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Analysis and Remediation of Fecal Coliform Bacteria Pollution in Selected Surface Water Bodies of Enugu State of Nigeria

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***Abstract*—**The assessment of surface waters in Enugu metropolis for fecal coliform bacteria was undertaken. Enugu urban was divided into three areas (A1, A2 and A3), and fecal coliform bacteria analysed in the surface waters found in these areas for four years (2005-2008). The plate count method was used for the analyses. Data generated were subjected to statistical tests involving; Normality test, Homogeneity of variance test, correlation test, and tolerance limit test. The influence of seasonality and pollution trends were investigated using time series plots. Results from the tolerance limit test at 95% coverage with 95% confidence, and with respect to EU maximum permissible concentration show that the three areas suffer from fecal coliform pollution. To this end, remediation procedure involving the use of saw-dust extracts from three woods namely; *Chlorophora-Excelsa* (C-Excelsa),*Khayan-Senegalensis*,(C-Senegalensis) and *Erythrophylum-Ivorensis* (E-Ivorensis) in controlling the coliforms was studied. Results show that mixture of the acetone extracts of the woods show the most effective antibacterial inhibitory activities (26.00mm zone of inhibition) against E-coli. Methanol extract mixture of the three woods gave best inhibitory activity (26.00mm zone of inhibition) against S-areus, and 25.00mm zones of inhibition against E-Aerogenes. The aqueous extracts mixture gave acceptable zones of inhibitions against the three bacteria organisms.

***Keywords*—**Coliform bacteria, Pollution, Remediation, Saw-dust

I. Introduction

ENUGU state commands an estimated population of over four million people. It has been the administrative capital of the then eastern region of Nigerian, and is now a state of its own. Its economic status is owed to the very large deposits of coal (that have been mined since 1961) and a very good number of industrial concerns [1]. On account of its position as the heart of eastern region of Nigeria, it is known to have more industries, Agric establishments and business concerns, when compared to other neighboring states The population of Enugu as a result of these economic lure, has doubled recently, owing to the migration of workers and their dependents from neighboring states to Enugu. Water supplies in Enugu state depend heavily on surface water bodies and generally water uses such as drinking, irrigation, industrial processes and domestic purposes place great demands on water status [2]. Even though water quality parameters discriminate against various water uses, each water use lead to specific and generally predictable impact on the condition of the aquatic environment and on the health implication to humans The records of many general hospitals and private clinics in Enugu state, show regular cases of water-borne diseases among the populace [3]. This might have a bearing to the health of Enugu streams and water bodies, as the people depend more on streams and rivers for their water use. There are undoubtedly good reasons to believe that the surface waters of Enugu state are polluted with fecal coliform group of bacteria, which are known to be indicators of the pathogens load of water. This work regards this as a big problem deserving urgent attention. Even though rivers and streams have natural self purification ability, the rivers do not flow for a good enough distance to enable this purification to happen [4]. This therefore calls for a scheme to be developed with respect to reduction of these bacteria within a short distance of their generation in the rivers. The analyses of coliform bacteria in Maryland river located in area 2 (A2) of this work show remarkable decrease in fecal coliform population near a timber market where saw-dusts are dumped into the river. It was therefore expedient to examine the solvent extracts of the types of woods predominantly found in the saw-dusts, for their anti-bacterial potentials. Many works exist in literature concerning the anti-bacterial potentials of a vast many plants with respect to drug development, but a few works have studied any relationship between plant extracts and fecal coliform bacteria water pollution control. Moreover, in Enugu state there has not been any profile describing the coliform pollution pattern of the rivers and streams. This work has addressed these problems.

EXPERIMENTAL Procedure

Sampling/Mapping of Study Areas

A total of three maps (fig.1-fig.3) were developed for this work from Enugu urban map. These maps show the rivers and streams, the chosen sampling sites, and the pollution indices at the sites. Fig.1 and fig.2 describe the sampling area designated as area-1 (A1) in this work. There are six sites in this area (S1-S6). Fig.3 describes the area named A2. This area has four sites (S1-S4). All the sites were chosen with respect to physical pollution factors in the various areas.

