

Isolation and Identification of Microorganisms Associated with the Poultry Environment

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ABSTRACT

Microbial assessment of water and air samples from Rufa'i Poultry Farm TalataMafara was carried out to investigate the pollution caused by the farm activities in the immediate environment. Water and air samples were collected during the rainy, early, and late dry seasons and were analyzed using standard methods. The Microbiological analysis revealed eighty-three (83) isolates from the samples with 26 bacterial species. *Enterobacterspp* (11%), *Brochothrix spp* (10%), *Citrobacter freundi* (7.2%), *Staphylococcus aureus* (7.2%), *Escherichia coli* (6%) and *Shigella spp* (5%) were in higher percentage. Water had the highest colony count of 4.12×10^7 cfu/ml in the rainy season while air has the highest load of 6.04×10^2 cfu in the late dry season. Thirteen (13) species were identified from a total of 337 fungal isolates recovered from the samples. The isolates with a high percentage of occurrence are *Aspergillus flavus* (28%), *Trichoderma spp.* (17.2%) and *A .niger* (11%). Most of these isolates are pathogenic while others are potential pathogens that can pose severe health risks especially to immune-compromised individuals in particular. Therefore, adequate measures should be put in place to curb the indiscriminate release of effluents into the environment.

Keywords: Microbial, Pathogens, Environment and Pollution.

INTRODUCTION

In recent decades, the poultry industry has made tremendous adjustments to meet the increasing demand for a safe supply of meat and eggs and this growth has been accompanied by structural changes within the sector, characterized by the emergence and growth of "land-independent" (industrial) farming establishments, and the intensification and concentration of poultry operations (Maheshwari, 2013). The production of poultry products results in hatchery wastes, manure (bird excrement), litter (bedding materials such as sawdust, wood shavings, straw, and peanut or rice hulls), and on-farm mortalities, various kinds of packaging for feed and pesticides, used ventilation filters, unused/spoilt medications and used cleaning materials (IFC, 2007). Most of these by-products can provide organic and inorganic nutrients that are of value if managed and recycled properly. However, they also give rise to potential environmental and human health concerns as the sources of elements, vectors for insects and vermin, and pathogenic micro-organisms. Specific concerns that are well documented include degradation of nearby surface and/or groundwater, resulting from increased loading of nutrients such as nitrogen and phosphorus (and potassium in some locations). Air quality issues are less well understood and include the effect of ammonia(NH₃), hydrogen sulphide(H₂S), odour, and dust particulates containing bacteria, bacterial toxins and chicken skin debris emitted from poultry production facilities directly into the atmosphere (AKanni and Benson, 2014).

The huge amounts of faecal waste produced by chicken farming, together with feathers, bedding and dead chickens (litter), are difficult to manage in landfills or as compost. In addition, Storage of the waste or over-fertilization of land with the chicken manure can cause runoff into streams, rivers, canals, lakes and ponds. Soil can also be a source of other pathogens from the disposal of dead chickens or when chicken manure is stored nearby or spread on top of fields (Blue, 2017). Pollution

of soil and water with nutrients and pathogens are generally caused by poor manure management and occurs where manure is stored. Poultry facilities are a source of the odour and attract flies, rodents and other pests that create local nuisances and carry disease (Maheshwari, 2013).

Disease-causing microorganisms in manure such as *E. coli* and *Salmonella* may be present and these pathogens are most likely transported to water with surface runoff and erosion or by direct animal access to surface water. Streams and lakes water used for drinking and recreational purposes provide great opportunities for transmitting these pathogens (Hunt, 2010; Jactone, 2010 and Anon, 2017). Lovett *et al.* (1971) reported 17 different types of fungi in poultry litter from commercial poultry enterprises; poultry litter is an ideal medium for the development of fungi. The most abundant of these fungi included *Penicillium*, *Aspergillus*, *Scopulariopsis* and *Candida*. Mycotoxins are produced by *Aspergillus* species (Westlake and Dutton, 1985). Manure handling, storage, and utilization in livestock and poultry production results in a wide range of air emissions, which may create air quality concerns, which include odour, atmospheric visibility, and respiratory health issues (Jactone, 2010). There is a need to investigate the immediate environment of the poultry farm in order to found the possible microorganism resulting from the commercial production of poultry.

