Distribution of *Escherichia coli* and total coliforms in relation to the physico-chemical characteristics of wastewaters in Vientiane capital, Lao PDR

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មូលន័យសង្ខេប

ការយល់ដឹងអំពីការឆ្លើយតបរបស់ *Escherichia coli* និង total coliforms ចំពោះលក្ខណៈគុណភាពទឹកនៅក្នុងសំណល់រាវមានសារៈសំខាន់ណាស់ដើម្បីគ្រប់គ្រងការបំពុលទឹក និងការពារការវិចរិលនៃប្រព័ន្ធអេកូឡូស៊ី។ យើងបានធ្វើការសង្កេតផលប៉ះពាល់នៃលក្ខណៈរូប-គីមីរបស់សំណល់រាវលើរបាយនៃ *E. coli* និង total coliforms នៅក្នុងប្រឡាយទឹកនានានៅទីក្រុងវៀងច័ន្ទ ប្រទេសឡាវ។ ទីតាំងសិក្សាចំនួន ៥កន្លែង និងប៉ារ៉ាម៉ែត្រទឹកលក្ខណៈរូប-លក្ខណៈគីមីចំនួន 23 ត្រូវបានប្រមូលសម្រាប់ការសិក្សានារដូវវស្សាក្នុងឆ្នាំ 2023។ ម៉ូឌែលវិភាគពហុអថេរ (Principal component analyses) ត្រូវបានប្រើដើម្បីកំណត់ទំនាក់ទំនងរវាងអថេររូប-គីមី ចំណែកឯម៉ូឌែលលីនេអ៊ែរ (Simple linear regression) ត្រូវបានប្រើដើម្បីវាយតម្លៃការឆ្លើយតបរបស់ *E. coli* និង total coliforms ទៅនឹងជម្រាលលក្ខណៈរូបនិងលក្ខណៈគីមីនៅក្នុងសំណល់រាវ។ ជាលទ្ធផល ទីតាំងសិក្សាទី4 ដែលស្ថិតនៅផ្នែកខាងកើតនៃទីក្រុងត្រូវបានរកឃើញថាមានកំហាប់ខ្ពស់បំផុតទាំង *E. coli* និង total coliforms (7,700 & 87,000 cfu/100ml រៀងគ្នា) ខណៈទីតាំងសិក្សាទី5 ដែលស្ថិតនៅភាគអាគ្នេយ៍នៃទីក្រុងមានកំហាប់ទាបបំផុត។ ម៉ូឌែលលីនេអ៊ែររបស់យើងបានបង្ហាញថា របាយនៃ *E. coli* និង total coliforms មានទំនាក់ទំនងវិជ្ជមានជាមួយនឹងការកើនឡើងនៃកំហាប់អាម៉ូញ៉ូម និងមានទំនាក់ទំនងអវិជ្ជមានជាមួយនឹងកំហាប់ជាតិប្រៃក្នុងទឹក។ អាម៉ូញ៉ូមជាសារធាតុចិញ្ចឹមយ៉ាងសំខាន់ចំពោះការលូតលាស់ និងការបន្តពូជរបស់បាក់តេរី ចំណែកឯកម្រិតជាតិប្រៃខ្ពស់បណ្តាលឱ្យកើតមានស្ត្រេសអ៊ីពែរអូស្មូស (hyperosmotic stress) និងបង្អាក់សកម្មភាពដំណើរការកោសិកាសំខាន់ៗដែលកំណត់ការបន្តពូជរបស់បាក់តេរី។ អាស្រ័យហេតុនេះ ការកំណត់កម្រិតជាតិប្រៃ និងកំហាប់អាម៉ូញ៉ូមដោយកាត់បន្ថយការបង្ហូរទឹកស្អុយដោយផ្ទាល់ពីលំនៅដ្ឋាន និងរោងចក្រឧស្សាហកម្មជាវិធានការដ៏សំខាន់មួយសម្រាប់ស្តារប្រព័ន្ធអេកូឡូស៊ីក្នុងទីក្រុងវៀងច័ន្ទ។

