

Assessment of Physicochemical Qualities, Heavy Metals Concentration and Bacterial Pathogens in Elechi Creek, Port Harcourt, Nigeria

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Abstract: *Escherichia coli* is one of the most frequent causes of many common bacterial infections, such as urinary tract infection (UTI), cholangitis, diarrhoea, bacteraemia, neonatal meningitis and pneumonia. Assessment of physicochemical parameters, heavy metals and bacterial pathogens in Elechi Creek, Port Harcourt, Nigeria was carried out. Two hundred and ten (210) water samples were collected for the analysis of physicochemical parameters, heavy metals and bacterial detection (72, 18 and 120, respectively). The physicochemical parameters were carried out periodically and seasonally; periodic variations were carried out in the morning and evening hours, while the seasonal variations were carried out in November (the end of rainy season), April (the start of rainy season) and July (the middle of rainy season). The heavy metals were analysed only seasonally. Potential hydrogen (pH), Temperature, Salinity, Total dissolved solid (TDS), Dissolved oxygen (DO) and Biological oxygen demand (BOD) were the physicochemical parameters analysed. The results showed that all the parameters, except the Temperature fell within the recommended values. For example, in the mornings, the highest mean Temperature value was 29.33 ± 0.21 , while the highest mean values in the evenings ranged from 29.80 ± 0.43 to 30.43 ± 0.49 . The highest mean values were obtained in the evening of April (30.43 ± 0.49), followed by the evening of November (29.93 ± 0.15). A significant difference was observed both in April and July ($p=0.019$; $t=-3.836$ and $p=0.011$; $t=-4.427$, respectively). The results obtained from the heavy metals (Cadmium, Mercury, Lead, Arsenic, Chromium and Nickel) analysis did not fall within the recommended values. According to the results of the Heterotrophic Plate Count (HPC), the highest mean values were obtained in the morning and evening of July (5.18×10^6) and 5.79×10^5 , respectively). The highest HPC observed in July could be as a result of heavy rainfall during the season as erosion carries and empties every dirt in the water body. The amount of nutrients from dump sites located very close to the water body may have contributed to the high HPC. Coliforms were reported in this study and the highest number was observed in the evening of July, day 1 (21 MPN/100 ml). *Escherichia coli* was isolated from all the water samples, which was not within the standard recommended values of zero by World Health Organization (WHO). The antibiotic susceptibility testing was performed on 128 isolates of *E. coli* and the results revealed that all the isolates of *E. coli* were susceptible to Meropenem, Imipenem, Tigecycline, Amikacin, Ciprofloxacin and Colistin, while 28(21.71 %) isolates were resistant to Cefoxitin. The Multiple Drug Resistance (MDR) isolates were 11 (8.60 %). Isolates that showed Multiple Antibiotic Resistance Index (MARI) of 0.0 were 97 (75.78 %). However, the isolates that showed MARI of 0.1 and 0.2 were 10 (7.81%) for each.

Keywords: *Escherichia coli*; Multiple Drug Resistance; Multiple Antibiotic Resistance Index; Seasonal; Periodic; Antibiotics; Heavy metals; Physicochemical parameters; Pollution; Water bodies; Antibiotics; Resistance

1. Introduction

Water quality or the quality of water has to do with the biological, chemical and physical, as well as radiological characteristics of water (Diersong et al., 2009). These characteristics of water affect the aquatic life, such as plants and animals dwelling in the water environment (UNDESA, 2105). It has been observed that pollutants from different sources are introduced into the water bodies without proper treatment in order to get rid of the harmful materials. In 2005, Fakayo denoted that an industrial development regularly has to do with the generation of industrial effluents, which can bring about pollution of water bodies.

Some researchers in Nigeria have observed that the concentrations of some heavy metals in water bodies are above the acceptable and permissible levels (Olayinka & Alo, 2004; Essoka & Umaru, 2006). When the concentration of heavy metals in water bodies is high, this could be toxic to the aquatic organisms; it could lead to a decline in fish and seafood population. The accumulation of heavy metals in aquatic food web is a potential threat to public health that is why they are regarded as priority pollutants. Increase in human activities in and around water bodies has been

reported to affect aquatic environment (Ezeronye and Ubalua 2005; Obaroh et al., 2012).

