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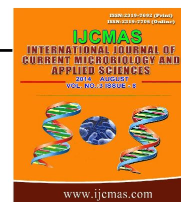

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Original Research Article

Distribution of Microorganisms in Water, Soils and Sediment from Abattoir Wastes in Southern Nigeria

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A B S T R A C T

This study was carried out to determine the distribution of microorganisms particularly those of public health importance in areas where abattoir activities are in operation. Abattoirs located at Egbu in Imo State and Trans-Amadi in Port Harcourt, Rivers State was selected for sampling using standard analytical methods. A total of twenty sampling points were established using Global Positioning System (GPS). Sampling was carried out both in wet and dry seasons. Result showed that soil samples from Trans-Amadi abattoir had highest levels of total coliform and total *Vibrio* counts of $2.9x10^7$cfu/g and $6.0x10^6$ cfu/g, respectively while surface water had the highest *Salmonella* and *Shigella* counts of $1.5x10^7$ cfu/ml. In Egbu abattoir, statistical analysis using ANOVA revealed a significant difference at 0.05 level in the total heterotrophic bacterial count from sediment and those from soil, waste water and surface water samples. The predominant bacterial genera identified were *Pseudomonas, Bacillus, Staphylococcus, Micrococcus, Lactobacillus, Streptococcus, Klebsiella, Vibrio, Salmonella, Escherichia coli, Citrobacter, Acinetobacter, Serratia, Proteus, Enterobacter, Shigella, Flavobacterium, Achromobacter* species. Pollution of water resources by abattoir wastes might lead to destruction of primary producers and this in turn leads to diminishing consumer populations in water. The consequences of such anthropogenic pollution can also lead to the transmission of diseases by water–borne pathogens. Therefore, continuous monitoring should be maintained in order to promote and maintain a safe working environment and ensure detection when abnormalities that could endanger both workers and environment occur.

Keywords

Abattoir wastes, Micro-organisms, soil, wastewater, sediment, public health

Introduction

The continuous drive to increase meat production for the protein need of the ever increasing world population has been accompanied by some pollution problems (Adesemoye *et al*., 2006; Nafarnda *et al*., 2012). In Nigeria, the abattoir industry is an important component of the livestock industry providing domestic meat supply to over 150 million people and employment opportunities for teaming population (Nafarnda *et al*., 2012). Adeyemi and Adeyemo (2007) reported that cities face

serious problems of high volume of wastes from abattoir due to inadequate disposal technologies and high cost of management. In Nigeria, adequate abattoir waste management is lacking in all public abattoirs such that large solid wastes and untreated effluents are common sites (Odeyemi, 1991; Adeyemo, 2002; Adebowale *et al*., 2010) unlike in developed countries where these facilities are adequately provided (Ogbonnaya, 2008). These abattoir wastes could be a source of embarrassment since conventional methods of waste management have been grossly neglected (Adedipe, 2002; Adeyemi and Adeyemo, 2007).

Wastewater or effluent generated from the abattoir is characterized by the presence of a high concentration of whole blood of slaughtered food animals and suspended particles of semi- digested and undigested feeds within the stomach and intestine of slaughtered and dressed food animals (Coker *et al*., 2001). In addition, there may also be the presence of pathogenic microorganisms, such as *Salmonella, Escherichia coli* (including serotype 0157:H7), *Shigella*, parasite eggs and amoebic cysts (Bull and Rogers, 2001; Adebowale *et al*., 2010) which are of public health importance.

Also, several pathogenic bacteria and fungi species has been isolated from abattoir wastewater and surface water; including *Staphylococcus, Escherichia coli, Streptococcus, Salmonella, Aspergillus, Mucor, Saccharomyces* and *Penicillium* species (Coker *et al*., 2001; Adesomoye *et al*., 2006; Adebowale *et al*., 2010). These pathogens might threaten public health by migrating into ground water or surface water; wind or vectors like animals, birds and arthropods can transmit diseases from these microorganisms (Mason, 1991; Meadows, 1995; Gauri, 2004 Raheem and Morenikeji, 2008).

Bacteria from abattoir waste discharged into water columns can subsequently be absorbed to sediments, and when the bottom stream is disturbed, the sediment releases the bacteria back into the water columns presenting long term health hazards (Sherer *et al*., 1992; Nafarnda *et al*., 2012). Pathogens present in animal carcasses or shed in animal wastes may include rotaviruses, hepatatitis E virus, *Salmonella* spp., *E.coli* 0157:H7, *Yersinia enterocolitica*, *Campylobacter* spp., *Cryptosporidium parvum*, and *Giardia lamblia* (Sobsey *et al*., 2002). Fecal wastes from domestic livestock in the abattoir are excreted on the floors of the animal pens, the accumulation of these fecal materials act as a collection basin for pathogenic microorganisms which may spread between animals and man leading to zoonoses (Adeyemo *et al.,* 2002). These zoonotic pathogens can exceed millions to billions per gram of feces, and may infect humans through various routes such as contaminated air, contact with livestock animals or their waste products, swimming in water impacted by animal feces, exposure to potential vectors (such as flies, mosquitoes, water fowl, and rodents), or consumption of food or water contaminated by animal wastes (Armand-Lefevre *et al*., 1998; Schlech *et al*., 2005).

In Rivers state, wastes generated from a slaughterhouse in Trans-Amadi abattoir, Port Harcourt, Nigeria are channeled directly into one of the tributaries of the River Niger. This act could introduce enteric pathogens e.g. *Bacillus* sp., *Escherichia* sp., etc and excess nutrients into the river, resulting to eutrophication (Odeyemi, 1991; Adeyemo *et al.,* 2002). These consequences of anthropogenic pollution during abattoir operations can lead to the transmission of diseases by water–borne pathogens, eutrophication of water bodies,

accumulation of toxic or recalcitrant chemicals in the soil, destabilization of ecological balance and negative effects on human health (Amisu *et al.,* 2003; Nafarnda *et al*., 2012). Abu-Ashour *et al* (1994) revealed that some bacteria also possess the ability to attach to solid/substrate surfaces by electrostatic hydrogen bonding and hydrophobic interactions. After attachment, they secrete slimy materials that can attract other organisms and nutrients to the interface. Attachment to surfaces benefits microorganisms in several ways both on nutritional and survival basis which invariably enhances bioremediation (Abu-Ashour *et al*., 1994). This study therefore is aimed at assessing the distribution of microorganisms in the various location sites where abattoir operations are carried out, considering the environmental and public health implications.

