



## Contributory pattern of paper mill effluents on the population and distribution of enteric pathogens in Owerinta River, Eastern Nigeria

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### Abstract

Wastewater discharge into freshwaters is a major source of pathogens. Relationship between bacterial pathogens in 3 paper mill effluents and recipient Owerinta River was determined under standard microbiological analysis. The THBC of effluents and River ranged from  $1.8 \times 10^5$  -  $7.2 \times 10^6$  cfu/ml and  $2.0 \times 10^3$  -  $5.5 \times 10^4$  cfu/ml while the TCBC ranged from  $3.1 \times 10^4$  -  $8.8 \times 10^4$  cfu/ml and  $1.0 \times 10^3$  -  $2.0 \times 10^4$  cfu/ml respectively. Trend in counts for effluents within samples was: Effluent I > Effluent - II > Effluent - III while within river samples was B > C > A. Isolates occurred thus: *Escherichia coli* (100%), *Klebsiella* spp. (83.3%), *Shigella* spp. (66.7%), *Salmonella* spp. (83.3%), *Proteus* spp. (33.3%), *Pseudomonas* spp. (83.3%), *Staphylococcus* spp. (66.7%), *Bacillus* spp. (100%), and *Citrobacter* spp. (11.1%). The effluents contributed to the bacterial load and presence of pathogens, and the counts were above established limits for drinking water.

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## Introduction

Many people struggle to obtain access to safe water. A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not the rule, and waterborne infections are common. Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrhea diseases (Fenwick, 2006). According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. From these, more than 50% are microbial intestinal infections, with cholera standing out in the first place (Cabral, 2010). Acute microbial diarrheal diseases are major public health problem in developing countries. People affected by diarrheal diseases are those with the lowest financial resources and poorest hygienic facilities. Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water (Sea *et al.*, 2000). Microbial waterborne diseases also affect developed countries. In the USA, it has been estimated that each year 560,000 people suffer from severe waterborne diseases, and 7.1 million suffer from a mild to moderate infections, resulting in estimated 12,000 deaths a year (Medema *et al.*, 2003).

In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal feces (Cabral, 2010). Waste water discharges in fresh waters and coastal seawaters are the major sources of fecal microorganism, including pathogens (WHO, 2008; Fenwick, 2006; George *et al.*, 2001; Grabow, 1996). This research therefore tried to ascertain the possible contribution of the effluents of the three different paper mill industries that discharge their effluents into Owerrinta River on the population and load of enteric bacterial pathogens and the potential consequences on the local inhabitants of Owerrinta who depend majorly on the River for source of water for domestic and agricultural irrigation.

## Materials and methods

### Study area

Owerrinta River is located within longitude 7°17'E and Latitude 5°18'N and, a part of Imo River, serves as a recipient of effluents from three paper mill industries (Effluent I - Star paper mill, Effluent II - Amec paper mill, and Effluent III - Industrial paper mill) closely sited together, and provides sand for excavators, source of fishes and water for domestic uses.

### Sample collection

Samples were collected in triplicates with the aid of sterile 1 liter water sampling cans. Collected samples were transported immediately to the laboratory. Effluent water samples were collected from discharge points before discharge into Owerrinta River. Owerrinta river samples were collected thus: upstream – 100metres before the first discharge point; discharge point – 20metres after the third discharge point; and downstream – 100metres after the third discharge point.

### Microbiological analysis

Sterilization of media was carried out by moist heat sterilization method using autoclave at 121°C, 15psi and for 15 minutes. Heat stable materials were sterilized using hot air oven at 160°C for 1 hour as described by Cruickshank *et al.* (1982). Heat labile materials were aseptically rinsed with alcohol and distilled water. The water samples were aseptically subjected to 10 fold serial dilutions to dilute the population of microorganism sufficiently in sterile blanks of 9ml peptone water and then plated to produce discrete colonies for easy enumeration. The media used include Nutrient agar, MacConkey agar, Eosin Methylene Blue agar, TCBS, and *Salmonella – Shigella* agar. All media were prepared as directed by the manufacturer. The method of Dubey and Maheshwari (2004) was adopted for the inoculation of media. Spread plates of appropriately diluted samples were incubated at 37°C for 24 hours for heterotrophic bacterial count (THBC) while total coliform bacterial count (TCBC) were determined after incubation at 45°C for 24 hours in MacConkey

agar. Identification of isolates was based on the scheme described by Cheesborough (1984).