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Abstract

Understanding the response of *Escherichia coli* and total coliforms to water quality characteristics in wastewater is crucial to controlling aquatic pollution and preventing ecosystem degradation. We investigated the effects of the physico-chemical characteristics of wastewater on the distribution of *E. coli* and total coliforms in canals in Vientiane city in Lao PDR. Five sites and 23 physico-chemical water parameters were selected for sampling during the wet season in 2023. Principal component analyses were used to define relationships between physico-chemical variables, whereas simple linear regression was applied to assess the responses of *E. coli* and total coliforms to physico-chemical gradients in the wastewater. As a result, site four, located in the east portion of the city, was found to have the greatest concentration of both *E. coli* and total coliforms (7,700 & 87,000 cfu/100 ml, respectively), whereas site five, in the southeast of the city, had the least. Our linear model showed that the distributions of *E. coli* and total coliforms were positively associated with increasing concentrations of ammonium and negatively correlated with salinity levels. Ammonium is an essential nutrient for bacterial growth and reproduction, whereas high-salinity environments cause hyperosmotic stress and inactivate crucial cellular processes, limiting bacterial reproduction. Consequently, limiting salinity and ammonium concentrations by reducing direct inputs of sewage water from households and industrial plants would be an important measure for ecosystem restoration in Vientiane city.

Keywords ammonium, ecosystem health, hyperosmotic stress, salinity, sewage water, Southeast Asia

Introduction

Water is one of the most significant natural resources on earth because all living things need water for life. Water covers ca. 71% of the world's surface, totalling 1,386 million cubic kilometres, but only around 2% of this is used by humans (Shankar & Ponnusamy, 2023). Human population growth, industrialization, agricultural practices, urbanization and rapid economic expansion have all contributed to massive discharges of wastewater into freshwater ecosystems. These are often contaminated and unsuitable for human consumption (Mallick *et al*., 2021). For instance, more than 80% of the wastewater produced globally is not collected or treated (UNESCO, 2020) and only 39% of the global population had access to properly run sanitary facilities in 2015 (UNICEF & WHO, 2019). Wastewaters are typically discharged into waterways, increasing dangers to human health, ecosystems and climate (ADB, 2021). The public and private wastewater disposal systems of many Southeast Asian countries require improvement because they are weak or nonexistent (USAID, 2010). This is partly because the untreated wastewaters also affect human health and aquatic life, especially through life-threatening diseases such as diarrhoea, cholera and typhoid. Diarrhea in particular is one of the most prevalent illnesses in underdeveloped nations (Yoneda *et al*., 2022).

Like many Southeast Asian countries, Lao PDR faces challenges in relation to sewage disposal (ADB, 2021). In urban areas of the country for example, large amounts of wastewater are dumped directly into the environment without treatment (Baetings & O'Leary, 2010). This is especially true in Vientiane city, where most of the wastewater generated in commercial and industrial districts is dumped straight into lakes, streams, marshes, drainage canals, and ultimately the Mekong River (Tilley *et al*., 2008). Additionally, most wastewater produced in residential areas (kitchens, bathrooms & laundries) is not treated before its release into the environment (Khattiyavong & Lee, 2019). This poses an issue because the majority of rural areas in Lao PDR directly use water from natural canal systems or small tributaries without treatment for residential use (Ribolzi *et al*., 2011). This occurs in many developing countries. For example, about 85,000 people in the floating community in Tonle Sap Lake of Cambodia dump household waste into the lake (Brown *et al*., 2010). This includes manure due to a lack of latrines and they also directly use the water in the lake in daily life. This has increased concentrations of *Escherichia coli* and total coliforms in the water surrounding the floating villages which exceed the World Health Organization safety guidelines (Brown *et al*., 2010).

Escherichia coli is a bacterium which commonly inhabits the lower guts of its primary hosts, which are warm-blooded animals (Hunter *et al*., 2005). The bacteria can also live for long periods of time in water, on algae, and in soils in tropical, subtropical and temperate environments (Desmarais *et al*., 2002; Byappanahalli & Fujioka, 2004; Ksoll *et al*., 2007). They are heterotrophic and only require simple carbon and nitrogen sources, plus phosphorus, sulphur and other trace elements for growth (Díaz *et al*., 2001). It is frequently adopted as an indicator for water contamination because it is a reliable indicator of potential health risks associated with faecal contamination (Basavaraju & Gunashree, 2022). Total coliforms are a type of bacteria that can occur throughout nature (Niyoyitungiye *et al*., 2020). Total coliform counts are crucial in wastewater treatment because the bacteria break down organic waste into water and biomass (Hunter *et al*., 2005). Their existence in water suggests it has been tainted by sewage, runoff, industrial waste and/or agricultural waste (APHA, 1999). While this does not necessarily indicate that they will cause disease, it does indicate that the water has been polluted and could be dangerous to human health (Ukpong & Udechukwu, 2015).

