of the Department of Environment and Natural Resources (DENR) that the Balili River, in its current state, has failed the Class A standard because the river is highly polluted (Hent 2019).

This study therefore aimed to assess the bacteriological quality of the water, soil, and vegetables, particularly lettuce, *Lactuca sativa* from selected La Trinidad, Benguet agricultural farms that have been identified to be irrigated with wastewater coming from Balili River. Using the physicochemical and bacteriological parameters of the river, the water quality of Balili River was also evaluated using a method of computation for the water quality index using missing parameters.

## 2 Materials and Methods

### 2.1 Study Site Description

The municipality of La Trinidad, situated in the Province of Benguet is popularly known as the vegetable capital of the Philippines. La Trinidad is approximately located 256 km north of Metro Manila. Barangay Balili, the site of collection in this

**Table 1** Agricultural farms from Barangay Balili, La Trinidad Benguet that were identified to be irrigated with Balili River wastewater based on interviews and reconnaissance trips

Site	Coordinates	Location/description	Vegetables and fruits found during sampling periods
Site 1	16° 27' 37'' N, 120° 35' 19'' E	Private agricultural farm across the Commission on Higher Education (CHED) Office	Lettuce, strawberries
Site 2	16° 27' 18'' N, 120° 35' 28'' E	Experimental University Farm of Benguet State University	Lettuce, broccoli, oranges
Site 3	16° 26' 28'' N, 120° 35' 39'' E	Private agricultural farm behind Apple Blossom Bakery	Lettuce, onions, mint

study is located on the southwestern part of the municipality of La Trinidad. According to the Department of Environment and Natural Resources (DENR) cadastral survey, this barangay has an estimated land area of 119.0164 ha or 1.4731% of the municipality's total land area. The three sampling sites in this present study, which have been verified to be vegetable farmlands irrigated with Balili River wastewater are located in Barangay Balili (Table 1).

Different water sources of La Trinidad flow out to the different creeks and tributaries going to Balili River, the main drainage of the municipality. Aside from being the main catchment in the valley, Balili River stretches or spans about 50 km all the way to the Province of La Union where it is known as the Naguilan River, and from there, this river flows out directly into the China Sea. Twelve major creeks have been recorded to drain from three directions in the municipality and are considered tributaries to the river. Balili River is characterized by a relatively steep to gradient, suggesting that the flow pattern of water run-off takes the form of a rapid stream which is a loss of the flow resource (CLUP 2019).

## 2.2 Sample Source

Samples of Balili River water, soil, and vegetables (lettuce, *Lactuca sativa*) were collected from three agricultural farmlands in Barangay Balili, La Trinidad, Benguet (Table 1). The sampling periods were done on a quarterly basis and were extended for a period of one year, from September 2016 to August 2017 with one type of vegetable specimen collected at each sampling time. Lettuce was the vegetable of choice since it was the vegetable found common to all three sampling sites. Water, soil and vegetable specimens were collected during each sampling time. Sampling period 1 was last September 2016, Sampling period 2 was last January 2017, sampling period 3 was last April 2017 while sampling period 4 was last August 2017.

Upon collection on field, the samples of vegetables were placed in sterile containers and transported to the laboratory and processed, where leaves and roots from the lettuce vegetable were separated. Two sets from these samples were prepared: the first set was analyzed without previous rinsing, whereas the second set was rinsed in tap water before being subjected to bacteriological analysis. The rinse from the vegetable using tap water was also subjected to analysis. After processing, the samples were then homogenized.

Ten (10) g of each soil sample was collected and weighed. Serial dilution was done using a previously prepared sterile phosphate buffer, and mixed for 1 min in a blender. Soil samples were then serially diluted before being plated out in a microbiological media for bacteriological examination and isolation of pure cultures of bacteria.

### **2.3 Bacteriological Examination**

#### *Water*

Fecal (FC) as well as Total Coliform (TC) were enumerated by the membrane filter technique. The diluted samples (1.0, 0.1 and 0.01  $\mu\text{L}$ ) were then filtered through sterile membrane filters (with pore size 0.045  $\mu\text{m}$ , with a diameter of 47 mm). The culture medium that was used for enumeration of FC and TC was M-endo broth.

#### *Vegetables and Soils*

The bacteriological examination of vegetables and soils were performed by the three-tube fermentation technique as described in the Standard Methods for the Examination of Water and Wastewater (APHA 2005).

### **2.4 PCR Amplification of *E. coli* Genomic DNA**

The Freeze-Thaw method was used for the isolation of crude DNA samples, and were used as a template for PCR analyses. PCR amplification of bacterial DNA was done using the designed *wecA* gene primers, these primers are used for the specific detection of *E. coli* DNA. The set of primers used were the following: forward primer, GGT GTT CGG CAA GCT TTA TCT CAG and reverse primer, GGT TAA ATT GGG GCT GCC ACC ACG. The PCR procedures used in this study were patterned after the methods described by Bayardelle and Zafarulla (2002). This *wecA* gene is important for enterobacterial common antigen (ECA) and is specific for detecting *E. coli* (Debroy et al. 2006).