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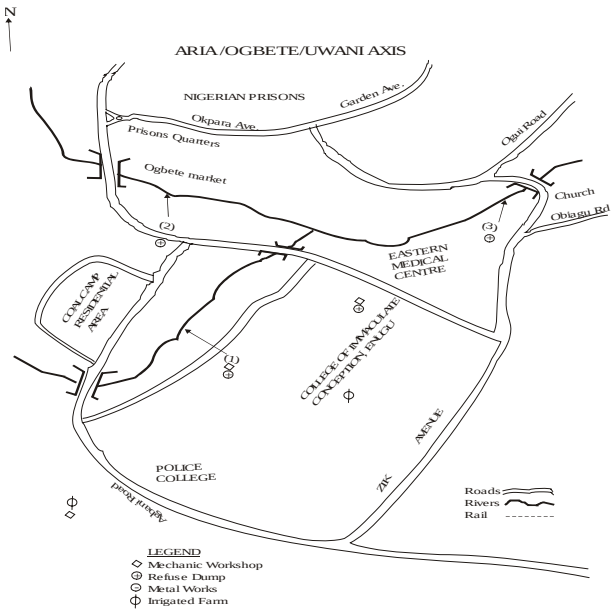

Fig. 1 Sub map of Enugu Urban Showing A1 map1

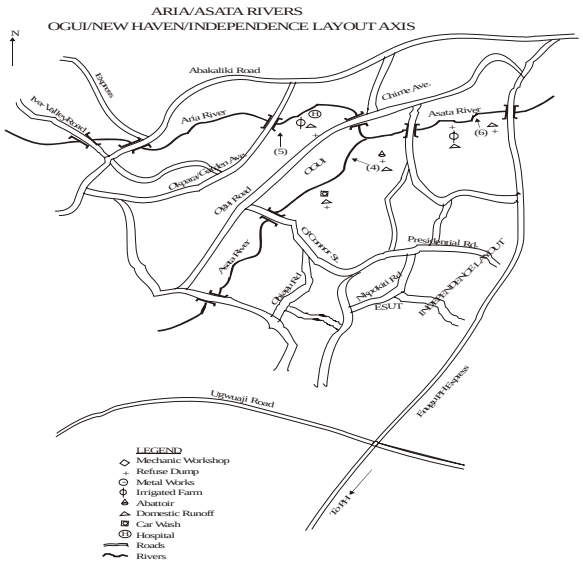

Fig. 2 Sub-map of Enugu Urban Showing A1 map2

A. Determination of Total Fecal coliform Bacteria

MacConkey agar Petri dishes were used for this experiment. Sterile forceps was used to place sterile membrane filter on the membrane filtration apparatus. Water sample was shaken and delivered to the filtration apparatus by the aid of a graduated cylinder. Vacuum was then applied to aid the filtration process. After the filtration, the membrane filter was removed with sterile forceps and rolled onto the media plate. The plates were then placed in an incubator set at 37^0C and incubated for 24hrs. The bacterial colony counting was done using a hand held magnifying lens (5X magnification) [5]..