MATERIALS AND METHODS

Study Area

The study was conducted at Rufa'i poultry farm, which is situated in the Gurbi area (a village) in Talata Mafara Local Government Area located on latitude 12°10'0" n - 12°40'0" n and 5°50'0" e - 6°2'0" e. the town has an elevation of 1037 ft (311.1m) above sea level. the town lies on the southern edge of the major irrigation project fed by the Bakalori dam.

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Air Sampling Procedure

Prepared nutrient (NA) and Potato Dextrose agar (PDA) in nine (9) (replicates plates) for the growth of bacteria and fungi were exposed and placed at three strategic places within the farmhouse for 15 minutes (Sekulka, 2007 and Musa *et al.*, 2017). The Petri dishes were covered and transferred to the Microbiology and Mycology laboratories of Usmanu Danfodiyo University Sokoto for microbial analysis.

Water Sampling Procedure

Water samples were collected from three sampling sites at 100m intervals (upstream, midstream, and downstream). Samples were collected three times (August 2017 to January 2018) during the rainy, early dry, and late dry seasons (Adewale *et al.*, 2013). Water was collected using sterile open-necked bottles dipped into the running river. The bottles were filled then screw-capped tightly and transferred to the laboratory for analysis (Bezuidenhout *et al.*, 2002).

Analysis of Air and Water Samples

Bacteriological properties were determined using procedures described by Bezuidenhout *et al.* (2002). All air sampling plates were incubated at 37°C for 24hrs. Bacterial isolates obtained were identified using standard methods; Presumptive colonies were confirmed by gram staining and biochemical reactions. The water samples were tenfold serially diluted in nine test tubes before inoculation (from the 6th and 7th tubes) on the prepared media. The result of microbial load count was recorded and compared with WHO drinking/domestic water standard. Presumptive colonies were confirmed by gram staining and biochemical tests (Booth *et al.*, 2006).

Fungal Study

All air and water samples were inoculated onto potato dextrose agar (PDA) and incubated for five to seven days. The pure culture was obtained and maintained by sub-culturing the isolates on the primary plates onto fresh PDA plates and incubated at room temperature for 5 days (Jha, 1995). Identification of the fungal isolates was carried out by comparison with mycological ATLAS and microscopy using lactophenol cotton blue under x10 and x40 objective lens (Cheesbrough, 2000). The water quality result was compared with the World Health Organisation and EPA drinking/domestic water standards.

RESULT AND DISCUSSION

The microorganisms detected in the poultry environment were bacteria and fungi. Table 1 and Figure 1 show the frequency of occurrence of bacterial isolates recovered from the two sample sites. Eighty-three (83) isolates were recovered from the samples (water and air). Out of the 83, the rainy season had the highest isolates from the water samples 17 (20.4%) this is in accordance with the work of Suma *et al.* (2014), which could be due to the low or neutral pH of the water 7.8 which favours the growth of microorganisms and the least was 11(13.2%) in the early dry season. The highest isolates from the air were during the late dry season 16 (19.2%), which may be due to the airy and dusty weather which increase the population of the microbes in the atmosphere. Though air does not contain any nutrients to support the growth and survival of microorganisms it can act as an important medium for carrying and spreading biological agents (Peplow, 2014). The least was 13(15.6%) during the early dry season.

Table 1: Frequency of Occurrence of Bacterial isolates from Rufa'i Poultry Farm (Water & Air)