Recent research regarding *E. coli* in the Mekong River basin includes the effects of hydrological regimes and land use on in-stream concentrations (Nakhle *et al*., 2021) and monitoring of *E. coli* at multiple scales from 2011 to 2021 (Boithias *et al*., 2022). Additionally, studies have assessed factors related to *E. coli* contamination of drinking water in suburban and rural areas of Lao PDR and Thailand (Vannavong *et al*., 2018) and contamination of communal tobacco waterpipes in Lao PDR (Sinclair *et al*., 2021). As the distribution of *E. coli* and total coliforms in relation to physical and chemical factors had yet to be investigated in the Vientiane city in Lao PDR however, we evaluated this by sampling during the wet season in 2023. Our primary aim was to provide baseline data regarding the distribution of *E. coli* and total coliforms along a major canal in the city. We also evaluate water quality properties and their variation with a view to improving management of water quality within the city.

Methods

Study sites

Five locations on canals were randomly selected for sampling in Vientiane city (Fig. 1). These canals receive large amounts of wastewaters from different areas which are untreated and discharged directly into the canal systems (Deevanhxay, 2022).

The first sampling location (WS1: 17.987347°N, 102.623586°E) was located on the northern edge of the city and comprised a waterway which connects the Nongtha Lake and the Thatlaung Lake (Fig. 2). Based on observations made during sampling, this receives wastewater from neighbouring households and the water surface is dark green and pungent smelling. The second sampling site (WS2: 17.986531°N, 102.636361°E) was also situated in the northern portion of the city. This had few aquatic plants, and the water was also dark and pungent smelling. The third sampling location (WS3: 17.975969°N, 102.648483°E) was located in the eastern section of the city near agricultural lands. This was characterized by sparse aquatic vegetation and the water was

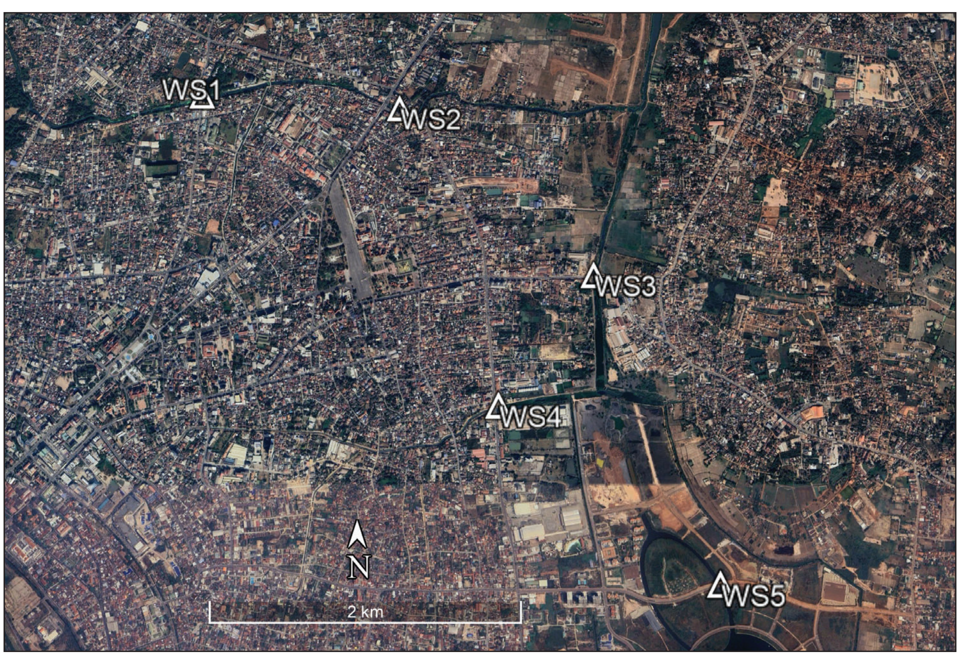

Fig. 1 Sampling locations (WS1–WS5) on wastewater canals in Vientiane city, Lao PDR

Fig. 2 Indicative images of wastewater canals sampled in Vientiane city, Lao PDR

dark, pungent and slow-flowing. The fourth sampling site (WS4: 17.968017°N, 102.642247°E) was situated in residential areas near the ASEAN Mall, whereas the fifth and final sampling site (WS5: 17.957536°N, 102.655458°E) was located near Thatluang Lake in the southeast of the city and had rather clear and clean water.