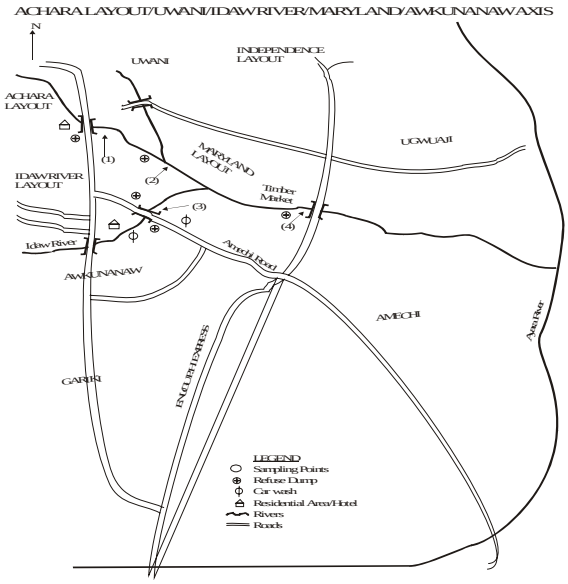

Fig. 3 Sub-map of Enugu Urban Showing A3

B. Determination of the Antibacterial Potential of Saw-dust

Materials used for the experiment are; Analytical balance, incubator, refrigerator, Autoclave, Bijou bottles, Petri-dishes, syringes, and cotton wool. Analytical grade reagents were used throughout. Shavings (saw dust) from mahogany (*chlorophora Excelsa*); Iyi (*Khaya Senegslensis*) and Iroko *(Erythrophylum Ivorensis*) were obtained from the Timbre market and used for the analysis. 250ml each of methanol, Acetone and distilled water were set aside for the experiment.10g each of the saw dust from the various woods were used for the extractions using acetone, methanol, and water in each case. Cold and hot water extracts were done within 24hrs. Soxhlet extractions using the organic solvents was done following the method described by [6]. For the cold water extraction, 10g of ground saw dust was put in 100ml distilled water, Stirred very well and allowed to stand for 24hrs; while the 10g sample and solvent were heated with stirring for 3hrs and allowed to stand for 24hrs, for the hot water extraction. After the extractions, the water extracts were reconstituted using sterile distilled water to concentrations of 250, 200, 150, 100, and 50mg/l. The organic extracts were diluted using 50% Dimethylsulphoxide (DMSO). 50ml aliquorts of the various wood extracts were taken and mixed in separate sterile bottles to obtain methanol extract mixture, Acetone extract mixture; cold water extracts mixture, and hot water extract mixture [7]-[9]. For the microbial tests using the extracts and mixtures**,** the coliform bacteria studied were *Escherichia coli* (E-coli), *Enterobacter Aerogenes* (E-Aerogenes), and *staphylococcus Aureus (S-Aureus).* The following procedure involving the cup plate agar diffusion method described by Aidan, et al. [10] was adopted. The pure culture organisms procured from ESUT teaching hospital were inoculated unto MacConkey agar plates (diameter; 15cm). A sterile cork borer was used to make wells (diameter; 6mm) on the plates. 0.1ml aliquorts of the extracts, extract mixtures, and

pure solvents (controls) were applied in each of the wells in the culture plates already streaked with the test organisms. The cultures were put in the incubator set at 370C and left to stand for 24hrs. Antibacterial activity was determined by measuring the zone of inhibition around each well. The minimum inhibitory concentrations for the various extracts and mixtures on the organisms were determined according to the method described by [11].

II. RESULTS AND DISCUSSION

The sampling and analyses of samples for the year 2006 started in January [Table I]. At this period, the dry season was still on. Most of the water bodies had moderate to low water levels. There was no noticeable increase in the turbidity levels of the sites, by mere physical examination. The amount of refuse at the banks of Fig.1, and Fig.2 rivers had increased obviously from the wastes generated from Christmas activities the previous month.

TABLE I
AVERAGE COLIFORM VALUES FOR THE SITES IN A-1, A-1B, AND A-2 (CFU/100ML) X 10^3

Month	2005			2006			2007			2008		
	A1	A1b	A2	A1	A1b	A2	A1	A1b	A2	A1	A1b	A2
Jan.	-	-	-	12.3		22.6	3.4	104	42	8.2	103	58
Feb.	-	-	-	8.10	96.8	28.3	3.6	112	38	9.93	111	63
March	-	-	-	5.32	122	32.4	5.8	82	34	9.24	90	42
April	10	73	31	8.31	106	42.0	6.2	73	26	8.45	94	55
May	22	12	38	7.42	132	43.0	6.4	80	25	7.03	82	43
June	28	38	29	8.11	110	61.0	5.7	79	58	11.2	81	50
July	24	47	40	8.22	120	48	6.0	98	26	10.3	98	54
Aug.	12	44	39	6.01	138	44	7.3	0.41	41	-	79	41
Sept.	16	41	26	5.22	142	56	-	91	53	9.46	63	73
Oct	20	36	29	4.83	141	52	-	84	43	10.5	74	68
Nov	8	56	58	601	133	-	7.7	86	0.21	10.4	92	65