Isolates	Rainy		Early dry		Late dry		Isolates No (%)
	Water	Air	Water	Air	Water	Air	
<i>B. anthracis</i>	0	0	0	0	0	3(18.7%)	3(4)
<i>B. cereus</i>	0	1(7.1%)	0	1(7.6%)	0	1(6.2%)	3(4)
<i>B. firmus</i>	0	1(7.1%)	0	0	0	1(6.2%)	2(2.4)
<i>B. laterosporus</i>	0	0	0	0	2(16.6%)	0	2(2.4)
<i>B. licheniformis</i>	0	1(7.1%)	0	0	0	1(6.2%)	2(2.4)
<i>B. mycoides</i>	0	0	0	0	0	1(6.2%)	1(1.2)
<i>B. pumilus</i>	0	0	0	0	0	1(6.2%)	1(1.2)
<i>Bacillus circulans</i>	1(%)	0	0	0	0	0	1(1.2)
<i>Brochothrix spp.</i>	2(11.7%)	3(21.4%)	1(9.0%)	2(15.6%)	0	0	8(10)
<i>Citrobacter freundii</i>	3(17.6%)	1(7.1%)	2(18.1%)	0	0	0	6(7.2)
<i>Enterobacter spp.</i>	1(5.8%)	2(14.2%)	4(36.3%)	0	0	2(12.5%)	9(11)
<i>Escherichia coli</i>	3(17.6%)	0	0	2(15.3%)	0	0	5(6)
<i>Klebsiella pneumonia</i>	1(5.8%)	0	0	0	4(33.3%)	0	5(6)
<i>Kurthia spp</i>	0	0	0	1(7.6%)	1(8.3%)	0	2(2.4)
<i>Lactobacillus spp</i>	0	0	0	2(15.3%)	0	0	2(2.4)
<i>Listeria spp.</i>	0	0	2(18.1%)	0	1(8.3%)	1(6.2%)	4(5)
<i>Proteus vulgaris</i>	2(11.7%)	0	0	0	2(16.6%)	2(12.5%)	6(7.2)
<i>S. carnosus</i>	0	0	0	1(7.6%)	0	0	1(1.2)
<i>S. caprae</i>	1(5.8%)	0	0	0	0	0	1(1.2)
<i>Salmonella typhi</i>	0	0	0	2(15.3%)	0	1(6.2%)	3(4)
<i>Serratia marcescens</i>	1(5.8%)	0	0	0	0	1(6.2%)	2(2.4)
<i>Shigella spp.</i>	0	2(14.2%)	0	0	2(16.6%)	0	4(5)
<i>Staphylococcus scohnii</i>	0	0	2(18.1%)	0	0	0	2(2.4)
<i>Staphylococcus arlettae</i>	1(5.8%)	0	0	0	0	0	1(1.2)
<i>Staphylococcus aureus</i>	1(5.8%)	3(21.4%)	0	1(7.6%)	0	1(6.2%)	6(7.2)
<i>Staphylococcus capitis</i>	0	0	0	1(7.6%)	0	0	1(1.2)
Total	17(20.4%)	14(16.8%)	11(13.2%)	13(15.6%)	12(14.4%)	16(19.2%)	83