Sample collection

A water sampler was used to collect samples at the five sites during the wet season on 25 and 26 May 2023. Duplicate water samples of 500 ml were collected at each site, for a study total of ten samples. These were stored at <4°C and transported for laboratory testing within 48 hours (Vail *et al*., 2003; Othman *et al*., 2015). All samples were stored in 50 ml polypropylene centrifuge tubes and wrapped with aluminum foil to avoid contamination and direct contact with light. To avoid under-detection of the bacteria under investigation, all bacteriological tests were conducted within a day of sample collection (Othman *et al*., 2015; Bonetta *et al*., 2022).

Enumeration of *E. coli* & total coliforms

To quantify colonies of *E. coli* and total coliforms in our water samples, we first diluted these with deionized water at three ratios: 1:10, 1:50 and 1:100 (Paretti *et al*., 2017). One millilitre of water was added onto a 3M Petrifilm Rapid *E. coli*/Coliform Count Plate, splashed thoroughly through the plate, and incubated at 37°C for 24 hours (Niyoyitungiye *et al*., 2020). This was followed by numeration of the colonies using a Petrifilm Plate Reader Advanced (3M, USA). In data interpretation, red colonies with or without gas generation indicate the total number of coliforms, whereas blue colonies with or without gas production indicate *E. coli* (Bird *et al*., 2021). Multiplying counts by the dilution factor for a given plate provides the result for that plate (Bird *et al*., 2021). We were only able to count colonies of bacteria in our 1:100 dilution samples and consequently only provide results for these samples. Colonies were not countable in our lower dilution (1:10 & 1:50) samples of wastewater because these contained many particles.

Measurement of physico-chemical parameters

Several water quality variables were measured at each site using a HI-9829 Multiparameter Portable Water Quality Meter (Hanna Instruments, UK). These included water temperature, dissolved oxygen, electrical conductivity, pH, total dissolved solids, oxidation-reduction potential and salinity. Other water quality parameters were measured in the laboratory. Turbidity was tested using a turbidimeter (TB 211 IR, Lovibond / Tintometer, Germany), whereas water appearance was measured using a portable water colour meter (Hanna, UK) and chemical oxygen demand was tested using the HR-COD closed-reflux potassium dichromate method with a MD600 photometer (Lovibond / Tintometer, Germany). Inorganic cations and anions (ammonium, sodium, nitrate, sulphate, phosphate, chloride, lithium, potassium, calcium, magnesium, and bromide) were measured via ion chromatography (IC Eco, Switzerland).

Data analysis

Simple descriptive statistics were used to summarize the distribution of *E. coli* and total coliforms across our sampling sites. A Principal Component Analysis (PCA) was performed to explore the relationship between physico-chemical variables and define gradients in these along the principal axes of the PCA (Vieira *et al*., 2012). Before computing the PCA, all data were standardized such that the mean value equalled zero and the standard deviation equalled one. This process makes the data unitless, prevents factors with large scales from introducing bias (Jolliffe, 1998) and creates a more accurate representation of the underlying structure of the data. A simple linear model was used to fit the responses of *E. coli* and total coliforms to variation of physico-chemical parameters and determine the most important parameters in this regard. All statistical analyses were performed in the R programme environment (R Core Team, 2022).

Results

Occurrence of *E. coli* & total coliforms

We recorded a total of 17,000 cfu/100 ml of *E. coli* and 163,900 cfu/100 ml of total coliforms in our samples. The highest occurrence of *E. coli* (7,700 cfu/100 ml) and total coliforms (87,000 cfu/100 ml) was observed in WS4, followed by WS2 with 3,300 cfu/100 ml of *E. coli* and 30,100 cfu/100 ml of total coliforms. Similar to WS2, samples from WS1 and WS3 contained less *E. coli* (3,200 & 2,800 cfu/100 ml respectively) and total coliforms (27,300 & 19,200 cfu/100 ml respectively). In contrast, WS5 contained the lowest density of *E. coli* (0 cfu/100 ml) and total coliforms (300 cfu/100 ml) compared to other sites. Ranges of *E. coli*, total coliforms and physico-chemical parameters recorded during the study are provided in Table 1.