## 2.5 *Identification of Bacterial Isolates and Phylogenetic Analyses*

Identification of bacterial isolates was done by submitting pure cultures for 16S ribosomal RNA gene sequencing to Macrogen, South Korea. Using the MEGA X (v. 10.1) (Debroy et al. 2006) software, the 16S rRNA gene sequences were then aligned using the BLAST software v. 2.6.1 (Tamura et al. 2013) of the National Center for Biotechnology Information (NCBI), in comparison with 16S ribosomal RNA sequences (Bacteria and Archaea) Database.

## 2.6 *Physicochemical Properties of Soil and Water from La Trinidad, Benguet*

Data on the physicochemical and bacteriological characteristics of Balili River water were obtained from the Environment Management Bureau of the Cordillera Administrative Region, (EMB-CAR) Ambient Monitoring and Technical Services Section of the Department of Environment and Natural Resources (DENR), Baguio City, Philippines.

## 2.7 *Water Quality Index Computation*

Water Quality Index (WQI) was computed using the physicochemical parameters and bacteriological data obtained from the EMB-DENR from the period of 2012–2017. The formula for computation for Water Quality Index with missing parameters was used (Srivastava and Kumar 2013).

$$WQI_{MP} = \sum WYQY / \sum WY \quad (1)$$

where

Y = available parameters

QY = q-values of available parameters

WY = weighting factors of available parameters.

The above Eq. 1, is actually based from a standard equation for computing the Water Quality Index given by the National Science Foundation (Srivastava and Kumar 2013; Gupta et al. 2017) as shown in Eq. 2:

$$WQI = \sum WXQX \quad (2)$$

$$\begin{aligned}
 \text{WQI} = & W_{\text{BOD}}Q_{\text{BOD}} + W_{\text{DO}}Q_{\text{DO}} + W_{\text{pH}}Q_{\text{pH}} + W_{\text{PHOSPHATE}}Q_{\text{PHOSPHATE}} \\
 & + W_{\text{NITRATE}}Q_{\text{NITRATE}} + W_{\text{FC}}Q_{\text{FC}} + W_{\text{TDS}}Q_{\text{TDS}} + W_{\text{TEMP.}}Q_{\text{TEMP.}} \\
 & + W_{\text{TURBIDITY}}Q_{\text{TURBIDITY}}
 \end{aligned}$$

where

WX = weight factors of the water quality parameters

QX = q-value of the water quality parameters

X = water quality parameters.

The average values of the above parameters (BOD, DO, pH, phosphate, nitrate, fecal coliform, total dissolved solids or TDS, temperature and turbidity) were computed per year for the six-year monitoring period (2012–2017) and values for  $\text{WQI}_{\text{MP}}$  were computed for each year using the Microsoft Excel Program.

### 3 Results and Discussion

#### 3.1 Water Quality Index of the Balili River

In the Philippines, there is no formulated and developed guidelines on water quality index. Only water quality standards given by the Department of Environment and Natural Resources (DENR) are used as a basis for acceptable values of physicochemical parameters. This was issued by DENR in an Administrative Order 34 series of 1990, or DAO, 2016 (Ichwana et al. 2016). This administrative order stipulates the water quality criteria or standards based on beneficial usage of the body of water or classification of freshwater and marine waters, which provides the basis for determining the suitability of water bodies for specific use (DAO 2016).

Results of WQI computation and interpretation of river water quality for the Balili River for the six-year monitoring period using the mathematical formula in Eq. 2 are shown in Table 2. The data indicate that the water quality of the Balili River is really

**Table 2** Computed water quality index with missing parameters for Balili River for the period of 2012–2017 using the physicochemical and bacteriological data from DENR-EMB

Year	$\text{WQI}_{\text{MP}}$	Water quality
2012	28.95	Bad
2013	22.07	Very bad
2014	20.58	Very bad
2015	28.06	Bad
2016	27.26	Bad
2017	23.21	Very bad



bad to very bad confirming that the said river is polluted and therefore not suited to be used for irrigation of vegetable farmlands. These results are in contrast to the study done in the rivers of Palawan, Philippines wherein the study also computed the WQI of the rivers using the WQI developed by the Canadian Council of Ministers of the Environment (Martinico-Perez et al. 2019). The study done in the rivers of Palawan described the rivers as having good to excellent water quality and thus can be recommended to be utilized for irrigation in agriculture and other purposes (Class D, classification) (Ichwana et al. 2016). After complete treatment, there were five (5) river monitoring stations that were found to have good to excellent ratings and can be considered as sources of drinking water (Class A, classification of DAO 2016; CCME 2001).

### ***3.2 Bacteriological Examination***

Overall, the results of our bacteriological analyses indicated that the level of fecal coliform and total coliform in the three sampling sites of this study were exceedingly high with values that ranged from >6000 to 13,000 MPN/100 mL for the four sampling periods. On average, the highest number of both total coliform and fecal coliform were observed during the fourth sampling period in May 2017 (Figs. 2 and 3).