Fig.2 rivers particularly received dead animal wastes and dung within the period and evidences of pollution was shown in the very foul smell spewing from the river. Physical examination of Fig.1 show monumental amount of used motor oil in the river. Most of these rivers receive effluents laden with oil, from road-side mechanics. Most of the farmers at A2 (fig.3) use animal dung to fertilize their crops and this had influence on the coliform level of the sites in the area [12], [13]. Activities at the University of Nigeria Teaching Hospital (UNTH), very close to A1-map1(fig.1) was waning from April, following the relocation of the hospital, solids and liquid pollutants were therefore minimal, and the only source of pollution for the sampling area seem to be the Akwata Market, and mechanic workshops.Problem with coliform group of bacterial was more obvious with A1-map1 rivers. A2 river also show evidences of high coliform load. These sampling areas play hosts to monumental domestic effluents, and at some points they are chocked by severe solid waste dumping. Traders from Artisan market and Ogbete market dump all forms of solid waste in the water bodies. There are very strong month by month and seasonal trends in coliform across the sampling areas. The total fecal coliform was higher during dry season than rainy season. At the beginning of the year 2008, the Enugu State Waste Management Authority (ESWAMA) had cleared the solid wastes dumped at various points of the rivers banks. The coliform count values did not reflect this activity of ESWAMA, as they were above tolerable values throughout the analysis months [Table I]. Even though, the state government had introduced a better solid waste disposal option, and hence no more solid wastes were dumped at the rivers banks, the impact has yet to reflect on the pollution parameters. Coliform bacteria were found at levels much higher than what is permitted for surface waters, in all the rivers. Effluents from Artisan market enter into Asata river in A-1 (Fig.2). There are chicken and goat slaughter points in the market and these contribute seriously to the pathogen load of the riverIn Area-2 (Fig.3) there was a sudden reduction in coliform between some sampling points in the rivers found in the area. Sampling points close to the Timber market were high in coliform, but points after the market were low in coliform. This was observed generally for the analyses years.Table VIII shows the Area-1 total coliform statistical analysis. The skewness coefficient of the data show good degree of skewness, but not enough to reject the assumption of normality]. Correlation and covariance tests reveal that the assumption of equal group variances is not out of place [14]. The correlation shows that the sites have some relationships close to linear, with each other. The box plot [fig 4] for sites-2, 3 4, 5 and 6 were all very small in length relative to site-1 box length. This shows that the distributional character of this parameter in this area is not reliable [15]. However, the normality of the logged data was not rejected by Kolmogorov Smirnov (KS) test. Based on this, the tolerance limit test was performed and result show that all the sites are heavily polluted. Fig. 4 shows the time plots over the years. For most of the sites, coliform levels decreased between 2005 and 2006, and maintained a nearly regular trend between 2006 and 2008.

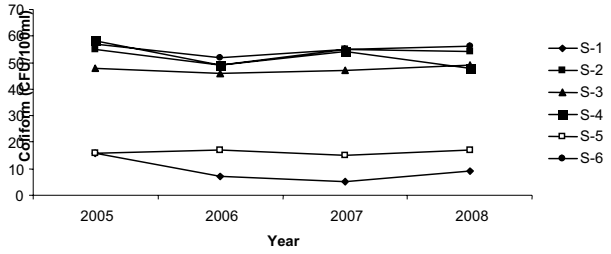

Fig. 4 Trends in Coliforms at Area-1

This might be traced to the activities of ESWAMA.Table IX shows the statistical results for coliform in Area-2. Correlation was not significant between S-2 and S-4. All other sites show significant correlations with each other. From the box plots, the population median of the sites differs greatly from each other, but the assumption of normality was good based on the relative lengths of the boxes [16], [17]. Logged data was certified normal by the KS test and was used for the tolerance limit test. Result for the tolerance limit show that all the sites are polluted with coliform over the years. Fig. 5 shows the

time series plot for coliform over the years at A2. Between 2005 and 2007, coliform levels increased in S-1, but decreased in the other sites. A regular trend was maintained between 2007 and 2008. Site-9 shows the highest severity of coliform pollution relative to the other sites.