According research, *E. coli* is one of the normal flora of the gut of endotherms, which are warm blooded animals; however, some of the strains are pathogenic (Kaper et al., 2004). The pathogenic strains of *E. coli* are grouped into six and they are common worldwide. These pathotypes are Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC) or Enterohaemorrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Ali et al., 2014). The bacterium is known to cause gastrointestinal diseases, especially among children in developing countries (Koneman et al., 2006; Ali et al., 2014). The main mode of acquiring diarrhegenic infections caused by *E. coli* is via person-to-person transmission and consumption of water and food contaminated with the wastes of endotherms (Ali et al., 2014). Although, strains of *E. coli* have been reported by researchers in Nigeria, however *E. coli* strains isolated from Elechi Creek and evaluation for their antibiogram profiling have not been reported. This is the first report on isolates of

E. coli form Elechi Creek with the evaluation for their antibiogram profiling.

2. Materials and Methods

Study area

This study was carried out in Rivers State, Nigeria. Elechi Creek is one of the known water bodies located in Obio / Akpo Local Government Area of the State. The choice of this water body was based on human activities around it and its close proximity to the market.

Study design

The climate of Rivers State is dominated by rainy season (April to November) and dry season (November to March). That is why this study was carried out in November (end of rainy season), April (start of rainy season) and July (middle of rainy season) on water samples collected from Elechi Creek located at Rivers East Senatorial District. The physicochemical parameters were carried out periodically and seasonally; periodic variations were carried out in the morning and evening hours, while the seasonal variations were carried out in November (the end of rainy season), April (the start of rainy season) and July (the middle of rainy season). The heavy metals were analysed, while the bacterial detection was analysed both periodically and seasonally.

Sample size

A total number of two hundred and ten (210) water samples were collected for the analysis of physicochemical parameters, heavy metals and bacterial detection (72, 18 and 120, respectively).

Collection of water samples for physicochemical parameters

At Elechi Creek in the months of November, April and July, water samples were collected in 72 separate containers (4 oz. (118.3 ml) sterile specimen containers) for the measurement of physicochemical parameters. Notwithstanding, the 72 water samples were collected for both seasonal and periodic variations. In a day, three water samples were collected in the mornings and three were collected in the evenings, so that in two days 12 water samples were collected. Then, in the three seasons a total of 36 water samples were collected from November 2016 to July 2017. Therefore, 72 water samples were collected from November 2016 to July 2018 (November 2016, April 2017, July 2017, November 2017, April 2018 and July 2018). Additionally, all, but Biological oxygen demand (BOD) were measured *in situ* using Horiba Water Checker (Model U-10) and mercury thermometer (APHA, 1998).

Collection of water samples for the measurement of heavy metal concentrations

In the months of November (2016/2017), April (2017/2018) and July (2017/2018), water samples were collected in 18 separate containers (4 oz. (118.3 ml) sterile specimen containers) for the measurement of heavy metal concentrations. The 18 water samples were collected for just seasonal variations. In each month, three water samples were collected from each location, so that in three months, 9 water samples were collected and throughout the sampling

period, 18 water samples were collected. In order to preserve the samples for the measurement of heavy metal concentrations, 5 drops (0.1 ml) of concentrated nitric acid (HNO₃) were added.

Collection of water samples for the detection of bacteria

A total of 120 water samples were collected aseptically in sterile bottles (4 oz. (118.3 ml) capacity) for bacteriological analysis from November 2016 to July 2018. After the collection, the water samples were put in a cooler filled with ice packs, transported immediately to the laboratory and processed within two hours. The sampling was carried out periodically (in the morning and evening hours) and seasonally (Greenberg, 1985).

Cultivation of microorganisms

Media such as Nutrient agar, MacConkey agar, MacConkey broth and CHROMagar for *Escherichia coli* and other coliforms (CHROMagar ECC) were prepared and used following manufacturers' instructions and directions. After the preparation of the media, the media plates were labelled clearly and stored appropriately in the refrigerator at 4-6 °C.

Data were acquired from HPC, TCC and Total *E. coli* Count (TE); the TCC and TEC were carried out using the Most Probable Number (MPN) technique, which was statistically determined by the use of MacCrady table.