Materials and Methods

Study Area

The study was carried out in abattoirs located at Egbu in Imo State; and Trans-Amadi in Port Harcourt, Rivers State. Egbu lies within longitude 05° 28.432′ - 05° 29.802′N and latitude 007° 03.200′ - 007° 04.215′E (Fig 1). This area Egbu has a tropical climate. The average relative humidity is about 80%. The inhabitants of the areas are mainly farmers, civil servants, petty traders and casual workers.

Port Harcourt is located on longitude 4° 48.442′ - 4° 49.444′N and latitude 007° 02.303′ – 007° 03.545′E. The climate of Port Harcourt falls within the sub equatorial climate belt. Temperature and humidity are high throughout the year. The area is marked by two distinct seasons, the wet and dry seasons, with 70% of the annual rain fall between April and August, while 22% is spread in the three months of September to November. However, the driest months are from December to March. The river located at the abattoir in Egbu in Imo state is popularly called the Otamiri River while the river in Trans Amadi abattoir in Port Harcourt is called the Oginigba creek.

Sampling Points

A total of twenty (20) sampling points were considered for the study. The sampling stations, sampling points' codes, sampling points' coordinates and types of samples collected are presented in Table 1. During sample collection, Global Positioning System (GPS) machine (Model GPS 76) was used for the location of the sampling points.

Collection of Samples

Soil Samples

Soil samples were collected from four different sampling points coded A, B, C and D from a depth of 0-15cm using soil auger. About 500g of bulked composite soil samples was collected from points A, B and C; then prepared using the method of Ekundayo and Obuekwe (1997). Soil sample from point D, which is about 400m from Egbu and Trans Amadi abattoirs served as control sample. The soil samples were collected into labeled polyethylene bags and transported to the laboratory in a cooler packed with ice blocks for analysis.

Surface Water samples

Surface water samples were collected using the method of Odokuma and Okpokwasili (1993). The collection was carried out using 4 litre plastic bottles previously sterilized with 70% alcohol 24 hours before the final collection. The bottles were rinsed 3 to 4 times with the water sample before the final

collection. The water samples were collected along the course of the river at two different points coded A and B. Point A is the immediate point of discharge of the abattoir wastes into the river, Point B is about 400m upstream from Point A. The sample from point A served as the test sample while that from point B served as the control sample. To collect the water sample, base of the sterilized sample bottle was held with one hand, the bottle was plunged about 30cm below the water surface with the mouth of the sample bottles positioned in an opposite direction to water flow. The bottle was filled with water sample leaving a gap of about 2cm and covered immediately as described by Onyeagba and Umeham (2004). Immediately after collection, the samples were labeled and transported to the laboratory in a cooler packed with ice blocks for analysis.

Sediment samples

Sediment samples were collected from the same sampling points where surface water samples were collected using a grab sampler. The sediment sample was scooped from the grab's cup and transferred into sterile sample bottle. The sample was labeled and then transported to the laboratory in a cooler packed with ice blocks for analysis.

Waste water Samples

Waste water samples were collected using the method of Adesemoye *et al.* (2006). Sterile 2.0 litre sample bottles were used to aseptically draw part of the abattoir waste water. The samples were collected at four different points coded A, B, C and D as the waste water was running off the drainage system. About 500ml of the sample collected from each point were pooled together to get a composite sample. Control samples were collected from water stored in buckets used for washing meat and utensils in the abattoirs. The samples were placed in a cooler containing ice blocks and transported immediately to the laboratory for analysis.

Preparation of Samples

Sediment and Soil samples were processed using the method of Adesemoye *et al*. (2006). Ten grams of the soil sample was weighed and added to 90ml of sterile distilled water to get an aliquot, similarly, ten grams of the sediment sample was added to 90ml of sterile distilled water to get an aliquot. One milliliter of the aliquots, waste water and surface water samples were then serially diluted using the ten-fold serial dilution method as described by Prescott *et al* (2005).

Microbiological Analysis

The presence of various microorganisms in the water samples from Otamiri River in Imo state and Oginigba creek from Trans Amadi abattoir in Port Harcourt were identified using standard procedures. One milliliter each of the waste water samples was separately added to 9 ml of 0.1% peptone water diluents to give a 10^{-3} dilution. After thorough shaking further serial 10- fold (v/v) dilutions were made by transferring 1 ml of the original solution to freshly prepared peptone water diluents to a range of 10^{-3} dilutions. Aliquots (0.1 ml) of various dilutions were transferred to plates of surface dried Nutrient agar in duplicate and inoculated by spreading with flamed glass spreaders and incubated at 37^{0}C for 24 hours. Aerobic bacteria were subjected to further identification according to determinative schemes of Cowan and Steel (1994).

Total heterotrophic bacterial counts: This was determined with the nutrient agar using the spread plate technique as described by Prescott *et al* (2005). Here 0.1ml of the serially diluted samples was each inoculated onto different sterile nutrient agar plates in triplicates. The plates were incubated for 24 hours at 37°C. After incubation, colonies that appeared on the plates were counted and the mean expressed as cfu/ml for surface water, wastewater and cfu/g for soil and sediment samples.

Total coliform counts: The method of Prescott *et al*. (2005) was adopted where 0.1 milliliter of the serially diluted samples were each inoculated onto different sterile MacConkey agar plates in triplicates, the inoculums were then spread evenly on the surface of the media using a sterile spreader. This was followed by incubation at 37°C for 24 hours, after which the colonies were counted and the mean total coliform count expressed as cfu/ml and cfu/g as applicable.

Total *Salmonella-Shigella* counts

This was determined with the *Salmonella-Shigella* agar using the spread plate method as described by Prescott *et al*. (2005). One milliliter of the serially diluted samples was inoculated onto sterile pre-dried *Salmonella-Shigella* agar plates in duplicates. The inocula were then spread evenly on the surface of the media using a sterile spreader. The plates were then incubated at 37°C for 24 hours, after which the colonies that developed were counted and the mean total *Salmonella-Shigella* counts recorded accordingly for water, sediment, soil and waste water samples.