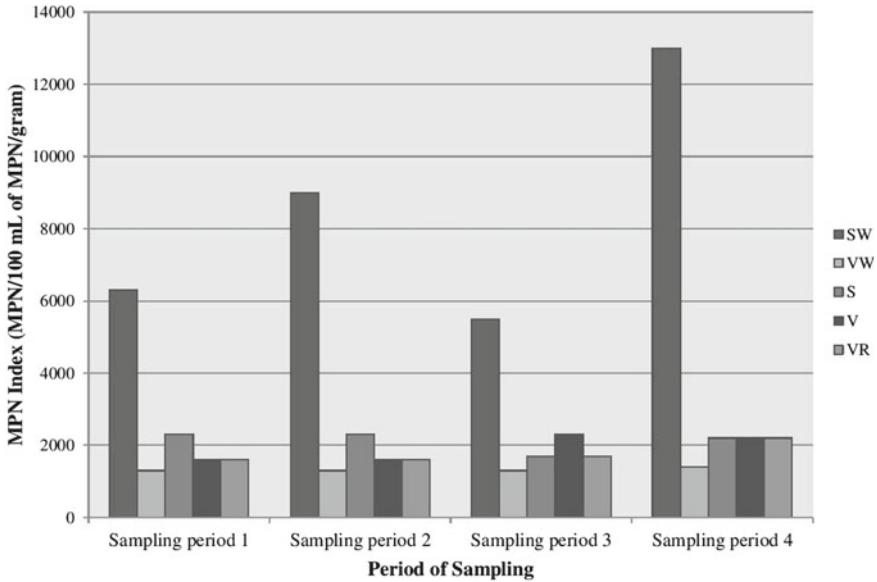
It was also observed that the water samples from the rinse of the vegetables harbored a large number of coliform bacteria, having as much as 1500 MPN/100 mL for total coliform and 2500 MPN/100 mL for fecal coliform. Accordingly, the highest coliform levels in the samples were also detected during the fourth sampling period.

After the three phases of presumptive, confirmatory, and completed tests in the vegetable and soil samples, the presence of coliform bacteria was confirmed. As shown in Fig. 2, most of the total coliform of the soil and unrinsed vegetable samples were above the detection limit of the method used (more than 2400 coliforms per gram) whereas the total coliform of rinsed samples does not appear to be far from these values. In general, the results show that total coliform and fecal coliform were also highest during the fourth sampling period (May 2017).

The high coliform values can be attributed to anthropogenic activities since there is a large number of residences along the river. And unfortunately, these residences directly discharge their sewerage wastes to the river. Also, as a means of livelihood, people living in the area engage in backyard piggeries that discharge animal wastes directly to the river (Hent 2019).

### ***3.3 Heavy Metal Content of Soil and Water***

The computed mean values of the five types of heavy metal studied and monitored for six (6) years by DENR-EMB for Balili River surface water were compared with the

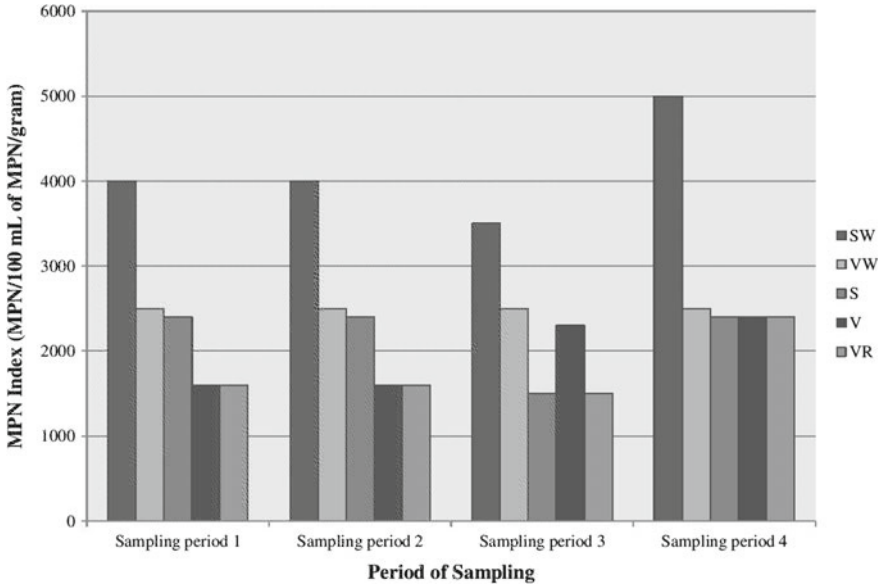


**Fig. 1** Average total coliform counts in Balili River wastewater, soil samples irrigated by the river, and the vegetable samples during the four sampling periods. SW = water samples from the three sites, VW = tap water, S = soil samples from the three sites, V = unrinsed vegetable samples, VR = rinsed vegetable samples