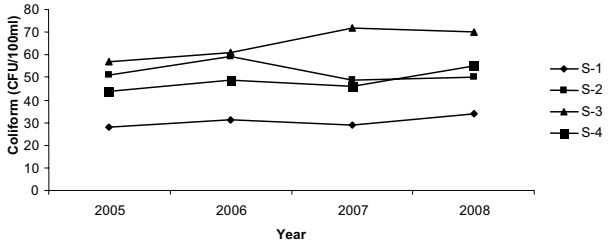

Fig. 5 Trends in Coliforms at Area-2

Tables II, III, and IV show the zones of inhibition of the methanol, acetone and water extracts respectively. It is very obvious from the tables that the mixture of extracts gave more zones of inhibition, than the individual extracts. With respect to methanol extract and E-Coil, it is clear that the mixture of extracts was more effective at inhibiting the growth of E-Coli than the individual wood extracts. However their zones of inhibition are still considered good enough to make them qualify as effective against E-Coli [18]- [22]. The mixture of extracts show some synergistic effect as its zone of inhibition of 24 was almost double the effect of the individual extracts. The growths of *E-Aerogenese* and *S-Aureus* were also

TABLE II
ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACTS: ZONES **OF** INHIBITION (MM)

Organism	*C-Excelsa*	*K-senegalensis*	*E –Ivorensis*	Mixture	Pure Methanol
E – Coli	13	20	13	24.0	2
E – Aerogenes	8	6	10	15	3
S –Aureus	20	12	12	26.0	2

TABLE III
ANTIBACTERIAL ACTIVITY OF ACETONE EXTRACT: ZONES OF INHIBITION (MM)

Organism	*C-Excelsa*	*K-Senegalensis*	*E –Ivorensis*	Mixture	Pure Acetone
E – Coli	15	10	10	26	3
E – Aerogenes	4	7	6	12	2
S –Aureus	14	10	23	25	2

TABLE IV
ANTIBACTERIAL ACTIVITY OF WATER EXTRACTS: ZONES OF INHIBITION(MM**)**

Organism	*C-Excelsa*		*K-Senegalensis*		*E –Ivorensis*		Mixture		Pure Water
	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	
E – Coli	4	5	4	6	3	4	8	10	0
E – Aerogenes	2	4	6	8	2	5	8	12	0
S –Aureus	0.0	3	2	3	0.0	2	2	5	0

inhibited by the individual methanol extracts and again the mixture gave best results (Fig 9). *E- Aerogenese* recorded the least zones of inhibition for both extracts and mixture. The result for acetone extracts and mixture did not deviate so much from what was obtained with the methanol extracts. The result for the water extracts and mixture was a far cry from what was obtained with the organic solvents but the zones of inhibition found, were good enough to believe that the water extracts are effective against the organisms [23]. This may explain why the mixture of saw dust at A1-S4 was reducing the population of total fecal coliform found in Maryland River. The mixture of saw dust soak in the river water, and the phytochemical matter responsible for the inhibition are extracted, and the coliforms are killed. Organic solvents may have offered better results (fig.9), as they are more effective in extracting the active principles than water [8]. Effort was made to overrule the belief that the organic solvents might be killing the bacteria, by running the pure solvents blanks as control. Results show insignificant zones of inhibitions for the pure solvents, supporting the fact that the wood extracts are responsible for the inhibitions. Prior to the determination of the inhibitory effects of the extracts, the minimum inhibitory concentrations (MIC) for the various wood extracts were determined by using concentrations of 50, 100, 150, 200 and 250 mg/ml of the extracts for the experiment. Results are shown on tables V, VI and VII. Generally, the extract mixtures gave lower MIC than the individual, extracts, showing better inhibitory ability [24]-[28].