Total *Vibrio* count

Total *Vibrio* count was determined with the thiosulphate citrate bile salt (TCBS) agar using the spread plate technique as described by Prescott *et al* (2005). One milliliter of the serially diluted samples were inoculated onto sterile pre-dried TCBS agar plates in triplicates and then spread evenly with a sterile bent glass rod. The plates were incubated at 37°C for 24 hours, after which the colonies that developed were counted and the mean recorded accordingly for surface water, wastewater, sediment and soil samples.

Total fungal counts

This was determined using the potato dextrose agar (PDA) onto which sterile streptomycin (50 mg/ml) had been added to suppress bacterial growth (Okerentugba and Ezereonye, 2003). The spread plate technique as described by Prescott *et al* (2005) was adopted. An aliquot (0.1ml) of the serially diluted samples were inoculated in triplicates onto sterile pre-dried PDA plates and then spread evenly with a sterile glass spreader. The plates were incubated at room temperature for about 3-5 days after which the colonies were counted and the mean of the count recorded accordingly.

Total hydrocarbon utilizing bacterial counts.

The population of hydrocarbon utilizing bacteria was determined by inoculating 0.1ml aliquot of the serially diluted samples onto mineral salt agar media using the spread plate technique as described by Odokuma (2003). The vapour phase transfer method of Mills and Colwell (1978) was adopted. It employed the use of sterile filter paper discs soaked in filter-sterilized crude oil which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were then aseptically transferred to the inside covers of the inoculated petri dishes and incubated for 5 days at room temperature. After the incubation period, mean of the colonies were recorded.

Fig.1 Map of Imo and Rivers states showing the study areas

Table.1 Identification of Sampling stations, points, coordinates and sample types in the study areas

Sampling Stations	Sampling Points	Sampling point Co-ordinates		Types of Samples
		Northing (N)	Easting (E)	
Egbu Abattoir I	A	05^{0} 28.432$^{/}$	007^{0} 03.200$^{/}$	Soil (Test sample)
	B	05^{0} 28.441$^{/}$	007^{0} 03.209$^{/}$	Soil (Test sample)
	C	05^{0} 28.582$^{/}$	007^{0} 03.312$^{/}$	Surface water and Sediment (Test samples)
	D	05^{0} 28.559$^{/}$	007^{0} 3.231$^{/}$	Soil (Control sample)
Egbu Abattoir II	A	05^{0} 29.651$^{/}$	007^{0} 04.205$^{/}$	Waste water
	B	05^{0} 29.668$^{/}$	007^{0} 04.215$^{/}$	Waste water
	C	05^{0} 29.705$^{/}$	007^{0} 04.285$^{/}$	Waste water
	D	05^{0} 29.802	007^{0} 04.918$^{/}$	Waste water
Otamiri River	A	$05^{0/}$28.426'	007^{0}03.179'	Surface water and Sediment(Test sample)
	B	05^{0}27.423'	007^{0}04.156'	Surface water and Sediment (control)
Trans-Amadi Abattoir I	A	04^{0} 48.886$^{/}$	007^{0} 2.707$^{/}$	Soil (Test sample)
	B	04^{0} 48.782$^{/}$	007^{0} 2.608$^{/}$	Soil (Test sample)
	C	04^{0} 48.615$^{/}$	007^{0} 2.405$^{/}$	Surface water and Sediment (Test samples)
	D	04^{0} 48.442$^{/}$	007^{0} 2.303$^{/}$	Soil (Control sample)
Trans- Amadi abattoir II	A	04^{0} 49.789$^{/}$	007^{0} 03.801$^{/}$	Waste water
	B	04^{0} 49.628$^{/}$	007^{0} 03.702$^{/}$	Waste water
	C	04^{0} 49.522$^{/}$	007^{0} 03.665$^{/}$	Waste water
	D	04^{0} 49.444	007^{0} 03.545$^{/}$	Waste water
Oginigba Creek	A	$04^{0/}$50.001'	007^{0}04.425'	Surface water and Sediment (Test samples)
	B	04^{0}50.111'	007^{0}04.225	Surface water and Sediment (Control)

Total hydrocarbon utilizing fungal counts

Total hydrocarbon utilizing fungi was determined by inoculating 0.1ml of the serially diluted samples onto mineral salt agar using the method of Odokuma (2003). Eight hundred milliliter of the mineral salt medium was supplemented with 70mg of Aureomycin hydrochloride in 200ml of sterile distilled water (Odokuma, 2003). The vapour phase transfer method of Mills and Colwell (1978) was adopted. It employed the use of sterile filter paper discs soaked in filter-sterilized crude oil which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were then aseptically transferred to the inside covers of the inoculated petri dishes and incubated for 5-10 days at room temperature. After the incubation period, mean of the colonies for the triplicate plates were calculated and recorded accordingly.

Results and Discussion

The results of the microbial counts obtained during the study period for Egbu abattoir in the rainy season are presented in Table 1 for the test and control samples respectively. Total heterotrophic count of 2.5×10^6 cfu/g was obtained from the sediment sample collected from Otamiri River in Egbu while the waste water sample had the least observed count of 1.2×10^6 cfu/ml during this period. Results further showed that the total fungal count of 6.0×10^5 cfu/g was obtained for the sediment sample which was observed to be significantly different from those obtained from soil, waste water and surface water samples (Table 1). Statistical analysis using ANOVA revealed a significant difference at 0.05 level of significance in the total heterotrophic bacterial count from sediment and those from soil, waste water and surface water samples.