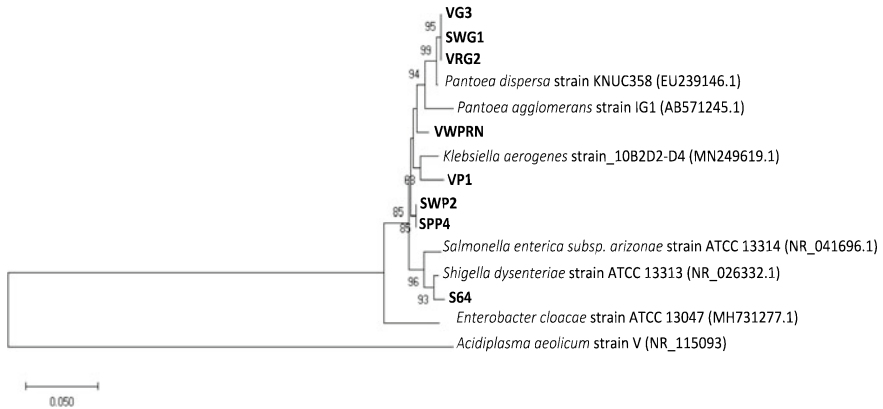
Aquatic Toxicity Reference Value (TRV) (Table 3). The TRV is defined as the dose above which significant ecological effects may occur to wildlife species after prolonged dietary exposure and below this value, it is expected that relevant effects will not occur (Cornell and Schwertmann 2003). The mean values of copper, cadmium, lead, and mercury exceeded the TRV, while the mean value of zinc ( $117.75 \mu\text{g/L}$ ) is very close to the TRV value ( $120 \mu\text{g/L}$ ). The computed mean values of the metal content follow this decreasing order:  $\text{Zn} > \text{Cd} > \text{Cu} > \text{Pb} > \text{Hg}$ . This means that the Balili River wastewater has high content for all metals analyzed (except for zinc) since the values obtained by EMB-CAR greatly exceeded the TRVs for cadmium, copper, lead and mercury. The high heavy metal content may be attributed to domestic sewage disposal and agricultural runoff from the vegetable and flower gardens of the area (Burriss et al. 2001).

### 3.4 Isolation and Identification of Bacteria and PCR Analyses

In this study, a total of eight morphologically distinct bacteria were successfully isolated from the river wastewater, agricultural soils, and vegetable samples. Results



**Fig. 2** Average fecal coliform counts in Balili River wastewater, soil samples irrigated by the river, and the vegetable samples during the four sampling periods. SW = water samples from the three sites, VW = tap water, S = soil samples from the three sites, V = unrinsed vegetable samples, VR = rinsed vegetable samples



**Fig. 3** Phylogenetic tree constructed using the MEGA software version X. At branched points, bootstrap values of >50% are indicated. Isolates in the present study were shown in bold, whereas the gene sequence from *Acidoplasma aeolicum* was used as the outgroup. The tree was constructed using MEGA software version X

**Table 3** Comparison of the means of the heavy metal content of Balili River wastewater analyzed by DENR, EMB-CAR for the period of 2012–2017 with the aquatic toxicity reference values

Heavy metal	Mean value for 6 years ( $\mu\text{g/L}$ )	Aquatic toxicity reference value ( $\mu\text{g/L}$ )*
Copper (Cu)	20.67	6.54
Cadmium (Cd)	111.55	0.66
Lead (PB)	13.00	1.32
Zinc (Zn)	117.75	120
Mercury ( Hg)	0.778	0.12

\*Source: Burris et al. (2001)

of Polymerase Chain Reaction (PCR) revealed that the *weca* gene primers used were able to detect enterobacterial DNA in the isolates. All of the isolates analyzed after PCR showed the presence of bands, with the products of the amplification at 763 bp (data not shown). *Enterobacteriaceae* is a group of Gram-negative bacteria which are rod-shaped and are usually found in the gastrointestinal tract of most animals.

Furthermore, comparison to sequence databases (Table 3) shows that the bacterial isolates exhibited up to 97–99% similarity with *Enterobacter hormaechei*, *Pantoea dispersa*, *E. coli*, *Enterobacter ludwigii*, and *Klebsiella variicola*, all of which belongs to the family Enterobacteriaceae. However, among the isolates of this present study, no strain of *E. coli* O157:H7 was detected. Instead, a different strain of the bacterium that was not further characterized was found.