Water properties

Three PCA axes were retained in our analysis and explained more than 96% of the total variance (Fig. 3). Dissolved oxygen (DO & ppmDO: Table 1), pH, oxidation-reduction potential, calcium, water temperature, colour and magnesium were strongly correlated on the first PCA axis, particularly dissolved oxygen. As such, the first PCA axis summarized variation in oxygen concentrations in our samples and indicated an oxygen gradient where concentration gradually reduced with nutrient accumulation (Fig. 3a). Seven variables were strongly correlated along the second PCA axis, specifically chloride, electrical conductivity, salinity, sodium, bromide, total dissolved solids and fluoride (Fig. 3a), whereas an opposite correlation was observed for ammonium. Chlo-

Table 1 Summary of physico-chemical parameters, *Esherichia coli* and total coliforms at study sites in Vientiane city, Lao PDR

Variable / Metric	Unit	Minimum	Maximum	Mean	SD
Chemical oxygen demand (COD)	mg/l	14.2	60	34.54	17.54
Water temperature (WT)	°C	29.58	32.76	30.87	1.28
pH	–	7.02	7.66	7.25	0.29
Oxidation-reduction potential (ORP)	mV	-201.1	158.5	-59.92	146.04
Electrical conductivity (EC)	μS/cm	410	750	616	126.01
Total dissolved solids (TDS)	mg/l	206	379	310.2	63.77
Salinity (Sal)	PSU	0.19	0.37	0.29	0.06
Dissolved oxygen (DO)	%	0	68	27.62	30.69
Dissolved oxygen (ppmDO)	mg/l	0	4.92	2.02	2.22
Turbidity (Tur)	NTU	13.6	49.3	28.98	16.39
Color	–	10	40	21	11.93
Lithium (Li)	mg/l	0	3.07	0.62	1.36
Sodium (Na)	mg/l	31.26	105.37	62.79	26.8
Ammonium ($NH4^+$)	mg/l	0.36	13.7	10.46	5.69
Potassium (K)	mg/l	6.01	8.68	7.39	1.11
Calcium (Ca)	mg/l	27.3	42.74	35.85	5.9
Magnesium (Mg)	mg/l	5.84	9.23	7.6	1.23
Fluoride (F)	mg/l	0.09	0.3	0.26	0.09
Chloride (Cl)	mg/l	28.57	137.68	82.37	38.92
Bromide (Br)	mg/l	0.1	0.35	0.26	0.09
Nitrate (NO3)	mg/l	0.05	0.16	0.1	0.05
Sulfate (SO4)	mg/l	0.24	28.88	9.35	11.8
Phosphate (PO4)	mg/l	0	1.3	0.59	0.65
Escherichia coli	cfu/100ml	0	7,700	34,00	2,759.52
Total coliforms	cfu/100ml	300	8,7000	32,780	32,466.86

ride, sodium and ammonium contributed significantly to the second axis and this was regarded as the salinity gradient in our samples. In other words, the concentration of chloride and sodium increased gradually in samples from WS1 to WS5 along the second axis (as observed in our PCA biplot: Fig. 3a), in contrast to the decreasing concentration of ammonium along the same axis. The third axis was characterized by nitrate, chemical oxygen demand, potassium, phosphate, turbidity, sulphate and lithium and was regarded as the nutrient gradient (Fig. 3b). Concentrations of nitrate and phosphate decreased gradually from WS1 to WS5 (as observed on the third axis of the PCA biplot: Fig. 3b), in contrast to increasing dissolved oxygen. This suggests water samples from WS1–WS3 possessed a relatively high concentration of nutrients, whereas lower concentrations were observed in samples from WS4 and WS5.

Response of *E. coli* & total coliforms to water properties

Results from our linear model indicated that the distribution of *E. coli* and total coliforms related negatively with the increase in salinity and positively with the increase in ammonium on the second axis (adjusted R^2=0.99) (Table 2, Fig. 4). In other words, a high density of *E. coli* and total coliforms were observed in water samples with low salinity and high ammonium concentrations. However, no response in *E. coli* and total coliforms was observed in relation to the oxygen gradient (first axis) (p=0.068) or the nutrient gradient (third axis) (p=0.072) (Table 2, Fig. 4).