Total hydrocarbon utilizing bacterial counts gave the highest and least values of 7.4×10^5 cfu/ml and 3.0×10^5 cfu/ml from surface water and waste water, respectively. While there was no significant difference in the total hydrocarbon utilizing bacterial count values obtained for sediment and waste water samples, there were significant differences between total hydrocarbon utilizing bacterial count from surface water and that from waste water, sediment and soil at 0.05 level of significance. Total hydrocarbon utilizing fungal count recorded the highest and least values of 2.5×10^5 cfu/ml and 1.8×10^5 cfu/g, respectively from surface water and soil samples. There was no significant difference observed in the values obtained from soil, sediment, waste water and surface water. There were significant differences in the total coliform counts from the soil and surface water samples analyzed accounting for the highest and lowest values of 2.0×10^6 cfu/g and $1.2 10^6$ cfu/ml, respectively. *Salmonella* and *Shigella* counts revealed that there was no significant difference between values of 1.0×10^6 cfu/g and 9.0×10^5 cfu/ml obtained for soil and waste water, respectively. Likewise there was no significant difference between the counts from sediment and surface water samples. Analysis also showed that while there was no significant difference in the total *Vibrio* counts of 3.0×10^5 cfu/g and 3.5×10^5 cfu/g obtained from soil and sediment, respectively, significant difference existed between these counts and that obtained from surface water.

From the test results in the dry season, soil sample was found to be richer in total heterotrophic bacteria while surface water had the least (Table 2). Results of analysis of variance (ANOVA) indicated a significant difference in the total heterotrophic bacterial count at 0.05level of significance between the samples analyzed. There was no significant difference in the total fungal counts of 3.0×10^5 cfu/g and 3.2×10^5 cfu/g from soil and sediment samples, respectively. Surface water had the highest fungal count of 6.0×10^5 cfu/ml, which was found to be significantly different from the values obtained from other samples. Total hydrocarbon utilizing bacterial count was found to have the highest value of 1.0×10^6 cfu/g in the sediment sample which was revealed through statistical analysis (ANOVA) at 0.05 levels to be significantly different from the values obtained for soil, surface water and waste water samples. Total hydrocarbon utilizing fungal count was observed to be more in the surface water with a value of 2.5×10^5 cfu/ml, while waste water had the least value of 8.0×10^4 cfu/ml. Analysis of variance (ANOVA) also showed a significant difference in the values obtained.

Table.1 Microbial counts of samples contaminated by Egbu abattoir wastes for Rainy season

SAMPLES	THBC		TFC		THUB		THUF		TCC		SSC		TVC	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
SOIL (cfu/g)	c 1.30X10^{6}	1.6X10^{6}	b 4.5X1O^{5}	4.0X10^{5}	b 5.4X10^{5}	3.0X10^{5}	a 1.8X10^{5}	2.5X10^{5}	a 2.0X10^{6}	8.0X10^{5}	a 1.0x10^{6}	3.0X10^{5}	a 3.0x10^{5}	2.5X10^{5}
SEDIMENT (cfu/g)	a 2.5x10^{6}	2.0X10^{6}	a 6.0x10^{5}	5.1X10^{5}	c 3.5x10^{5}	3.0X10^{5}	a 2.0x10^{5}	3.0X10^{5}	b 1.5x10^{6}	1.0X10^{6}	b 4.0x10^{5}	3.0X10^{5}	a 3.5x10^{5}	3.0X10^{5}
WASTE WATER (cfu/ml)	c 1.2x10^{6}	9.5X10^{5}	c 2.5x10^{5}	3.0X10^{5}	c 3.0x10^{5}	2.5X10^{5}	a 2.0x10^{5}	2.5X10^{5}	d 1.0x10^{6}	6.0X10^{5}	a 9.0x10^{5}	0.00	c 0.00	0.00
SURFACE WATER (cfu/ml)	b 2.0x10^{6}	1.5X10^{6}	b 4.0x10^{5}	3.0X10^{5}	a 7.4x10^{5}	0.00	a 2.5x10^{5}	2.0X10^{5}	c 1.2x10^{6}	7.0X10^{5}	b 3.0x10^{5}	3.0X10^{5}	b 1.3x10^{5}	0.00

Means in the same column with the same letter are not significantly different at 5% level of significance according to LSD test.

Table.2 Microbial counts of samples contaminated by Egbu abattoir wastes for Dry season

SAMPLES	THBC		TFC		THUB		THUF		TCC		SSC		TVC	
	Test	control	Test	control	Test	control	Test	control	Test	control	Test	control	Test	control
SOIL (cfu/g)	a 2.8X10^{6}	1.9X10^{6}	b 3.0X10^{5}	3.0X10^{5}	c 3.6X10^{5}	3.1X10^{5}	b 1.8X10^{5}	1.0X10^{5}	b 1.5X10^{6}	1.1X10^{6}	a 8.0X10^{5}	4.0X10^{5}	b 2.5X10^{5}	3.0X10^{5}
SEDIMENT (cfu/g)	b 2.5X10^{6}	2.0X10^{6}	b 3.2X10^{5}	4.5X10^{5}	a 1.0X10^{6}	6.5X10^{5}	c 1.0X10^{5}	1.5X10^{5}	c 1.0X10^{6}	7.6X10^{5}	b 2.5X10^{5}	3.0X10^{5}	a 3.5X10^{5}	2.5X10^{5}
WASTE WATER (cfu/ml)	c 2.2X10^{6}	1.7X10^{6}	c 1.5X10^{5}	0.00	d 2.0X10^{5}	3.5X10^{5}	d 8.0X10^{4}	0.00	a 2.0X10^{6}	1.0X10^{6}	c 1.2X10^{5}	2.5X10^{5}	c 1.0X10^{5}	0.00
SURFACE WATER (cfu/ml)	d 2.0X10^{6}	1.5X10^{6}	a 6.0X10^{5}	2.5X10^{5}	b 7.4X10^{5}	5.0X10^{5}	a 2.5X10^{5}	2.5X10^{5}	d 3.4X10^{5}	5.0X10^{5}	c 1.0X10^{5}	3.0X10^{5}	c 1.0X10^{5}	0.00

Means in the same column with the same letter are not significantly different at 5% level of significance according to LSD test.