Analysis of water samples for physicochemical parameters

The physicochemical parameters, such as the pH, Temperature, Salinity, Total dissolved solids and Dissolved oxygen were analysed *in situ* using Horiba Water Checker (Model U-10) and the Lovibond CM-21 Tintometer. Biological oxygen demand was analysed with mercury thermometer; was not analysed *in situ*.

Analysis of water samples for heavy metals

Heavy metals concentrations were determined by the use of Atomic Emission Spectrophotometer (Agilent Technologies 4210, MP-AES, USA); the water samples were treated with 0.1 ml of concentrated nitric acid (APHA, 2005).

Antibiotic sensitivity testing (AST)

Sensitivity of *E. Coli* isolates to Meropenem (10µg), Imipenem (10µg), Tigecycline (15µg), Cefotaxime (30µg), Gentamicin (30µg), Cefoxitin (30µg), Amoxicillin/Clavulanic acid (30µg), Amikacin (30µg), Ciprofloxacin (5µg) and Colistin(10 µg) (all from Oxoid Co. UK) was determined on Nutrient agar using the Kirby-Bauer-CLSI (Clinical and Laboratory Standards Institutes) modified disc agar diffusion (DAD) method as described by Isenberg (1998) (Table 8). Results were interpreted in accordance with the Clinical and Laboratory Standards Institutes (CLSI) guidelines (CLSI, 2008).

3. Results

Table 1 shows results of the periodic variations in November, morning and evening hours are presented. The values obtained for Potential hydrogen (pH), Temperature, Salinity, Total dissolved solid (TDS), Dissolved oxygen (DO) and Biological oxygen demand (BOD) are presented

in the Table. Statistically, there was a significant difference in TDS and DO ($p < 0.001$; $t = -11.363$).

Morning and evening values of physicochemical parameters in April were compared and the results are shown in Table 2. According to the statistical analysis, there was a significant variation in: pH ($p = 0.678$; $t = -0.451$), Temperature ($p = 0.002$; $t = -3.836$), Salinity ($p < 0.002$; $t = 6.849$), TDS ($p < 0.001$; $t = -8.794$) and DO ($p < 0.004$; $t = -6.047$).

In July, morning and evening values were compared and the values obtained are shown in Table 3. There was a significant difference in the Temperature, Salinity, TDS and DO. There was no significant difference among the rest of the obtained values between the morning and evening periods.

Values of the Heavy metal are shown in Table 4. The variance in the mean were compared statistically using the ANOVA and according to the result; there was no significant variation among the heavy metals.

Additionally, heterotrophic plate count (HPC) was carried out. According to the results, the highest HPC was observed in the evening of July (middle of rainy season) (Table 5). Moreover, total coliform count and total *E. coli* count were carried out. According to the results, the two groups of microorganisms were isolated and the highest number of coliform was observed in the evening of day 4 (21 MPN/100 ml) in July (Table 6 and Table 7). In addition, the results of the total *E. coli* count are shown in Table 6 and 7.

Tables 9 to 11 shows results of the antibiotics susceptibility test. Ten (10) antibiotics were selected from 7 antibiotic Class (Carbapenems, Glycylcycline, Cephalosporin, Aminoglycoside, Beta-lactamase, Fluoroquinolone and Polymyxins). According to the results, resistance among Beta-lactamase and Cephalosporin were most frequent than the others. Twenty eight (28) isolates were resistant to Cefoxitin, representing Cefoxitin resistance of 21.71 % ($n = 128$). However, none of the isolates of *E. coli* was resistant to Meropenem, Imipenem, Tigecycline, Amikacin, Ciprofloxacin and Colistin.

Table 1: Periodic Variations of Physicochemical Parameters in November

*(This was done in replicate)

	pH	Temperature (°C)	Salinity (%)	Total Dissolved Solid(mg/l)	Dissolved Oxygen (mg/l)	BOD (mg/l)
Morning	6.73±0.02	29.33±0.21	6.82±0.12	8.14±0.29	4.16±0.51	5.15±0.22
Evening	6.84±0.18	29.93±0.15	7.32±0.30	6.78±0.36	4.52±0.50	6.78±0.30
p-value	0.36	0.16	0.05	0.007	0.002	0.44
t-value	-1.044	-4.025	-2.716	5.130	-7.579	-0.867
Remarks	NS	NS	NS	S	S	NS