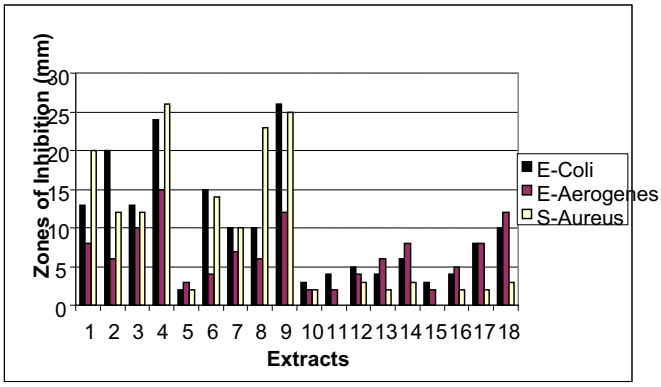

Fig.6 Relative Antibacterial Activities of the Wood Extracts

X-axis Key:
1=*C-Excelsa* (methanol); 2=*K-Senegalensis* (Methanol); 3=*E-Ivorensis*(methanol); 4=Methanol extracts mixture; 5=Pure Methanol; 6=*C-Excelsa*(Acetone); 7=*K-Senegslensis*(Acetone); 8=*E-Ivorensis*(Acetone); 9=Acetone Extracts Mixture; 10=Pure Acetone; 11=*C-Excelsa*(Cold water); 12=*C-Excelsa*(Hot water); 13=*K-Senegalensis*(cold water); 14=*K-Senegalensis*(hot water); 15=*E-Ivorensis*(cold water); 16=*E-Ivorensis*(hot water); 17=Cold water extracts mixture; 18=Hot water extracts mixture.

TABLE V
MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE EXTRACTS OF *C- EXCELSA* (MG/ML)

	Water Cold	Hot	Water Mixture Cold	Hot	Methanol	Methanol Mixture	Acetone	Acetone Mixture
E-Coli	200	150	150	100	150	100	150	50
E-Aerogens	200	200	200	150	150	150	150	100
S-Aureus	-	200	150	150	200	100	100	50

TABLE VI
MIC OF THE EXTRACTS OF K-SENEGALENSIS (MG/ML)

	Water Cold	Hot	Water Mixture Cold	Hot	Methanol	Methanol Mixture	Acetone	Acetone Mixture
E-Coli	100	200	150	150	150	150	150	100
E-Aerogenes	200	200	100	100	200	100	100	100
S-Aureus	200	200	200	150	100	100	100	50

TABLE VII
MIC OF THE EXTRACTS OF *E-IVORENSIS* (MG/ML)

	Water Cold	Hot	Water Mixture Cold	Hot	Methanol	Methanol Mixture	Acetone	Acetone Mixture
E-Coli	200	150	150	150	100	50	50	50
E-Aerogenes	200	200	150	100	100	100	150	50
S-Aureus	-	200	150	100	100	150	150	100

It is therefore clear from this study that both aqueous and organic solvents extracts of *E-Excelsa, K-Senegalensis* and *E-Ivonesis* are effective in reducing the population of total fecal coliforms found in surface waters[29]. The organic extracts offered the best results, but the experiment was conceived based on water as the medium of extraction. It may be possible for the saw dust from these woods to be used in constructing screens that can be put in the path of surface waters polluted with coliform group of bacterial, to reduce the concentration of these organisms. Further work should be in the area of adapting this scheme for a vast many disease causing water-borne pathogens.

IV CONCLUSIONS

The Enugu State metropolis surface waters are polluted with fecal coliform bacteria. This work has shown this from the four year (2005-2008) chemical analysis and statistical evaluation of Enugu urban surface waters for the bacteria. This work has also shown that extract mixtures of woods (from locally available plants) can be used to effectively reduce the fecal coliform bacteria load of the water bodies.