#### *Water, Soil, and Vegetable*

Two types of bacteria, isolated from Balili River wastewater were found to be closely related to the bacteria *Enterobacter hormaechei* and *Enterobacter ludwigii*. Members of the genus *Enterobacter* are known to cause hospital-acquired infections, such as lung, urinary tract, intra-abdominal, meningeal, and surgical site infections. On one hand, *Enterobacter hormaechei* is a species of bacterium that belongs to the *Enterobacter cloacae* complex. Bacteria under this group are mostly found in clinical specimens and are known to cause bloodstream infections like sepsis and potent producers of a wide spectrum of beta-lactamases (Islam et al. 2015). On the other hand, one study reported one case of *Enterobacter ludwigii* infection that was acquired through surgery (Townsend et al. 2008). Disturbingly, this study also found that this species is resistant to the drug carbapenem, indicating that it could possibly be a multiple drug-resistant bacterium. With this said, the researchers of the present study speculate about the possibility of the presence of hospital wastes in Balili River wastewater.

It has been suggested that soils in tropical environments are efficient in supporting the growth of coliform bacteria (Khajuria et al. 2013). A study showed a higher number of isolated *E. coli* in organic farms as compared to their conventional counterparts (Byappanahally and Fujioka 1998). This is possible enough because some of the sampling sites in the present study were once conventional farms that have been converted into organic farms.

**Table 4** Comparison of isolated coliform bacteria from Balili river wastewater, soil samples irrigated by the river, and the vegetable samples to sequence databases

Sample code	Closest relative	GenBank accession number	Similarity (%)
SWP2 <sup>a</sup>	<i>Enterobacter hormaechei</i>	CP017179.1	99
SWG1 <sup>a</sup>	<i>Pantoea dispersa</i>	AB907780.1	99
VWPRN <sup>b</sup>	<i>Enterobacter ludwigii</i>	CP017279.1	99
SPP4 <sup>c</sup>	<i>Enterobacter hormaechei</i>	CP017179.1	99
SG4 <sup>c</sup>	<i>Escherichia coli</i>	KC985144.1	97
VP1 <sup>d</sup>	<i>Klebsiella variicola</i>	CP010523.2	99
VG3 <sup>d</sup>	<i>Pantoea dispersa</i>	AB907780.1	99
VRG2 <sup>e</sup>	<i>Pantoea dispersa</i>	AB907780.1	99

<sup>a</sup>Isolated from Balili River Wastewater

<sup>b</sup>Isolated from the tap water from the rinse of vegetable samples

<sup>c</sup>Isolated from the soils

<sup>d</sup>Isolated from the unrinsed vegetable samples

<sup>e</sup>Isolated from the rinsed vegetable samples

This is because organic fertilizers contain manure that may have pathogenic bacteria (Mukherjee et al. 2004) hence, readily explaining the detection of higher coliform levels in these areas.

While the genera *Enterobacter* and *Escherichia*, comprise most of the known soil-derived coliforms (Johannesen et al. 2004), their presence in the agricultural soils irrigated with Balili River wastewater should be considered significant. Although no Shiga-toxin producing strain of *E. coli* (*E. coli* O157:H7) was isolated in the present study, the presence of *E. coli* alone stresses the danger of directly introducing these pathogens from wastewater to these agricultural fields.

Often misidentified as *Klebsiella pneumoniae*, the bacterium *Klebsiella variicola*, which has been obtained from the vegetable samples in the present study, is an enteric pathogen that is frequently isolated from clinical specimens and various species of plants (Heaton and Jones 2007). Members of the genus *Klebsiella* exhibit a wide array of virulence factors such as the presence of bacterial capsule and metabolic versatility, allowing them to thrive in large spectra of environments and demonstrate infective potential. Like *K. pneumoniae*, *K. variicola* is known to cause bloodstream infections in humans and cattle. Alarmingly though, *Klebsiella variicola* was found to cause higher mortality rates in patients as compared to the former (Rosenblatt et al. 2004).

Another bacterium isolated in the present study is *Pantoea dispersa*, one of the very few species of the genus *Pantoea* that is capable of growing up to 41 °C. While members of the genus *Pantoea* have been rarely reported to cause infections in humans, they are known to be plant pathogens, specifically causing leaf spots in okra *Abelmoschus esculentum* (Maatallah et al. 2014; Mehar et al. 2013). For instance, the bacterium *Pantoea agglomerans* has been found to colonize and aggregate with the bacterium *Salmonella enterica* on the phylloplane (leaf surface) of cilantro (Falkow

et al. 2006). Because of their ability to degrade the leaf surface and increase the release of plant nutrients, the presence of plant pathogens like *Pantoea* per se increases the likelihood of the entry of enteric pathogens like *Salmonella* (Brandl and Mandrell 2002).

Several scientific studies have suggested that bacterial contamination of vegetables can happen at any time during production but the irrigation using wastewater, with the edible parts of the plants being directly applied, has the greatest potential to cause contamination (Wells and Butterfield 1999; Pescod 1992). The general morphology of the lettuce leaves, having much of their surface exposed to the irrigation water and soil, as well as the method of irrigation both account for the high total coliform and fecal coliform in the samples. According to a study, the contamination of soil and plants grown in substrates irrigated with coliform-contaminated water is also highly dependent on the survival capabilities of the pathogens (Buck et al. 2003). Normally, bacterial pathogens survive for two months but this can extend up to five months given the favorable moist condition and protection against the heat from the sun (Gerba and Smith 2005). In this study, vegetable samples, as well as the water and soils, were collected on the same day or few days after wastewater irrigation. Hence, this account could readily explain the detection of fecal coliforms in the samples.