Table 2: Periodic Variations of Physicochemical Parameters in April

*(This was done in replicate)

	pH	Temperature (°C)	Salinity (%)	Total Dissolved Solid(mg/l)	Dissolved Oxygen (mg/l)	BOD (mg/l)
Morning	6.78 ± 0.03	29.33 ± 0.15	6.85 ± 0.04	7.90 ± 0.11	4.76 ± 0.08	6.27 ± 0.31
Evening	6.82 ± 0.18	30.43 ± 0.47	5.16 ± 0.42	9.30 ± 0.25	5.11 ± 0.38	7.66 ± 0.24
p-value	0.676	0.019	0.002	0.001	0.004	0.200
t-value	-0.451	-3.836	6.849	-8.794	-6.047	-1534
Remarks	NS	S	S	S	S	NS

Table 3: Periodic Variations of Physicochemical Parameters in July

*(This was done in replicate)

	pH	Temperature (°C)	Salinity (%)	Total Dissolved Solid(mg/l)	Dissolved Oxygen (mg/l)	BOD (mg/l)
Morning	6.73 ± 0.04	29.33 ± 0.15	6.29 ± 0.07	7.63 ± 0.06	3.77 ± 0.07	5.00 ± 0.04
Evening	6.82 ± 0.18	29.80 ± 0.10	5.40 ± 0.23	6.59 ± 0.14	3.14 ± 0.43	6.62 ± 0.47
p-value	0.47	0.011	0.003	< 0.001	0.004	0.068
t-value	-0.883	-4.427	6.407	11.888	-5.945	2.477
Remarks	NS	S	S	S	S	NS

Table 4: Seasonal Comparison of Heavy Metals among the Seasons

*(This was done in replicate)

	Cadmium (Cd) mg/l	Mercury (Hg)	Lead (Pb) mg/l	Arsenic (As) mg/l	Chromium (Cr) mg/l	Nickel (Ni) mg/l
November (A)	0.021 ± 0.002	0.387 ± 0.051	0.035 ± 0.005	0.017 ± 0.003	0.215 ± 0.027	0.104 ± 0.007
April (B)	0.019 ± 0.002	0.366 ± 0.034	0.031 ± 0.002	0.012 ± 0.003	0.192 ± 0.019	0.983 ± 0.006
July (C)	0.022 ± 0.003	0.333 ± 0.022	0.032 ± 0.003	0.013 ± 0.004	0.184 ± 0.012	0.103 ± 0.005
p-value	0.347	0.286	0.348	0.268	0.228	0.523
F-value	1.268	1.553	1.265	1.655	1.914	0.724
Remarks	NS	NS	NS	NS	NS	NS
Post hoc (Tukey)						

A and B					
A and C					
B and C					

Table 5: Periodic and Seasonal Comparisons of Heterotrophic Plate Count (HPC) (CFU/ml)

	November (10 ⁵)	April (10 ⁵)	July
Morning (n=10)	4.03 ± 0.97	4.68 ± 1.34	5.18 ± 1.09 (10 ⁶)
Evening (n=10)	4.49 ± 1.20	4.85 ± 1.19	5.79 ± 0.84 (10 ⁶)

*(This was done in replicate)

Table 6: Periodic Variations of Total Coliform Count (TCC) and Total *E. Coli* during the Morning Hours

Days	November		April		July	
	TCC MPN/100 ml	TCC MPN/100 ml	TCC MPN/100 ml	TEC MPN/100 ml	TCC MPN/100 ml	TEC MPN/100 ml
1	15	3 (20.0 %)	11	3 (27.3 %)	14	3 (21.4 %)
2	7	3 (42.9 %)	11	4 (36.4 %)	20	7 (35.0 %)
3	11	3 (27.3 %)	7	3 (42.9 %)	11	3 (27.3 %)
4	9	4 (57.1 %)	9	4 (44.4 %)	11	3 (27.3 %)
5	15	3 (20.0 %)	7	3 (42.9 %)	9	4 (44.4 %)

*(This was done in replicate)

Table 7: Periodic Variations of Total Coliform Count (TCC) and Total *E. coli* during the Evening Hours