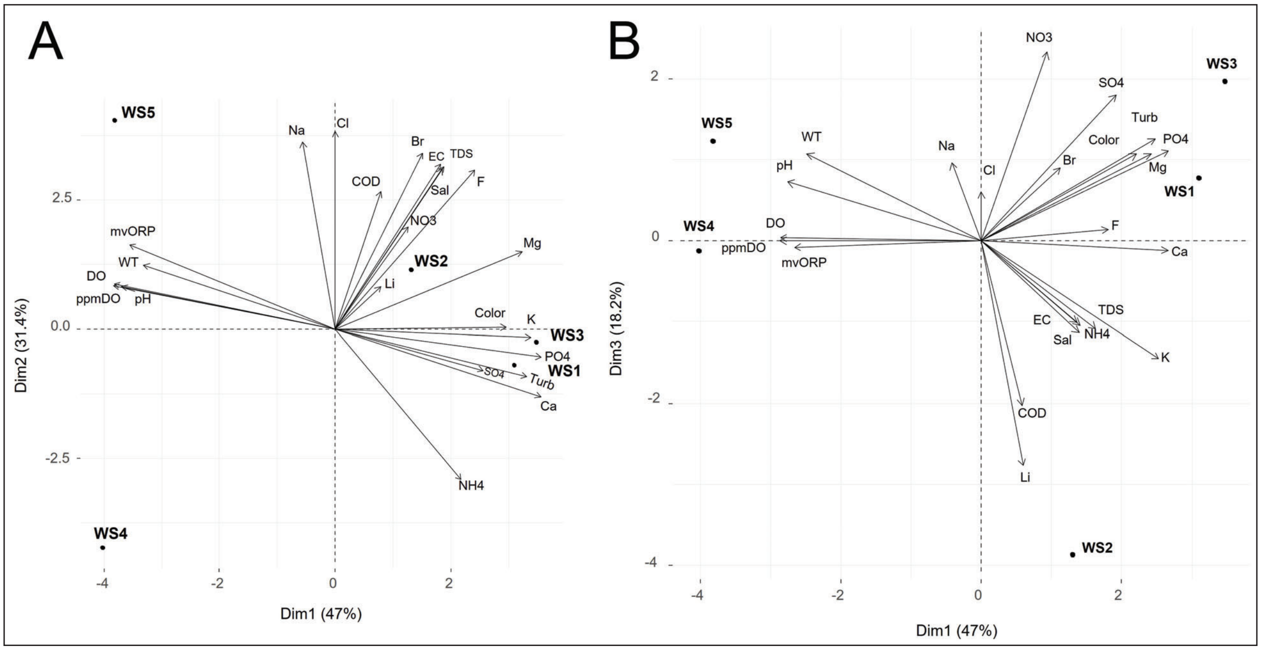

Fig. 3 Principal component analyses showing the relationship of physico-chemical variables on the A) first and second axes and B) first and third axes. The length of the arrows indicates the importance of individual contributions to variations of water quality. Abbreviations and units for each factor are provided in Table 1