However, based on the results of the DNA analysis shown in this present study, the researchers were intrigued by the presence of *Enterobacter hormaechei* on both river wastewater samples and soil samples and as well as the occurrence of *Pantoea dispersa* on both river wastewater samples and vegetable samples. As alluded to earlier, soil and vegetable samples in this study were collected on the same day or probably only a day or two after irrigation. Interestingly, *Pantoea dispersa* was also detected in the rinsed vegetable samples. With this, the researchers suspect that the bacteria isolated from the soil samples and vegetable samples could be a combination of recently established bacteria and naturally occurring soil as well as plant bacteria.

Furthermore, the researchers of this present study were also taking into account the possibility of internalization of bacteria. Studies about the adherence of pathogens to plant surfaces and modes of internalization are not new to the scientific field. A number of studies have already reported that there are some enteric pathogens that have the ability to survive on the phylloplane, and may have the possibility of being taken up the plant root systems and gain entry to the edible portions of plants like lettuce, apples, and tomatoes (Johannesen et al. 2004; Islam et al. 2002; Wachete et al. 2004; Seo and Frank 1999; Solomon et al. 2002; Burnett et al. 2000). Aside from that, these pathogens may also enter through plant structures like lenticels and wounds (Guo et al. 2001; Janisiewicz et al. 1999).

The phylogenetic tree that was constructed to establish the evolutionary similarity on the 16s rDNA sequences of the isolates and those in Genbank databases is shown in Fig. 3. Using the MEGA Software, Isolates VG3, SWG1, and VRG2 were shown to be very similar to *Pantoea dispersa* while isolate VWPRN was closely grouped with both *Pantoea agglomerans* and *Pantoea dispersa*. Isolates SWP2, and SPP4 were closely associated with *Samonella enterica* while isolate S64 had similar sequences with *Shigella dysenteriae*. Isolate VP1 was the only isolate with similar sequences

with *Klebsiella aerogenes* (Fig. 3). This clustering confirmed the alignments that was obtained using the BLAST algorithm.

## 4 Conclusion

Because of the increasing awareness of the health benefits of natural and organic products, the consumption of fresh vegetables on the agricultural market is on the rise. The findings of this study stress the danger of vegetable crops and soil contamination with pathogenic bacteria because of the use of the untreated Balili River wastewater for irrigation. Being consumed raw, lettuce, *Lactuca sativa* is a very high-risk crop for coliform contamination. Bacteria, such as *Enterobacter*, *Pantoea*, *Escherichia*, and *Klebsiella*, all of which have been isolated in this study are just a few examples of opportunistic pathogens in the mammalian gut, and they were confirmed to be present in the river wastewater, vegetable samples, and agricultural soils through isolation techniques and PCR analyses. Moreover, high numbers of fecal coliforms were determined and these fecal coliforms were isolated in this study from river water, vegetables, and soil. Computation of the Water Quality Index of Balili River done in this study using the physicochemical and bacteriological characteristics of the river for six years gave index values or WQI values ranging from 21 to 28. These WQI values mean that the Balili River is actually in very bad condition and therefore very much polluted. This study therefore, confirms the report that the water quality of Balili River has worsened through the years.

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# The Effects of Sea Level Rise on Salinity and Tidal Flooding Patterns in the Guadiana Estuary



Lara Mills, João Janeiro, and Flávio Martins

**Abstract** Sea level rise is a worldwide concern as a high percentage of the population accommodate coastal areas. The focus of this study is the impact of sea level rise in the Guadiana Estuary, an estuary in the Iberian Peninsula formed at the interface of the Guadiana River and the Gulf of Cadiz. Estuaries will be impacted by sea level rise as these transitional environments host highly diverse and complex marine ecosystems. Major consequences of sea level rise are the intrusion of salt from the sea into fresh water and an increase in flooding area. As the physical, chemical and biological components of estuaries are sensitive to changes in salinity, the purpose of this study is to further evaluate salt intrusion in the Guadiana Estuary caused by sea level rise. Hydrodynamics of the Guadiana Estuary were simulated in a two-dimensional numerical model with the MOHID water modeling system. A previously developed hydrodynamic model was implemented to further examine the evolution of salinity transport in the estuary in response to sea level rise. Varying tidal amplitudes, freshwater discharge from the Guadiana River and bathymetries of the estuary were incorporated in the model to fully evaluate the impacts of sea level rise on salinity transport and flooding areas of the estuary. Results show an overall increase in salinity and land inundation in the estuary in response to sea level rise.