Days	November		April		July	
	TCC MPN/100 ml	TCC MPN/100 ml	TCC MPN/100 ml	TEC MPN/100 ml	TCC MPN/100 ml	TEC MPN/100 ml
1	11	3 (27.3 %)	20	7 (35.0 %)	21	7 (33.3 %)
2	9	4 (44.4 %)	11	3 (27.3 %)	11	3 (27.3 %)
3	11	3 (27.3 %)	9	4 (44.4 %)	14	3 (21.4 %)
4	9	3 (33.3 %)	7	3 (42.9 %)	9	4 (44.4 %)
5	15	3 (20.0 %)	14	3 (21.4 %)	7	3 (42.9 %)

*(This was done in replicate)

Table 8: Antibiotics, Strength, Zone of Inhibition and Classes of all the Antibiotics Used

Antibiotic	Strength	Resistant Zone Diameter	Class of Antibiotics
Meropenem	10µg	≤ 13 mm	Carbapenems
Imipenem	10µg	≤ 13 mm	Carbapenem
Tigecycline	15µg	≤ 12 mm	Glycylcycline
Cefotaxime	30µg	≤15 mm or≤17 mm	Cephalosporin
Gentamicin	30µg	≤ 12 mm	Aminoglycoside
Cefoxitin	30µg	≤ 14 mm	Cephalosporin
Amoxicillin / Clavulanic acid	30µg	< 13 mm	Beta-lactamase
Amikacin	30µg	≤ 14 mm	Aminoglycoside
Ciprofloxacin	5µg	≤ 15 mm	Fluoroquinolone
Colistin sulphate	10µg	≤ 12 mm	Polymyxins

Table 9: Antibiotic Resistance of *E. coli*

Antibiotics	Elechi Creek(128)
Meropenem	0 (0 %)
Imipenem	0 (0 %)
Tigecycline	0 (0 %)
Cefotaxime	14 (10.85 %)
Gentamicin	10 (7.75 %)
Cefoxitin	28 (21.71 %)
Amoxicillin/ Clavulanic acid	25 (19.38 %)
Amikacin	0 (0 %)
Ciprofloxacin	0 (0 %)
Colistin	0 (0 %)

Table 10: Multidrug Resistance (MDR) Pattern of the Isolates of *E. Coli* with MARI of >0.2 (n=11)

Multidrug Resistance (MDR) Pattern	Number of Isolate (n=11)	Percentage (%)
CTX, FOX, AMC	5	45.45
CN, FOX, AMC	1	9.09
CTX, CN, FOX, AMC	4	36.36
CTX, CN, FOX, AMC, AK	1	9.09
Total	11	100

Table 11: Antibiotic Resistance Index of *E. coli* from Elechi Creek

MAR Index	Number of Isolates (n=128)	(%) MARI
0.0	97	75.78
0.1	10	7.81
0.2	10	7.81
0.3	6	4.69
0.4	4	3.13
0.5	1	0.78
Total	128	100

4. Discussion

All the results from this study were compared with the standard recommended values according to World Health Organization (WHO) (WHO, 1993). Generally, all the parameters fell within the recommended values for water bodies. However, the mean Temperature values obtained were higher both in the mornings and evenings throughout the course of this research. Although, the mean values of the other parameters are within the recommended values, the parameters were still compared statistically using the Student's T-test, Analysis of Variance (ANOVA) and the

Tukey test (Post Hoc). Statistically, there was a significant difference in TDS and DO ($p < 0.001$; $t = -11.363$).

The results of the periodic and seasonal variations of the physicochemical parameters are shown in Table 1, 2 and 3. According to this study, in the mornings, the highest mean Temperature value was 29.33 ± 0.21 , while the highest mean values in the evenings ranged from $29.80 \pm 30.43 \pm 0.49$. The highest mean values were obtained in the evening of April (30.43 ± 0.49), followed by the evening of November (29.93 ± 0.15). A significant difference was observed both in April and July ($p = 0.019$; $t = -3.836$ and $p = 0.011$; $t = -4.427$, respectively). This could be as a result of the increased heat during the start and end of rainy season; there will be increased rainfall during the middle of rainy season. Also, this high temperature could cause changes in aquatic life, including microorganisms and consequently, become detrimental to them if the rates remain high. This report is in conformity with the findings of other researchers (Fafioye *et al.*, 2005; Obunwo *et al.*, 2011; Olorode *et al.*, 2015). They stated that the Temperature rose between 1.00 pm and 3 pm throughout the course of their experiment; they also presumed it to be time of high insolation.