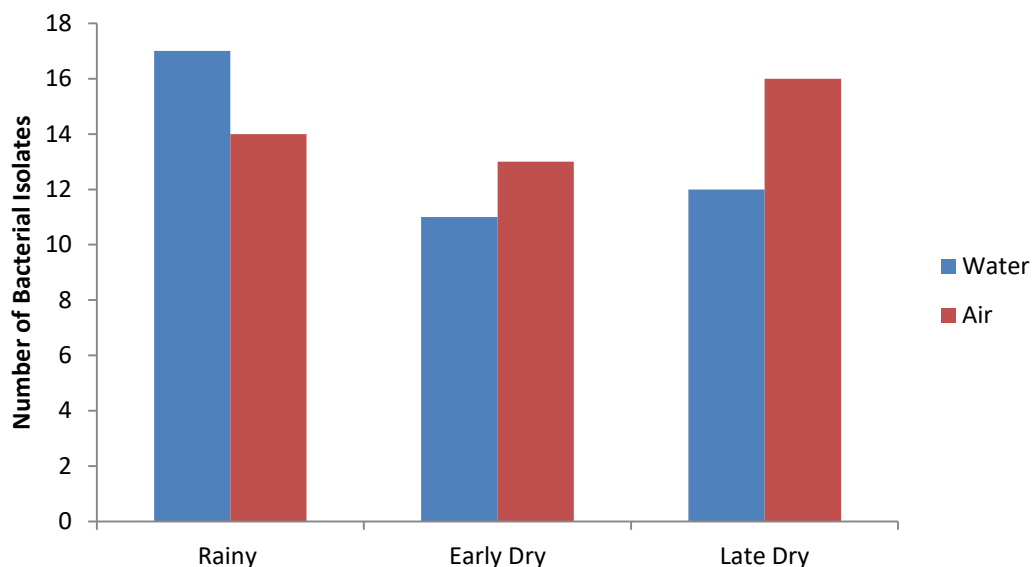


Figure 1: Frequency of Occurrence of Bacterial Isolates

The study demonstrated the occurrence of 26 heterotrophic bacterial species belonging to 15 genera from both water and air six(6) of the species were detected in the water samples (*B. laterosporus*, *B. circulans*, *K. pneumonia*, *S.caprae*, *S. cohnii*, and *S. arletteae*) while, 10 species were isolated from the air (*B. anthracis*, *B. cereus*, *B. firmus*, *B. licheniformis*, *B. mycoides*, *B. pumitis*, *Lactobacillus spp*, *S. capilosus*, *S. typhi* and *S. capitis*) and 10 from both air and water (*Brochothrix spp*, *C. freundi*, *Enterobacter spp*, *E. coli*, *Kurthia spp*, *Listeria spp*, *P. vulgaris*, *S.marcescens*, *Shigella spp* and *S. aureus*). Most of these species are pathogenic such as *K. pneumonia*, *Listeria spp*, *S. aureus*, *E. coli*, *Salmonella*, *Shigella spp*, *B. anthracis*, *B. cereus*, *B. pumitis*, *B. circulans*, *S.caprae*, and *S. cohnii*. Others are opportunist (*Brochothrix spp*, *C. freundi*, *Enterobacter spp*, *P. vulgaris*, *B. licheniformis*, *S.marcescens* and *S.capitis*). In water samples, 16 of these bacterial species such as *B. laterosporus*, *K. pneumonia*, *S. caprae*, *S. arletteae*, *Brochothrix spp*, *C. freundi*, *Enterobacter spp*, *E. coli*, *Listeria spp*, *P. vulgaris*, *Shigella spp*, and *S. aureus* were detected which indicated the incidence of water contamination as some of these species are indicators of faecal contamination (Webster *et al.*, 2004). *Enterobacter spp* were the most commonly isolated bacteria with 9 isolates (11%) and occurred in all three seasons but highest in the early dry season. The second most frequent isolate was *Brochothrix spp* 8(10%) and isolated more in the rainy season. Followed by *C. freundi* with 6 isolates (7.2%) that occurred mostly in the rainy season, *P. vulgaris* 6(7.2%) isolated most in the late dry. *S. aureus* 6(7.2%) was highest in the rainy season. *K. pneumoniae* 5(6%) was higher in the late dry season. *E. coli* 5(6%) occurred higher in the rainy season. with 3 isolates and the least in the early dry season. *Listeria spp* occurred 4(5%) but higher in the early dry season and *Shigella* was identified 4 (5%) with

2 isolates in the rainy season and 2 in the late dry season.

Heterotrophic count(HPC) measures a range of bacteria that are naturally present in the environment (EPA, 2002). The result of heterotrophic bacterial count from both water and air is presented in Table 2. The total bacterial counts for all the water samples were generally high exceeding the limit of 1.0×10^2 cfu/ml which is the standard limit of heterotrophic count for drinking water(EPA, 2002). The high total heterotrophic count is indicative of the presence of high organic dissolved salts in the water. These sources of bacterial contamination in this study include the poultry wastes which are channelled into the water body and surface runoff. Water samples from the rainy season had the highest microbial load 7.3×10^6 cfu/ml while the early dry season had the least 2.8×10^6 cfu/ml. From the air, the viable count ranges from 8.7×10^1 cfu to 1.72×10^2 cfu the highest microbial load was obtained during the late dry season while the least was in the early dry season.

The mean viable count of total heterotrophic bacteria from water and air ranges from 7.3×10^2 to 2.8×10^6 cfu/ml and 8.7×10^1 to 1.72×10^2 cfu respectively was all above WHO and EPA standard of 1.0×10^2 cfu/ml. The presence of such high bacteria counts and other indicator organisms such as *Staphylococcus aureus* indicate contamination of water source. Therefore, everything possible should be done to prevent pollution of the drinking water, special attention being given to the safe disposal of excrement and creating awareness especially for rural communities to be sure of the water they use for domestic activities. Environmental Protection Agency (EPA) establish heterotrophic plate count as a primary standard, which is based on health considerations.