**Keywords** Guadiana Estuary · Numerical model · Sea level rise

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# 1 Introduction

## 1.1 *Sea Level Change*

Global mean sea level is rising at a rate of approximately 3.2 mm per year (Church et al. 2013). According to the most recent report from the Intergovernmental Panel on Climate Change (IPCC), the rate of global mean sea level rise has been increasing over the last two centuries and continues to accelerate. Sea level rise will affect coastal areas, which should be a concern considering 10% of the world's population live within 10 m elevation of the present sea level (Carrasco et al. 2016). Direct consequences of sea level rise on coastal areas include an increase in flooding area, an increase in erosion, an increase in salinity and changes in ecosystems (Nicholls et al. 2011). Of relevance to the present study is the impact of sea level rise on estuaries, where rivers intersect with sea and freshwater mixes with saltwater.

## 1.2 *Effects of Sea Level Rise on Estuaries*

Salt intrusion is a direct consequence of sea level rise in estuaries (McLean et al. 2001). Estuarine circulation is mainly driven by freshwater flow, tides and density differences (Garel et al. 2009). A study by Chua and Xu (2014) found a stronger longitudinal salinity gradient in estuaries due to sea level rise, which in turn drives a stronger gravitational circulation. The increase in salinity will cause the water to become denser and thus, increase the stratification of the water column. Changes in estuarine stratification and circulation will further cause oxygen depletion (Hong and Shen 2012). These effects are detrimental to ecosystem services and marine habitat as estuaries hold highly diverse and complex ecosystems (Sampath et al. 2015). The objective of this study is to evaluate the evolution of hydrodynamics and salinity transport in response to various sea level rise scenarios in a major estuary in the Iberian Peninsula, the Guadiana Estuary.

## 1.3 *Physical Characteristics of the Guadiana Estuary*

The Guadiana Estuary is formed at the interface of the Guadiana River and the Gulf of Cadiz. The head of the Guadiana River begins in Spain and extends 810 km south toward the Gulf of Cadiz (Delgado et al. 2012). From its mouth in front of Vila Real de Santo António, the Guadiana Estuary extends 80 km north to its tidal limit near Mértola, Portugal. Because of its narrow width and moderate depth the Guadiana Estuary is considered a rock-bound estuary where the volume of water entering the estuary during the flood tide is larger than the freshwater discharge (Garel 2017). Tidal and riverine processes are the dominant forces in the estuary and the action

of the waves is considered negligible (Garel et al. 2009). The Guadiana Estuary is characterized by a semidiurnal mesotidal regime (Sampath and Boski 2016) with an average neap tidal range of 1.28 m and an average spring tidal range of 2.56 m (Garel et al. 2009). The western margin of the estuary is characterized by a salt marsh sheltered by a littoral sand spit that drains into the estuarine channel (Garel et al. 2009). The eastern margin is composed of barrier islands and sand spits separated by extensive salt marshes that drain to the Gulf of Cadiz through a tidal inlet (Garel et al. 2009). The flow rate from the Guadiana River varies drastically from less than 10–4660 m<sup>3</sup>/s. The construction of over 100 dams for water storage and irrigation since the 1950s has strongly reduced the freshwater flow rate (Garel et al. 2009). Of importance is the Alqueva Dam located 60 km upstream the head of the estuary (Garel and D'Alimonte 2017). This large reservoir was completed in 2002 and since then the freshwater flow into the estuary has been reduced from a yearly average of 143–16 m<sup>3</sup>/s (Garel et al. 2009). The reduction in flow rate also has an impact on residence time, the time it takes a particle to exit the system (Oliveira et al. 2006). The residence time of salinity for a river discharge of 8 m<sup>3</sup>/s ranges between 6 and 60 days, but when the river flow is high the residence time ranges from a few hours to 9 days (Oliveira et al. 2006). Thus, the discharge of the Guadiana River has a large effect on the horizontal distribution of salinity in the estuary. The Guadiana Estuary is well-mixed when the freshwater discharge from the Guadiana River is low and is partially stratified for higher discharges. When there is a higher tidal amplitude and lower discharge from the Guadiana River, tidal processes control the water circulation of the estuary and the estuary becomes well-mixed (Garel and D'Alimonte 2017). The estuary is weakly stratified when it is neap tide and the river discharge is low (Garel and D'Alimonte 2017). In the latter case, density-driven processes control the mixing of the estuary. The water column is stratified only under extreme conditions (Basos 2013).