Results of the heavy metals are presented in Table 4. The results obtained from the heavy metals (Cadmium, Mercury, Lead, Arsenic, Chromium and Nickel) analysis did not fall within the recommended values. The highest mean values were obtained in November (end of rainy season) than the other months. During the end of rainy season, the rate of rainfall reduces. This could cause an increase in the concentrations of heavy metals in water bodies as there will be no rainfall to reduce their levels (Bouka *et al.*, 2019). The variances in the mean were compared statistically using the ANOVA and according to the result; there was no significant variation among the heavy metals. High levels of heavy metals have been reported in Rivers State and outside the State (Ukpaka & Chuku, 2012; Abarshi *et al.*, 2017; Ollor *et al.*, 2018). Some of these researchers reported high levels of Pb, Cd, Zn, Ni, Cr and As (Ukpaka & Chuku, 2012). Water pollution with heavy metals has been revealed to cause changes in the composition of microorganisms that live in the water environment (Muller *et al.*, 2001; Kaci *et al.*, 2015).

According to the results of the Heterotrophic Plate Count (HPC), the highest mean values were obtained in morning and evening of July (5.18 ± 1.09 and 5.79 ± 0.84 , respectively). Rainfall during the middle of rainy season may have been responsible for the high number of bacteria observed. The reason being that as rainfalls, particles, including bacteria are emptied into the water body with runoffs. Several other researchers have reported increase in heterotrophic bacteria during the rainy season (July) (Christensen, 1987; Isobe *et al.*, 2004; Nwabueze, 2011).

Coliforms were reported in this study and the highest number was observed in the evening of July, day 1 (21 MPN/100 ml). *Escherichia coli* was also isolated from all the water samples, which was not within the standard recommended values of zero by WHO (WHO, 1993). Previously, the presence of *E. coli* in water bodies has been reported (Ollor *et al.*, 2018; Amosu *et al.*, 2018).

One hundred and twenty eight (128) confirmed isolates of *E. coli* were evaluated for their antibiogram profiling. In accordance with the results, all the isolates of *E. coli* were susceptible to Meropenem, Imipenem, Tigecycline, Amikacin, Ciprofloxacin and Colistin. According to the results, resistance among Beta-lactamase and Cephalosporin were most frequent than the others. Twenty eight (28) isolates were resistant to Cefoxitin, which represents Cefoxitin resistance of 21.71 % ($n = 128$).

According to this study, Multiple Drug Resistance (MDR) isolates were 11 (8.60 %). Moreover, 97 (75.78 %) isolates showed a Multiple Antibiotic Resistance Index (MARI) of 0.0 (susceptible to all antibiotics that were tested). Finally, the results revealed that 10 (7.81 %) isolates showed MARI of 0.1 and 0.2. The isolation of isolates of *E. coli* with MARI of > 0.2 were 11 (8.60); this may be due to the frequent use of antibiotics in Nigeria and how polluted the Water body is. The high Temperature of the Water body and high concentrations of the heavy metals observed in this current study may have as well added to the resistance level of the isolates of *E. coli* recovered from the Water body. In 2006, Tambekar and his co-researchers stated that bacteria from an environment where antibiotics are used unmonitored, often produce MARI greater than 0.2 (Tambekar *et al.*, 2006).

5. Conclusion

Now that it is obvious that there are high concentrations of heavy metals in Elechi Creek, in order to sustain a healthy water body ecosystem and prevent health problems to the locals, this study therefore recommends that the concentrations of Heavy metals be monitored regularly. Additionally, as it has also been confirmed that pathogenic microorganisms, such as antibiotic resistant *E. coli* are present in water bodies; it is recommended that the Fishes and Seafood caught from river environments be properly cooked. People should not eat Seafood, such as raw Crayfish; bathing in Water bodies should also be minimized. People should as well prevent food-and water-borne illnesses by cooking fishes and seafood to a minimum of 45°C and 145°F for 15 minutes, respectively and keep raw and cooked seafood separately to avoid cross-contamination.

Moreover, nationally, regionally and globally, the management and utilization of natural resources should be improved, so that the amount of wastes and pollutants that are generated by human activities would be reduced on a large scale. Finally, the building of toilets on water bodies and the discharge of untreated or improperly treated sewage should be discouraged.

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