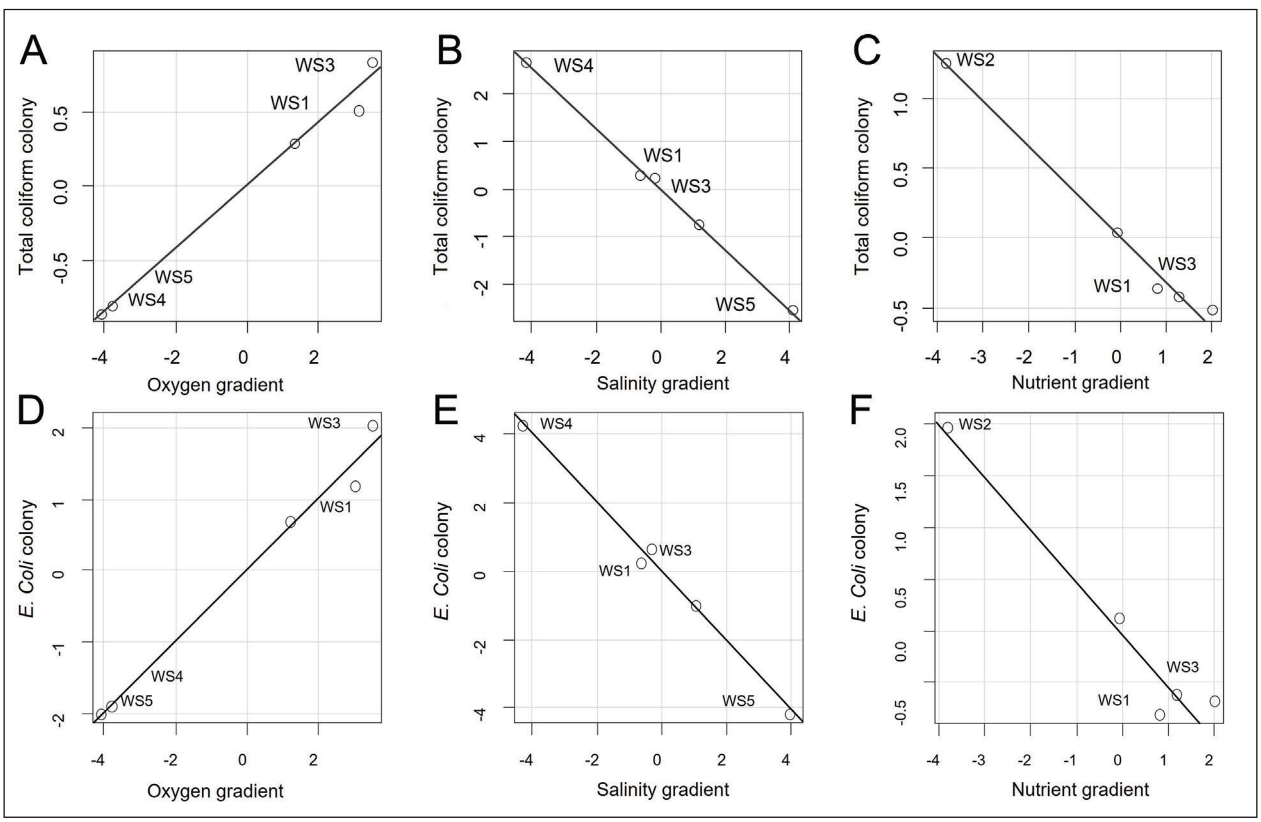

Fig. 4 Relationship of total coliforms to A) oxygen, B) salinity, and C) nutrient gradients and relationship of *E. coli* to D) oxygen, E) salinity, and F) nutrient gradients

Table 2 Regression models of *Esherichia coli*, total coliforms and water parameters (axis 1, 2 & 3) in Vientiane city, Lao PDR

Linear model	*p* value
Simple	
E.coli ~Axis1 (DO gradient)	0.089
E.coli ~Axis2 (Salinity gradient)	0.054
E.coli ~Axis3 (Nutrient gradient)	0.142
TotColi~Axis1 (dissolved oxygen gradient)	0.068
TotColi~Axis2 (Salinity gradient)	0.028
TotColi~Axis3 (Nutrient gradient)	0.072

Discussion

Effects of water properties on *E. coli* & total coliforms

Our study appears to be the first to document the effect of the physico-chemical characteristics of wastewater on the distribution of *E. coli* and total coliforms in Vientiane city. Although our sample size was small, this nonetheless contributes to knowledge on this subject within the city. In summary, we found that concentrations of *E. coli* ranged from 0 to 7,700 cfu/100 ml, whereas concentrations of total coliforms ranged from 300 to 87,000 cfu/100 ml, which is higher than levels reported by Boithias *et al*. (2022) in the Mekong River basin. We also found *E. coli* was negatively correlated with salinity and positively correlated with ammonium across our study sites.

The highest concentrations of *E. coli* and total coliforms were recorded at WS4 near the ASEAN Mall (7,700 and 87,000 cfu/100 ml, respectively). This may be due to the site being characterized by slow-flowing water, fine sediments and various aquatic plants. Studies have shown that *E. coli* and total coliforms grow in water, sediments, soil and on water plants in various climatic regions (Byappanahalli & Fujioka, 1998; Byappanahalli *et al*., 2006; Ishii *et al*., 2006; Beversdorf *et al*., 2007). WS4 also had lower salinity and higher ammonium concentrations. This is consistent with studies elsewhere that suggest the quantity of *E. coli* and total coliforms is negatively correlated with salinity (Troussellier *et al*., 1998). Further, large amounts of organic waste in water result in enormous quantities of bacteria because the high nutrient (ammonium) concentrations promote their growth (Othman *et al*., 2015).