## 2 Methods

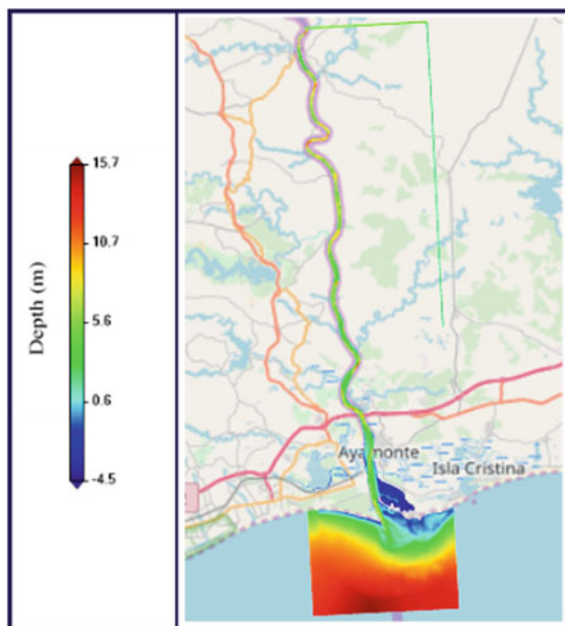
### 2.1 MOHID

Hydrodynamics and water properties of the Guadiana Estuary were simulated with the MOHID water modeling system in response to different scenarios of sea level rise. MOHID is programmed in ANSI FORTRAN 95 in order to produce object-oriented models integrating hydrodynamic processes for different marine systems (MARETEC 2017). The present study used MOHID in 2D, as the Guadiana Estuary is classified as a well-mixed estuary (Garel and D'Alimonte 2017). The Guadiana Estuary can become stratified, but only under freshwater flows of 1000 m<sup>3</sup>/s (Fortunato et al. 2002). The present study ran simulations for freshwater flows of 10 and 100 m<sup>3</sup>/s as these are typical conditions of the system after the closure of the Alqueva Dam. This further justifies the use of a two-dimensional model, allowing for long

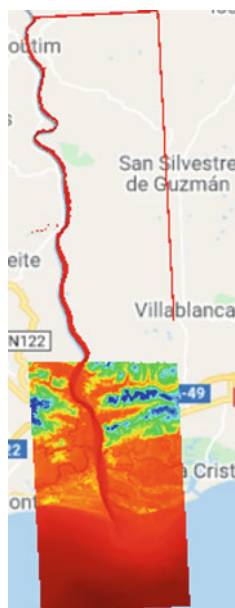
(one to two month) simulations, which would have had prohibitive computational costs with a three-dimensional model. MOHID solves Navier–Stokes equations with the hydrostatic approximation, using the finite volume method over a generic geometric grid. In this work, hydrodynamic and Eulerian transport modules were used. The finite volume method allows for the transport equations to be applied to the entire cell volume at specific points in discrete time (Neves et al. 2000). Of relevance are the results of a study by Mills et al. (2019) as the present study is a continuation of the work and methodologies used to simulate hydrodynamics and evolution of salt transport with respect to sea level rise. These authors used MOHID in 2D to simulate the hydrodynamics of the Guadiana Estuary considering different sea level rise scenarios up to the year 2100 along with varying freshwater flow rates of the Guadiana River (Mills et al. 2019). Results of the model demonstrated an overall increase in salinity in the estuary as well as flooding area around the estuary with respect to sea level rise. Sea level rise led to a reduction in velocity in the main channel, most likely due to an increase in depth and water volume (Mills et al. 2019). The model considered only the present bathymetry as well as a tidal amplitude equivalent to the average tidal amplitudes. The horizontal distribution of salinity in estuaries is dependent on several factors. One major variable is the balance between spring and neap tidal cycles and freshwater flow (Vargas et al. 2017). It is thus the aim of this work to expand upon the methodologies of Mills et al. (2019) to determine how the horizontal distribution of salinity varies between spring and neap tide as well as across a bathymetry that will allow flooding of the surrounding marshes.

## 2.2 *Model Setup*

The present study implements the same general setup of the model by Mills et al. (2019) and uses the same Cartesian computational grid of  $1400 \times 350$  cells with a resolution of 30 m. This computational mesh was chosen as it provides the most appropriate spatial resolution without incurring excessive calculation time. The calculation time for each simulation is high, especially when the river discharge is low. Two months of simulation time is required due to the high residence time when the freshwater flow rate is low (Oliveira et al. 2006). The hydrodynamic MOHID model of the Guadiana Estuary has been previously validated, calibrated and used in several studies (Morais et al. 2012; Lopes et al. 2003). The present model consists of two separate bathymetries: (1) a bathymetry in which coastal management strategies are implemented to keep the coastline as it is (Fig. 1) and (2) a bathymetry that allows for geomorphological changes caused by sea level rise and thus allows flooding around the estuary (Fig. 2). The first bathymetry is the current bathymetry of the Guadiana Estuary and was computed by triangular interpolation of measured bathymetric data on the Cartesian grid of  $1400 \times 350$  cells (Mills et al. 2019). The second bathymetry was computed by Sampath et al. (2011) in a behavior-oriented model. This bathymetry allows the surrounding saltmarshes and low-lying areas of



**Fig. 1** Bathymetry of the Guadiana Estuary corresponding to a maintained coastline on a mesh of  $1400 \times 350$  cells with a resolution of 30 m (Mills et al. 2019)



**Fig. 2** Bathymetry of the Guadiana Estuary corresponding to an unmaintained coastline ( $1400 \times 350$  cells with a resolution of 30 m). The grey space is land (Sampath et al. 2011)