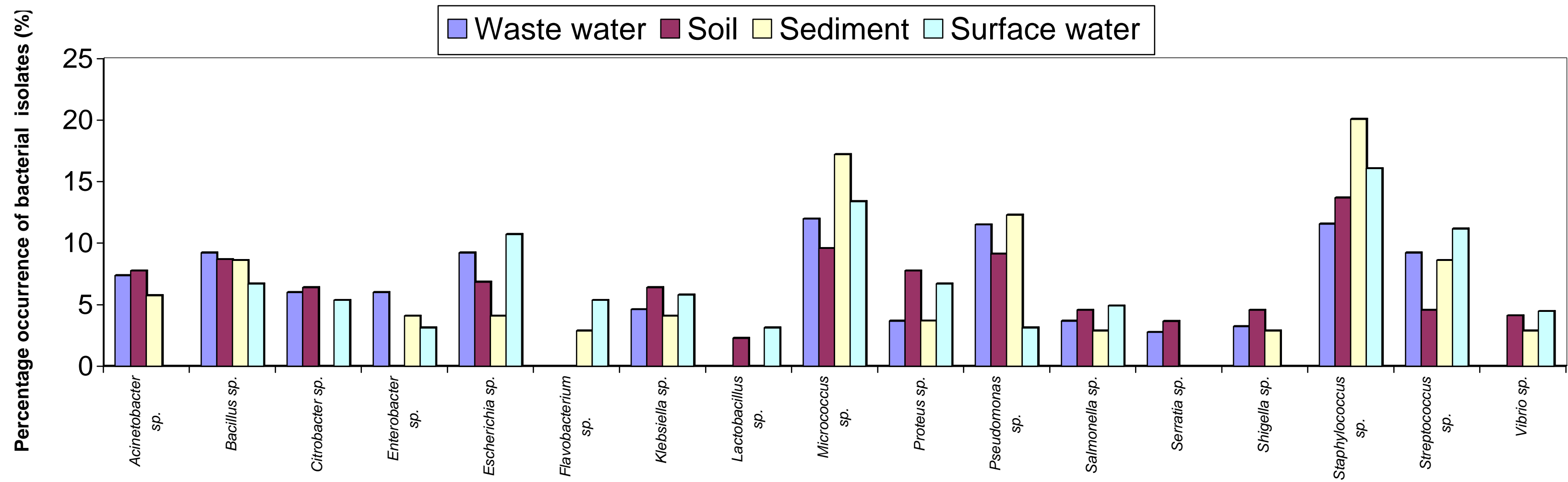

Fig.1 Percentage occurrence of bacterial isolates from samples contaminated by Egbu abattoir wastes for Rainy Season

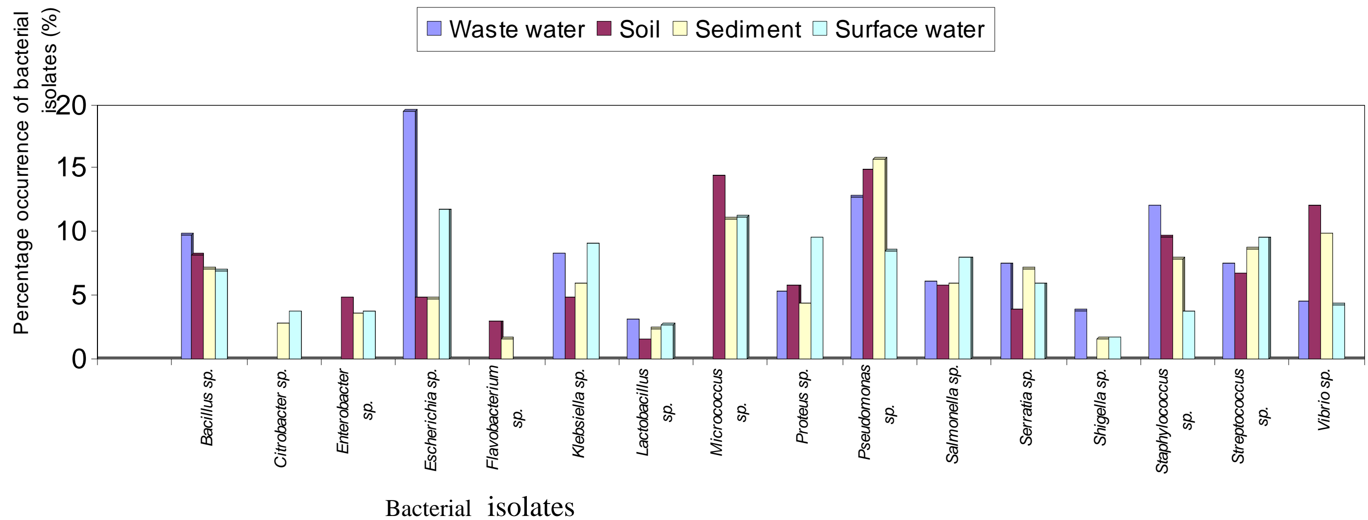

Fig.2 Percentage occurrence of bacterial isolates from samples contaminated by Egbu abattoir wastes for Dry Season

Table.3 Microbial counts of samples contaminated by Trans-Amadi abattoir wastes for rainy season

SAMPLES	**THBC**		**TFC**		**THUB**		**THUF**		**TCC**		**SSC**		**TVC**	
	Test	**Control**	**Test**	**control**	**Test**	**control**	**Test**	**control**	**Test**	**control**	**Test**	**control**	**Test**	**control**
SOIL (cfu/g)	a $2.8X10^{7}$	$2.2X10^{7}$	a $1.3X10^{7}$	$1.0X10^{7}$	b $5.2X10^{6}$	$3.5X10^{6}$	ba $1.1X10^{6}$	$2.0X10^{6}$	a $2.5X10^{7}$	$1.4X10^{7}$	b $9.5X10^{6}$	$4.0X10^{6}$	a $6.0X10^{6}$	$3.0X10^{6}$
SEDIMENT (cfu/g)	a $3.0X10^{7}$	$2.5X10^{7}$	b $8.0X10^{6}$	$5.0X10^{6}$	d $1.3X10^{5}$	$6.0X10^{6}$	a $2.0X10^{6}$	$2.0X10^{6}$	c $2.1X10^{7}$	$1.2X10^{7}$	c $6.0X10^{6}$	$4.0X10^{6}$	c $2.0X10^{6}$	$3.0X10^{6}$
WASTE WATER (cfu/ml)	a $2.1X10^{7}$	$1.0X10^{7}$	c $3.0X10^{6}$	$3.0X10^{6}$	c $3.3X10^{6}$	$2.5X10^{6}$	b $1.0X10^{6}$	0.00	d $1.5X10^{7}$	$5.0X10^{6}$	d $4.0X10^{6}$	$3.0X10^{6}$	c $1.0X10^{6}$	0.00
SURFACE WATER (cfu/ml)	a $2.8X10^{7}$	$2.4X10^{7}$	c $3.0X10^{6}$	$4.0X10^{6}$	a $1.0X10^{7}$	$6.0X10^{6}$	b $9.0X10^{5}$	$1.5X10^{6}$	b $2.5X10^{7}$	$8.0X10^{6}$	a $1.5X10^{7}$	$7.0X10^{6}$	b $3.6X10^{6}$	$3.2X10^{6}$

