

## Survival of Some Indicators and Bacterial Pathogens in A Waste Stabilization Pond.

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### Abstract

The survival of four species of bacteria was studied in a Waste Stabilization pond of the Waste Treatment plant of Ahmadu Bello University, Zaria. The four cultures used were: - *Escherichia coli*, *Streptococcus faecalis*, *Salmonella typhi* and *Vibrio cholera*. Of these, *E. coli* showed greater persistence, surviving for 26 days and a half-life of 5 days. *S. faecalis* and *S. typhi* survived for 18 days each with a half life of 4 days while *V. cholera* showed the least persistence of 6 days and half life of 2 days. The result suggests the need to increase the retention time for the treatment facility to 28 or more as against the 24 days presently in use.

**Key Words:** Coliform, Pathogen, Sewage.

### Introduction

The occurrence of faecal coliform in water is regarded as the single most important indicator of public health hazard from infectious agents. However, to isolate or determine its survival time, the organism must survive in that environment for an indefinite time. In this regard, the faecal coliform bacteria have been found to be among the first of the micro-organisms to die in the aquatic environment<sup>1</sup>. While other bacteria persist and so maintain the health hazard in the absence of the indicator bacteria<sup>2</sup>.

The International reference center for waste disposal<sup>3</sup> reported that the irrigation of crops with waste-water has become a common practice in many parts of the world; waste water together with excreta is also used for aquaculture for fish production and fertilisation of edible macrophytes water chestnut, waterlotus. Therefore cholera and typhoid fever caused by *Vibrio cholera* and *Salmonella typhi* can effectively be transmitted by the irrigation of vegetables with untreated wastewater.

As a result of the health hazards constituted by these organisms in association with waste water especially where such waters are used for the irrigation of crops, it has become necessary to study the survival of such organisms in these environments.

### Materials and Methods

#### Bacteria and Media.

All cultures used for this study were obtained from the Department of Microbiology, Ahmadu Bello University, Zaria.

Purified cultures of *E. coli* was identified by the

following cultural characteristics: growth on brilliant green lactose bile broth, characteristic colonies with metallic sheen on Eosin Methylene Blue (EMB) agar and characteristic tests for the production of Indole, Methyl Red and Voges Proskauer and citrate (IMVIC) utilization reactions. *S. faecalis* was characterised by their ability to blacken bile aesculin medium, and by their characteristic growth on M-enterococcus medium.

Cultures of pathogenic species of *S. typhi* and *V. cholerae* were characterised by standard biochemical tests such as:- production of small amount or hydrogen sulphide, Lysine decarboxylation, Ornithine decarboxylation, methyl red and a characteristic K/A (H<sub>2</sub>S) reaction on Triple Sugar Iron (TSI) agar. *S. typhi* and *V. cholera* were characterised by a typical growth on thiosulphate citrate bile sucrose (TCBS) medium, starch hydrolysis and haemolysis on blood agar. All cultures used were grown in Brain Heart Infusion (BHI) broth for 24 hrs at 37°C. After incubation, the cells were harvested by centrifugation at 3000rpm for 10mins. The cells were therefore diluted with sterile distilled water before introducing into dialysis sacs. The dialysis sac allows the water and solutes in the environment to diffuse readily through the sacs and to interact with the bacterial suspension.

A significant advantage of this system over procedures in which bacteria are studied in a limited and unchanging water is that a continuous flow of fresh water and solution come in contact with the bacteria under investigation. This characteristic allows the system to be responsive to physical and chemical

changes that may occur in the surrounding<sup>2</sup>.

The sacs were then tightened to strings labelled accordingly before suspending in the waste water (waste stabilization pond) which has a retention time of 24 days.

### Counting of Cells

The population density of the individual bacterial species were enumerated using the surface plate method: Using their selective or differential media: Eosine methylene blues agar for *E. coli*, M-enterococcus agar *S. faecalis*, Thiosulphate citrate bile sucrose for *V. cholera* and *salmonella-shegella* agar *S. typhi*.

### Results

#### Survival of pathogens

*Salmonella typhi* showed greater persistence of 18 days with a half life of 4 days while *V. cholera* persisted for only 6 days with a half life of 2 days

#### Survival of Indicator Organisms

*Escherichia coli* showed greater persistence of 26 days with a half life of 5 days while *S. faecalis* persisted for 18 days with a half life of 4 days

Fig 1 also shows the survival curve of all the 4 organisms.

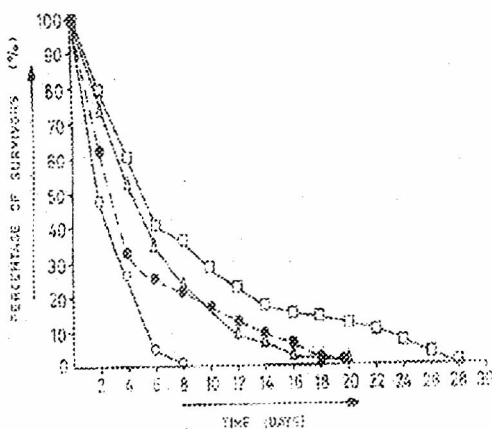


Fig. 1 Survival curve of four organisms - *E. coli*, *S. typhi*, *S. faecalis* and *V. cholerae* in a waste treatment pond.

○ *V. cholerae*, ● *S. typhi*, △ *S. faecalis*, □ *E. coli*

### DISCUSSION

The result of this experiment describing the survival of indicator bacteria in wastewater has demonstrated that the faecal coliform (*E. Coli*) remained viable for a longer period when compared to the persistence of *S. Faecalis*. Similar results have been observed by other workers<sup>4</sup>, when they studied the population dynamics of these organisms in dialysis sacs that were immersed in sea water. In another study, however, it was observed that the enterococci as a group persisted for longer periods than do the coliforms in a waste treatment lagoon<sup>5</sup>.

The observation of having greater persistence of one organism or group of organisms over the other could be due to insufficient washing or cultures and excessive population of the bacteria. This is evident by the fact that higher die-off rates were observed where there were higher population densities in the sacs than when there was a lower population density. From the initial survival experiments useful information has been gained regarding the survival potential of the indicator bacteria compared with the enteric pathogens<sup>2</sup>. Many workers<sup>6,7,8,9</sup> have made similar studies in both fresh and salt water. For example, great interest has been noted in the relationship between *Salmonella* and indicator bacteria. It has been found that the *Salmonella* survived better in water than the coliform bacteria<sup>7</sup>, while<sup>6,8,9</sup> have noted greater persistence of the indicator bacteria especially the coliforms. However, the results of these studies are difficult to compare due to the differences in the test systems and organisms used. The results of the present study indicates that the survival of *S. faecalis* and *E. coli* is comparable to that of *S. typhi* though *E. coli* showed greater persistence.

The concentration of organic and inorganic materials in the wastewater might have favoured the persistence of *E. Coli*, *S. Faecalis* and *S. Typhi* as suggested by Marsharipov<sup>10</sup>. In contrast, *V. cholerae* has a much higher die-off rate which does not correspond with that of *S. typhi* another enteric pathogen<sup>6</sup>.

The two indicator bacteria used for the study, *E. coli* and *S. faecalis* are both good indicators of sanitary significance because of the good correlation they show when compared with the survival of *S. typhi* in wastewater.

### Acknowledgement.

We are grateful to Mr. A.A. Odewumi of the Department of Microbiology, Ahmadu Bello University Zaria-Nigeria for providing the cultures used in this study. We also acknowledge the contributions of Dr. Charles Okuofu. Finally, we are thankful to Miss Jane Wetmang for typing the manuscript.

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