## Results

The mean result of total heterotrophic bacterial count (THBC) and total coliform bacterial count (TCBC) for the effluent samples and Owerinta River are as shown in Table 1. The THBC of the effluents ranged from  $1.8 \times 10^5$  -  $7.2 \times 10^6$  cfu/ml while the TCBC ranged from  $3.1 \times 10^4$  -  $8.8 \times 10^4$  cfu/ml and, the THBC of Owerinta Point ranged from  $2.0 \times 10^3$  -  $5.5 \times 10^4$  cfu/ml while the TCBC ranged from  $1.0 \times 10^3$  -  $2.0 \times 10^4$  cfu/ml. The trend of variations in bacterial count for effluent samples showed: Effluent- I > Effluent - II > Effluent - III while for river sample, it showed: B > C > A. These results were above the limits of USEPA Maximum Contaminant Levels (MCLS) of < 100cfu/ml in drinking water (USEPA, 2003).

**Table 1.** Mean total bacterial count of triplicate paper mill effluents and Owerinta River samples.

Samples	THBC (cfu/ml)	TCBC (cfu/ml)
Upstream (A)	$2.0 \times 10^3$	$1.0 \times 10^3$
Point of discharge (B)	$5.5 \times 10^4$	$2.0 \times 10^4$
Downstream (C)	$3.2 \times 10^4$	$2.1 \times 10^3$
Effluent I	$7.2 \times 10^6$	$5.0 \times 10^4$
Effluent II	$1.0 \times 10^6$	$8.8 \times 10^4$
Effluent III	$1.8 \times 10^5$	$3.1 \times 10^4$

Table 2 shows the % distribution of bacterial isolates from the different effluent and River samples at Owerinta Point of Imo River. These organisms include *Escherichia coli* (100%), *Klebsiella* spp. (83.3%), *Shigella* spp. (66.7%), *Salmonella* spp. (83.3%), *Proteus* spp. (33.3%), *Pseudomonas* spp. (83.3%), *Staphylococcus* spp. (66.7%), *Bacillus* spp. (100%), and *Citrobacter* spp. (11.1%). This implied that *Escherichia coli* and *Bacillus* spp. were isolated from all the River and effluent samples. *Citrobacter* spp. was isolated from one out of 6 samples.

The occurrence of total bacterial isolates from each sample showed upstream (55.6%), discharge point (77.8%), downstream (100%), Effluent I (66.7%),

Effluent II (55.7%) and Effluent III (44.4%). This means that all the isolates were isolated from downstream samples, while 5 out of the 9 isolates were isolated from Effluent III sample.

**Table 2.** Distribution of bacterial isolates from paper mill effluents and river samples.

Sampling point	<i>E. coli</i>	<i>Klebsiella</i>	<i>Shigella</i>	<i>Salmonella</i>	<i>Proteus</i>	<i>Pseudomonas</i>	<i>Staph.</i>	<i>Bacillus</i>	<i>Citrobacter</i>	%Occurrence <sup>b</sup>
Upstream	+	+	+	+	-	-	-	+	-	55.6
Discharge Point	+	+	+	+	-	+	+	+	-	77.8
Downstream	+	+	+	+	+	+	+	+	+	100
Effluent I	+	-	+	+	-	+	+	+	-	66.7
Effluent II	+	+	-	+	+	+	-	+	-	55.7
Effluent III	+	+	-	-	-	+	+	+	-	44.4