Means in the same column with the same letter are not significantly different at 5% level of significance according to LSD tes

Table.4 Microbial counts of samples contaminated by Trans-Amadi abattoir wastes for dry season

SAMPLES	THBC		TFC		THUB		THUF		TCC		SSC		TVC	
	Test	Control	Test	control	Test	control	Test	control	Test	control	Test	control	Test	control
SOIL (cfu/g)	a $3.0X10^{7}$	$2.5X10^{7}$	a $1.5X10^{7}$	$1.1X10^{7}$	d $3.2X10^{6}$	$3.0X10^{6}$	b $3.5X10^{6}$	$3.0X10^{6}$	a $2.9X10^{7}$	$1.2X10^{7}$	a $1.7X10^{7}$	$8.0X10^{6}$	a $5.0X10^{6}$	$3.5X10^{6}$
SEDIMENT (cfu/g)	a $2.8X10^{7}$	$2.2X10^{7}$	b $1.2X10^{7}$	$1.0X10^{7}$	a $1.4X10^{7}$	$6.0X10^{6}$	a $5.0X10^{6}$	$3.5X10^{6}$	a $2.5X10^{7}$	$1.0X10^{7}$	b $9.4X10^{6}$	$6.0X10^{6}$	a $5.0X10^{6}$	$3.0X10^{6}$
WASTE WATER (cfu/ml)	a $2.5X10^{7}$	$1.5X10^{7}$	c $3.5X10^{6}$	$3.0X10^{6}$	c $5.0X10^{6}$	$4.0X10^{6}$	c $2.0X10^{6}$	$2.0X10^{6}$	b $1.1X10^{7}$	$7.5X10^{6}$	c $2.5X10^{6}$	$3.0X10^{6}$	b $1.5X10^{6}$	0.00
SURFACE WATER (cfu/ml)	a $2.5X10^{7}$	$2.0X10^{7}$	c $3.0X10^{6}$	$3.0X10^{6}$	b $8.5X10^{6}$	$7.0X10^{6}$	d $1.8X10^{5}$	$2.0X10^{6}$	ab $2.0X10^{7}$	$9.0X10^{6}$	b $1.0X10^{7}$	$5.0X10^{6}$	b $2.1X10^{6}$	$3.0X10^{6}$

Means in the same column with the same letter are not significantly different at 5% level of significance according to LSD test.

While waste water had the highest value for total coliform count of 2.0×10^6 cfu/ml, surface water samples had the least count of 3.4×10^5 cfu/ml. It was also observed that waste water and surface water samples showed no significant difference in the S*almonella-Shigella* and total *Vibrio* counts. But while *Salmonella-Shigella* count had the highest value of 8.0×10^5 cfu/g from the soil samples, sediment sample had the highest *Vibrio* count of 3.5×10^5 cfu/g.
T-test revealed that there were significant differences between the total heterotrophic bacterial counts of test and control samples from sediment and surface water samples, soil and waste water samples. There was no significant difference however in the total fungal counts of tests and controls of sediment and soil samples from Otamiri River. While there was difference statistically at 0.05 level of significance using the t-test in the total hydrocarbon utilizing bacterial counts obtained from the test and control samples of sediment, soil and surface water.

Results of Microbial counts obtained from samples collected from Trans-Amadi abattoir during the rainy season are presented in Table 3. From the test results, sediment samples from Oginigba Creek in Trans Amadi had the highest values for total heterotrophic bacterial count and total hydrocarbon utilizing fungal count of 3.0×10^7 cfu/g and 2.0×10^6 cfu/g, respectively. Further analysis revealed that the soil and surface water had more and equal levels of total heterotrophic bacterial and total coliform counts than other samples. While the soil had higher levels of total fungal count and total V*ibrio* count of 1.3×10^7 cfu/g and 6.0×10^6 cfu /g, respectively, *Salmonella-Shigella* count had higher value of 1.5×10^7 cfu/ml in the surface water samples. In all, waste water had the least values for all the microbial groups enumerated. It was further ascertained through statistical analysis (ANOVA) at 0.05 confidence limit that while there was no statistical difference in the total heterotrophic bacterial counts values from the samples, there was a statistical difference in the values obtained for total hydrocarbon utilizing bacterial, total coliform and *Sallmonella-Shigella* counts. However, while there was statistical difference between total fungal and total *Vibrio* counts from soil and other samples, there was no statistical difference in the total fungal counts from the waste water and surface water samples. The same was applicable to the total *Vibrio* counts obtained from sediment and waste water.
T-test at 0.05 confidence limit showed that there was no statistical difference in the total heterotrophic and total hydrocarbon utilizing bacterial counts from test and control of sediment samples from Oginigba Creek, while there was a significant difference in the counts from soil samples, surface water and waste water samples. Total fungal counts from sediment and soil samples showed significant difference, while that from surface water and waste water samples did not show any significant difference.

From the Trans Amadi abattoir, the soil samples had more total heterotrophic bacteria, total fungi and total *Vibrio* counts of 3.0×10^7 cfu/g, 1.5×10^7 cfu/g and 5.0×10^6 cfu/g, respectively while total hydrocarbon utilizing bacteria had 3.2×10^6 cfu/g in the dry season. Lower counts of total heterotrophic bacteria, total fungi, total coliform, S*almonella-Shigella* and total V*ibrio* counts, were recorded for both sediment and surface water samples, while waste water also had low values for all the microbial groups enumerated within the same abattoir. There was statistical difference in the total coliform count value from waste water and those from soil and

sediment samples. Furthermore, total V*ibrio* counts from the soil and sediment showed no significant difference; likewise no significant difference existed between total V*ibrio* count from the waste water and that from surface water. Microbial count results also indicated statistical difference in the total hydrocarbon utilizing bacterial values obtained from test and control samples of sediment, while counts from test and control samples of soil, surface water and waste water did not show any significant difference.