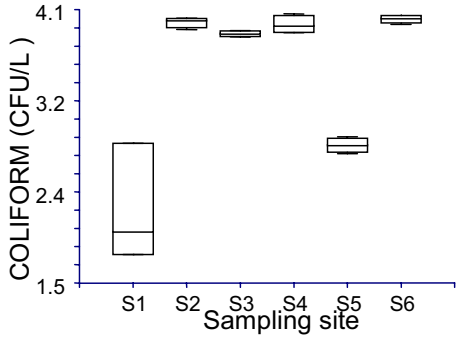

Fig.4 Box Plot for Coliform distribution in Area-1

TABLE VIII
SUMMARY STATISTICS FOR NATURAL LOG OF COLIFORM DATA IN A-1

Site	MEAN	StDv	VAR	SKEW	KURT	N
Site-1	2.20	0.46	0.21	1.10	1.30	4.0
Site-2	3.98	0.05	0.00	-1.77	3.11	4.0
Site-3	3.87	0.03	0.00	-0.42	-3.36	4.0
Site-4	3.96	0.09	0.01	0.48	-2.98	4.0
Site-5	2.81	0.06	0.00	-0.23	-0.40	4.0
Site-6	4.01	0.04	0.00	-1.15	1.26	4.0

Site	MIN	1st Q	MED	3rd Q	MAX	IQ-R	R	MID-R
Site-1	1.77	1.88	2.10	2.52	2.83	0.64	1.06	2.30
Site-2	3.91	3.95	4.00	4.01	4.02	0.06	0.11	3.96
Site-3	3.84	3.85	3.87	3.89	3.90	0.05	0.06	3.87
Site-4	3.88	3.89	3.95	4.03	4.06	0.14	0.18	3.97
Site-5	2.73	2.76	2.81	2.86	2.89	0.10	0.15	2.81
Site-6	3.96	3.99	4.02	4.04	4.05	0.05	0.08	4.01

CORRELATIONS (Numeric Variables)

Site	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6
Site-1	1.00	0.35	0.67	0.52	0.21	0.64
Site-2	0.35	1.00	0.73	0.65	-0.40	0.92
Site-3	0.67	0.73	1.00	0.25	0.31	0.93
Site-4	0.52	0.65	0.25	1.00	-0.72	0.56
Site-5	0.21	-0.40	0.31	-0.72	1.00	-0.06
Site-6	0.64	0.92	0.93	0.56	-0.06	1.00

TABLE IX
SUMMARY STATISTICS TABLES FOR COLIFORM DATA IN A-2

Site	MEAN	StDv	VAR	SKEW	KURT	N
S-1	30.25	2.87	8.25	0.85	-1.29	4.0
S-2	52.00	4.08	16.67	1.76	3.23	4.0
S-3	64.50	7.05	49.67	-0.14	-5.02	4.0
S-4	48.00	5.10	26.00	1.06	1.50	4.0

Site	MIN	1st Q	MED	3rd Q	MAX	IQ-R	R	MID-R
S-1	28.00	28.00	29.50	32.50	34.00	4.50	6.00	31.00
S-2	49.00	49.50	50.50	54.50	58.00	5.00	9.00	53.50
S-3	57.00	58.50	65.00	70.50	71.00	12.00	14.00	64.00
S-4	43.00	44.50	47.00	51.50	55.00	7.00	12.00	49.00

CORRELATIONS (Numeric Variables)

Site	S-1	S-2	S-3	S-4
S-1	1.00	0.17	0.32	0.96
S-2	0.17	1.00	-0.58	-0.05
S-3	0.32	-0.58	1.00	0.58
S-4	0.96	-0.05	0.58	1.00

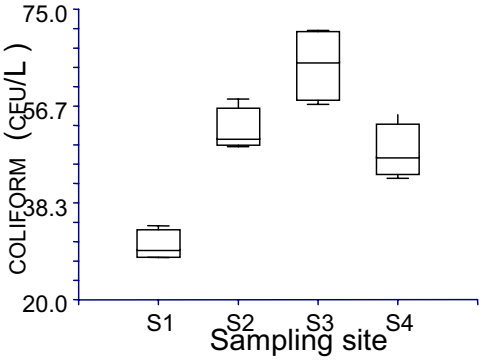

Fig 5 Box Plot for COLIFORM distribution in Area-2

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