%Occurrence<sup>a</sup> 100 83.3 66.7 83.3 33.3 83.3 66.7 100

11.1

a = % occurrence of individual isolate across the samples

b = % occurrence of total isolates from each sample

+ = Present and - = absent

## Discussion

The result of analysis of the impact of paper mill in Owerinta River showed that the river naturally had high coliform count, though the effluent impacted on it by increasing the count. This might be due to the presence of fecal microbial and biodegrading organisms in the effluent discharged from the industries into the River. This is supported by the report of Cabral (2006). The trend shown by the coliform counts of the different effluents implied that highest coliform discharge resulted from Effluent -I, followed by Effluent - II and then Effluent - III. The trend of coliform counts of water samples showed that the most impacted was the discharge point followed by the downstream. This might be due to the discharge of contaminated effluents at the discharge point and possible gradual recovery of the River at the downstream from the contamination. This is agreement with the works of Rechenburg *et al.* (2006). Because these coliform counts were above the limits of USEPA Maximum Contaminant Levels (MCLS) of < 100cfu/ml in drinking water (USEPA,

2003), both the River and effluents might be sources of water borne pathogens and possibly lead to water borne diseases upon usage. This corroborates the report of Health Canada (2006).

*Escherichia coli* (100%) was isolated from all the River and effluent samples. This indicated the recent fecal contamination of both the River and effluent samples. This corroborates the reports of Health Canada (2006) and Cabral (2010). The result also shows that effluent samples might have contributed to the fecal coliform load of River water samples, since the industries discharged their effluents into the River. This is supported by Schaffter and Parriarx (2002).

*Klebsiella* spp (83.3%) are ubiquitous enteric pathogens in the environment and was isolated from all the River samples and two effluent samples. This corroborates the works of Grimont *et al.* (2005). *Shigella* spp (66.7%) are also members of *Enterobacteriaceae* and were isolated from all River samples and Effluent I. Its isolation from these samples is implicative of fecal contamination. This was in agreement with the reports of Stockbine and Maurelli (2005), and Tetteh and Beuchat (2003). The implication of the presence of *Shigella* spp. in the samples is the risk of possible outbreak of shigellosis. This is supported by the work of Emch *et al.* (2008).

The presence of *Salmonella* spp. in some samples might be due to contamination from sewage and runoffs from agricultural lands. This was supported by the reports of WHO (2008) and Arvanitidon *et al.* (2005) *Salmonella* spp. are responsible for Salmonellosis (Le Minor, 2003). *Proteus* spp. (33.3%) are enteric pathogens associated with feces of both animals and man. It might have been introduced into the River from industrial effluent discharge of Effluent –II. This is supported by the report of Wilson (2005). *Pseudomonas* spp. (83.3%) are ubiquitous in nature (Prescott *et al.*, 2005). Their presence in all the effluents and at the discharge point and downstream samples might be due to their

ability to degrade wide varieties of molecules (mineralization). They might be associated with the microbial breakdown of effluent chemical components. This corroborates the works of Nwaugo *et al.* (2006) and Amund (2000).

*Staphylococcus* spp. (66.7%) isolated from effluents (I and II), discharge point downstream samples implied possible contamination from effluent sources and bodies of people swimming and excavating sand at the River points. This is in agreement with the report of Kayser *et al.*(2005). Possible species of *Staphylococcus* that are pathogenic to man include *S. aureus*, *S. epidermidis* and *S. saprophyticus* (Prescott *et al.*, 2005).

*Bacillus* spp. (100%) were present in all the samples which might be because they survive in a wide range of environmental conditions. This was supported by Prescott *et al.* (2005). Some species of *Bacillus* might be pathogenic e.g. *B. anthracis* (Prescott *et al.*, 2005), while *Bacillus subtilis* and *B. cereus* might be involved in biodegradation of pollutants (Nwaogu *et al.*, 2008 and Nwaugo *et al.*, 2006).

The presence of *Citrobacter* spp. (11.1%) in the downstream sample might be due to discharges into the river from sewage, soil and food wastes (Frederiskseen and Sogaard, 2003). The presence of *Citrobacter* spp. especially *C. freundii* in the sample might imply possibilities of meningitis with high morbidity and mortality potentials to exposed individuals. This is supported by the work of Donovan *et al.* (2008).

### Conclusion

This study has implicated industrial wastewater as major source of water borne pathogens in freshwater systems which might expose users especially in Nigeria to related diseases and their consequences. Treatment of wastewater from industries should include disinfection to avoid transmission of pathogenic organisms to over dependent teaming population of users.

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