Microorganisms are said to be ubiquitous and are known for essential functions which include; decomposition of organic materials, bioaccumulation of chemicals and biogeochemical cycling of elements. Their presence, abundance and growth in the environment are greatly influenced by factors such as pH, temperature, pressure, availability of nutrients and salinity (Ogbonna and Ideriah, 2014).

The result in Table 1 revealed that sediment from the Otamiri River (contaminated by Egbu abattoir) contained more total heterotrophic bacteria and fungi than the soil, waste water and surface water during the rainy season. This might be as a result of increased microbial load washed into the river from the soil by the rain and the fact that more nutrients are brought in by the rain through leaching of the soil which eventually settles at the bottom of the river, leading to increased nutrient levels which encouraged rapid multiplication of bacteria and fungi present. During the dry season, the soil had higher total heterotrophic bacterial count of 2.8×10^6 cfu/g than the other samples. This, according to Adesemoye *et al* (2006) could be as a result of destabilization of the soil ecological balance arising from contamination with abattoir wastes. Another possible reason for this high bacteria count in the soil during this period could be as a result of accumulation of wastes which are sources of microbial nutrients, since there was no rain to wash them off. The surface water had total heterotrophic fungal count of 6.0×10^5 cfu/ml which was higher than that from other samples. This could probably be due to high contamination of the river by air-borne fungal spores. Generally, the counts of bacteria and fungi from sediment, waste water and surface water samples from the test samples are higher than those from controls which signified contamination of the test samples by untreated abattoir wastes. However, counts of bacteria and fungi from test and control soil samples were almost equal which could be as a result of droppings from cattle that move around the abattoir and within the neighbourhood.

Coliforms were isolated from all the samples collected from the two abattoirs. The presence of this physiologic group in these samples is an indication of feacal contamination of the samples (Prescott *et al.,* 2005). This is possible since the cow dung is indiscriminately deposited within and around the abattoir. Through surface run-off, some of the feacal materials are carried to the nearby water body, leading to the presence of coliforms in such water body. Statistically, difference existed at 0.05 confidence limit in the level of total coliforms in all the samples from Egbu in the rainy and dry seasons, than the Trans-Amadi abattoir in the rainy season, this could be as a result of uneven contamination of these samples with fecal materials from the cows during these periods. However, the situation was not the same with the level of coliform counts in the samples from Trans-Amadi abattoir in the dry season. This could be as a result of even contamination of the samples with cow dung.

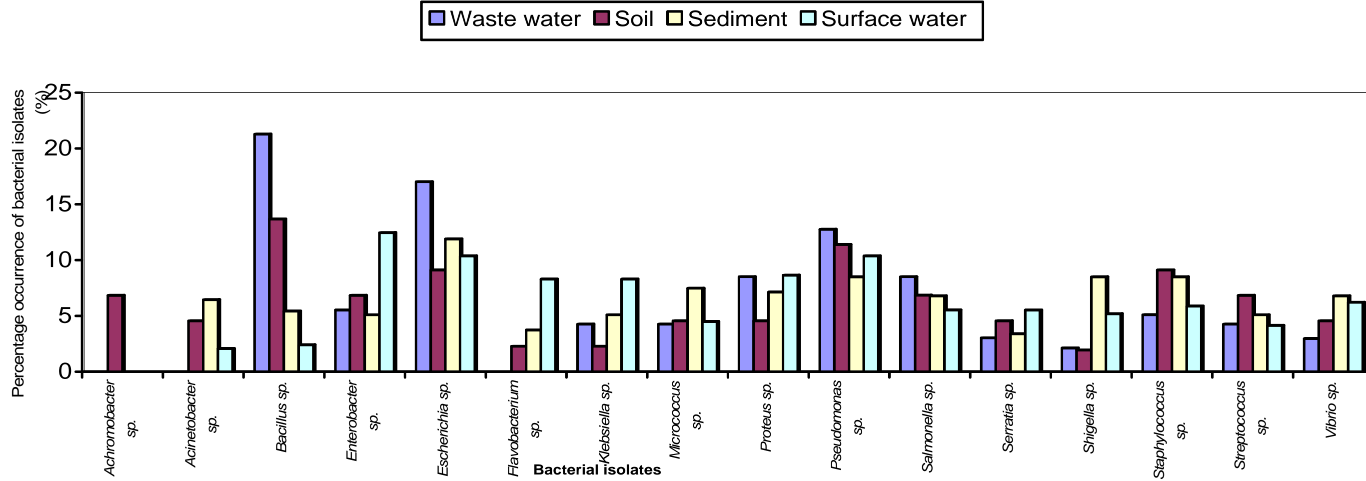

Fig.3 Percentage occurrence of bacterial isolates from samples contaminated by Trans Amadi abattoir wastes for rainy season

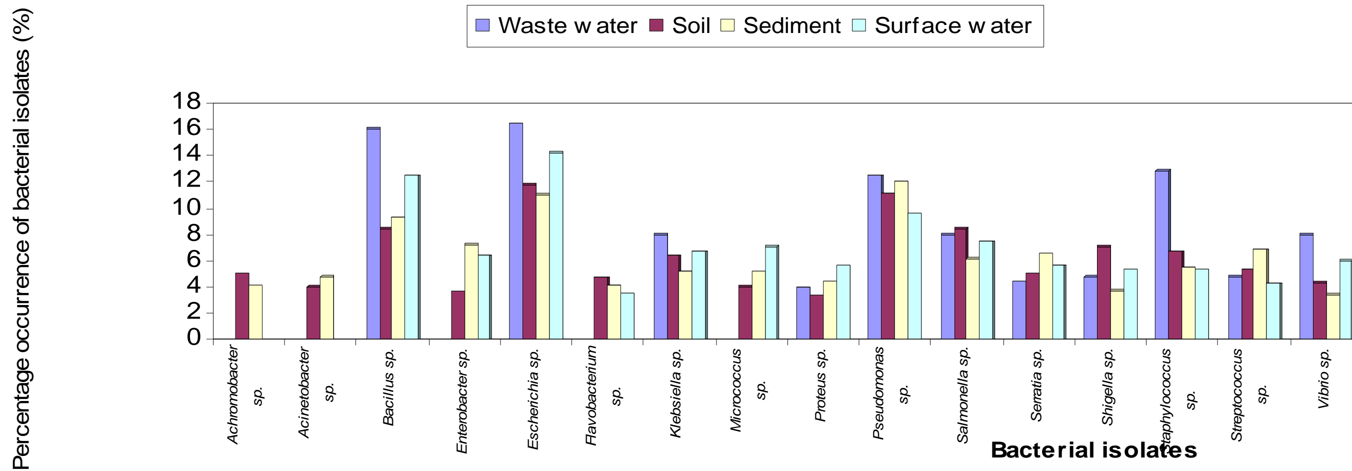

Fig.4 Percentage occurrence of bacterial isolates from samples contaminated by Trans Amadi abattoir wastes for dry season