Our fifth sampling site (WS5) had the lowest quantities of *E. coli* and total coliforms (0 and 300 cfu/100 ml, respectively). This might be due to the fact this site was far from residential areas, with few potential sources of water pollution apparent in the immediate environment. High levels of salinity and low nutrients were also recorded at WS5 which we regard as having the best water quality among our study samples.

Based on relationships revealed in a PCA, we grouped our predictor variables into three axes to identify the factors that determine variation in the distribution of *E. coli* and total coliforms. We found that these were negatively correlated with salinity. High saline conditions in water cause hyperosmotic stress, and deactivation of crucial cellular processes decrease the survival rates of *E. coli* and total coliforms (Troussellier *et al*., 1998). This is because when saline concentrations are higher in the water than in bacterial cells, it causes the water within the cells to leave, damaging these and causing them to shrink and possibly die (Troussellier *et al*., 1998; Kaushal *et al*., 2018). Consequently, the dispersion of *E. coli* and total coliforms is limited in water with higher salt concentrations. These in turn can result from pollutants created by various human activities including agriculture, urbanization and household waste (Corsi *et al*., 2010; Steele & Aitkenhead-Peterson, 2012; Kaushal *et al*., 2018).

We also found that *E. coli* and total coliforms were positively correlated with ammonium concentrations. Ammonium in wastewater typically results from cooking in residential areas, ammonia synthesis, various industrial processes and agricultural drainage (Gupta *et al*., 2015; Purwono *et al*., 2017). Ammonium ions are a major source of nitrogen pollution in wastewater and lead to increased oxygen demand and biological eutrophication (Sprynskyy *et al*., 2005). Excessive concentrations can also impair aquatic life, with 0.2 mg/l being harmful to fish and aquatic animals (Haralambous *et al*., 1992; Randall & Tsui, 2002). However, ammonium is also a vital ingredient for growth and reproduction of *E. coli* and total coliforms (Reitzer, 2003). Relatively high ammonium concentrations in subtropical and tropical conditions allow *E. coli* and total coliforms to survive and develop (Winfield & Groisman, 2003; Othman *et al*., 2015), because they use these to create amino acids, which are the building blocks of proteins. Importantly, ammonium is also needed for the synthesis of adenosine triphosphate, the cell's energy currency (Reitzer, 2003; van Heeswijk *et al*., 2013).

Implications for wastewater treatment

Among the water properties tested, salinity and ammonium had the greatest influence on concentrations of *E. coli* and total coliforms. Higher salinity was associated with decreases in both groups of bacteria, whereas higher ammonium levels were associated with increases.

As such, management of salinity and ammonium levels provide options for reducing *E. coli* and total coliforms. Chlorine can also be applied to remove these bacteria from wastewater. Because of its simplicity, low cost, and efficiency, the addition of chlorine to water is the most widely used approach globally (Aguilar-Ascon, 2019; Iordache & Woinaroschy, 2020). Reducing ammonium concentrations could also reduce *E. coli* and total coliforms from wastewater. Options for removing ammonium include ion exchange and adsorption, biological technology, air stripping, reverse osmosis, chemical precipitation and microwave radiation (Huang *et al*., 2017; Han *et al*., 2020). Those used most often to treat ammonium in wastewater include ion exchange and adsorption and biological technologies (Gupta *et al*., 2015; Huang *et al*., 2017). The former are often proposed due to their simplicity, effectiveness (with removal rates of 80–95%), short removal times, low expense and environmentally friendliness (Gupta *et al*., 2015; Mazloomi & Jalali, 2016) and have proved effective in multiple studies e.g., Balci & Dinçel (2002), Jorgensen & Weatherley (2003), Saltali *et al*. (2007), Mazloomi & Jalali (2016), Millar *et al*. (2016), Otero *et al*. (2024).

Allied to the above, efforts could be made to reduce waste inputs into urban waters (including filtration) and/or rehabilitation could be undertaken to remove organic compounds or nutrients and increase oxygen levels. Increased nutrients in water ultimately decrease oxygen levels and high levels of oxygen typically indicate water is healthy (Shamsudin & Fazalul, 2016). Treatment of wastewater prior to its discharge into natural water systems is critical for protecting the environment, public health and aquatic ecosystems. As significant gaps remain in our understanding of the distribution of *E. coli* and total coliforms in Vientiane city, future studies should seek to: i) expand the number of sampling sites; ii) undertake sampling during both the wet and dry seasons to determine the extent of seasonal variation; and, iii) increase the frequency of sampling in each season to ensure the data are representative.

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