Salmonella and *Shigella* were present in the samples collected from the abattoirs. Their presence was not astonishing since they co-habit with coliforms in the intestinal tract of warm blooded animals. Cow dung could be a good source of coliforms around the abattoirs. Statistically, there was little or no difference at $p < 0.05$ in the level of *Salmonella-Shigella* counts from all the samples collected from the Otamiri River and Ogingba creek abattoirs in the rainy and dry seasons. This could be as a result of even distribution of these organisms during these seasons. The soil had higher counts of 3.0×10^4cfu/g and 3.7×10^4 cfu/g, respectively within the periods. This could be due to random and uncontrolled deposition of wastes including cow dung within and around the abattoir environment. There was statistical difference at 0.05 confidence limit in the level of *Salmonella-Shigella* counts from samples collected both in the rainy and dry seasons from Trans-Amadi abattoir. Surface water from Oginigba Creek and soil samples had higher counts of 1.5×10^7 cfu/ml and 1.7×10^4 cfu/g in both the rainy and dry seasons respectively. This can be as a result of increased surface run-off from the abattoir into the river in the rainy season and accumulation of organic wastes including cow dung in the soil around the abattoir in the dry season respectively. However, there was much difference in the counts of *Salmonella-Shigella* obtained in the test and control samples from Trans-Amadi abattoir, with the test samples giving higher counts than the control samples. This is probably due to domestic activities that take place at different points around these abattoirs which can equally be a means of contaminating the environments outside these abattoirs with pathogenic organisms like *Salmonella* and *Shigella*.

Vibrio was isolated from most of the samples. This indicates that the sampling points were impacted by human activities. However, *Vibrio* was not isolated from waste water from Egbu abattoir during the rainy season. This is an indication that these samples were not impacted by human activities. It is equally possible that the samples were contaminated with organic wastes that were devoid of *Vibrio* species

The relatively high incidence of *Klebsiella, Enterobacter, Escherichia, Salmonella, Shigella, Citrobacter, Serratia* and *Proteus* around the test sampling points may be connected with high rate of cattle defecation near the sites. The introductions of wastes from the abattoir and the surface run-off into the sites and nearby rivers during the rains are also contributory factors (Ezeronye and Ubalua, 2005). The presence of these isolates in this study gives credence to these findings. The isolation of *E.coli* and other coliforms is an indication of recent human contamination of the sampling points, and is of great public health concern (Ezeama and Nwamkpa, 2002). The presence of *Bacillus* sp supports the finding by Ezeronye and Ubalua (2005). The organism is mostly a soil inhabitant and its presence could be as a result of contamination from overland run-off. The presence of *Pseudomonas, Acinetobacter, and Lactobacillus* around the abattoir is possible since they have been reported to be agents of meat spoilage (Frazier and Westhoff, 2003). Occurrence of *Pseudomonas* sp as a heterotrophic and hydrocarbon utilizing bacteria has been reported (Loureiro *et al*. 2005). The presence of *Pseudomonas* sp. within the abattoir environment is possible. This is probably due to the presence of hydrocarbons (PAHs) within the abattoir. This observation supports the report by Faria and Bharathi (2006) that *Pseudomonas* sp is widespread in the environment and concluded that they could contribute to the

oxidation of hydrocarbons in the environment. The same reason is applicable to *Achromobacter* and *Acinetobacter,* which according to Leahy and Colwell (1990) are among the hydrocarbon degraders. The incidence of *Staphylococcus* in this study is in agreement with the report by Chen *et al* (2001) who reported that *Staphylococcus* is naturally found in the hides of cattles. The recovery of *Flavobacterium* which is said to be authochthonous to the environment, agrees with the report of Austin (1988). The isolation of *Streptococcus* sp. and *Micrococcus* sp in this study agrees with the report of Adeyemo *et al* (2002), who isolated these organisms from abattoir environments.

Abattoir wastes pollution has adverse impacts on aquatic environment thus triggering algal blooms (eutrophication), depletion of dissolved oxygen, destruction of habitat and fish kills, thereby reducing the population of fishes and other aquatic organisms (Meadows, 1995; Chukwu *et al*., 2008). Some of the consequences of abattoir pollution are transmission of diseases by water borne pathogens, eutrophication of natural water bodies, accumulation of toxic or recalcitrant chemicals in the soil, destabilization of ecological balance and negative effects on human health (McLaughlin and Mineau, 1995; Sinha, 1997; Bridges et val., 2000; Boadi and Kuitunen, 2003; Amisu et al., 2003). Potential health risks from waterborne pathogens can exist in water contaminated by abattoir effluents (Cadmus *et al.,* 1999), runoff from feedlots (Miner *et al*., 1966), dairy farms (Janzen *et al*., 1974), grazed pastures (Doran and Linn 1979; Kunkel *et al*., 1983), fallow and sod amended with poultry litter (Giddens and Barnet, 1980), grassland treated with dairy manure (McCaskey *et al*., 1971), and sewage sludge treated land (Dunigan and Dick, 1980). Pollution of water resources by abattoir wastes might lead to destruction of primary producers and this in turn leads to diminishing consumer populations in water. The direct repercussion of this is diminishing fish yield hence human diet suffers. Therefore, continuous monitoring should be maintained in order to promote and maintain a safe working environment and ensure detection when abnormalities that could endanger both workers and environment occur.

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