Table 2: Total Heterotrophic Bacterial Colony Count (CFU) from Water and Air Samples

Seasons	Sample/Type	Colony (cfu)
Rainy	A/water	6 x 10 ⁵
	B/water	4.92 x 10 ⁷
	C/water	7.3 x 10 ⁶
	J/air	2.58x10 ²
	K/air	8.7x10 ¹
	L/air	5.22x10 ²
Early dry	D/water	3.32 x 10 ⁶
	E/water	4.12 x 10 ⁷
	F/water	4.26 x 10 ⁷
	M/air	6.1x10 ¹
	N/air	1.72 x10 ²
	O/air	2.21 x10 ²
Late dry	G/water	3.6 x 10 ⁷
	H/water	5.9 x 10 ⁷
	I/water	2.8 x 10 ⁶
	P/air	4.53 x10 ²
	Q/air	6.04 x10 ²
	R/air	3.63 x10 ²
WHO Standard	Drinking water	1.0 x 10 ²
EPA Standard	Drinking water	1.0 x 10 ²

Table 3 shows the variety of fungi isolated from the air and water samples during the three seasons (rainy, early and late dry seasons). Thirteen species (13) of fungi belonging to 8 genera and 4 classes (Eurotiomycetes, Sordariomycetes, Dothideomycetes and Zygomycetes) were identified from the samples. Eight (*A. flavus*, *A. oryza*, *B. westling*, *C. herbarum*, *F. solanum*, *P. italicum*,

S. fusca and *Trichoderma*) out of the 13 species were obtained in the water while seven were obtained in the air (*A. flavus*, *A. penicilloides*, *A. niger*, *F. oxysporium*, *M. racemosus*, *S. candida* and *Trichoderma spp*) and two (*A. flavus* and *Trichoderma spp*) were found in both water and air samples.

Table 3: Frequency of Occurrence of Fungi Isolated from Water and Air Samples

Isolates	Rainy		Early Dry				Late Dry				Isolates No (%)		
	Water		Air		Water		Air		Water			Air	
	No.	%	No	%	No	%	No	%	No	%		No	%
<i>A. flavus</i>	14	23.8	20	33.3	10	20	17	32.7	16	25.8	16	28.5	93(28)
<i>A. oryza</i>	4	8.5	0	0	6	12.2	0	0	5	8.0	0	0	15(4.4)
<i>A. penicilloides</i>	0	0	2	3.3	0	0	3	5.8	0	0	1	1.8	6(1.8)
<i>A. niger</i>	0	0	14	23.3	0	0	12	23	0	0	10	17.9	36(11)
<i>B. swestling</i>	1	1.7	0	0	5	10.2	0	0	3	4.8	0	0	9(2.6)
<i>C. herbanum</i>	10	16.9	0	0	5	10.2	0	0	8	12.9	0	0	23(6.9)
<i>F. solani</i>	7	11.9	0	0	5	10.2	0	0	10	16.1	0	0	22(6.5)
<i>F. oxysporium</i>	0	0	6	10.0	0	0	5	9.6	0	0	10	17.9	21(6.2)
<i>M. racemosus</i>	0	0	3	5.0	0	0	1	1	0	0	5	8.9	9(2.6)
<i>P. italicum</i>	7	11.9	0	0	4	8.7	0	0	5	8.0	0	0	16(4.7)
<i>S. fusca</i>	4	6.8	0	0	7	14.3	0	0	8	12.9	0	0	19(5.6)
<i>S. candida</i>	0	0	3	5.0	0	0	4	7.7	0	0	3	5.3	10(2.96)
<i>Trichoderma Spp.</i>	11	18.6	12	20	7	14.3	10	19	7	11.3	11	19.6	58(17.2)
Total	58	17.2	60	17.8	49	14.5	52	15.4	62	18.3	56	16.6	337

The fungi isolates (337) obtained in this study as shown in Table 3 and Figure 2 of the result indicated the late dry season had the highest number of isolates from the water sample, which may be due to the conducive environment with a moderate amount of water (balance condition) as the river always receives water from Bakalori dam, which allows it to have water all year round, in addition with the little breeze it makes an enabling environment for Fungal growth, this is contrary to the findings of Dilafroza *et al.* (2014) where a

maximum number of the fungal species was found during Rainy season. The least was seen in the early dry, which could be possibly due to the transition period from rainy season to dry season, therefore, microbial population falls.

While from the air, the concentration of the fungi varied considerably from season to season, this will be attributed to physical and chemical changes in the atmosphere. The rainy season has more isolates possibly

because fungi appreciate humid weather which makes the atmosphere damp like with water vapour, this was in accordance with the work of Dong *et al.* (2013) who stated that the levels of bacteria and fungi in the air are

significantly higher in the wet season than in dry season which is due to the optimal weather conditions and high humidity.

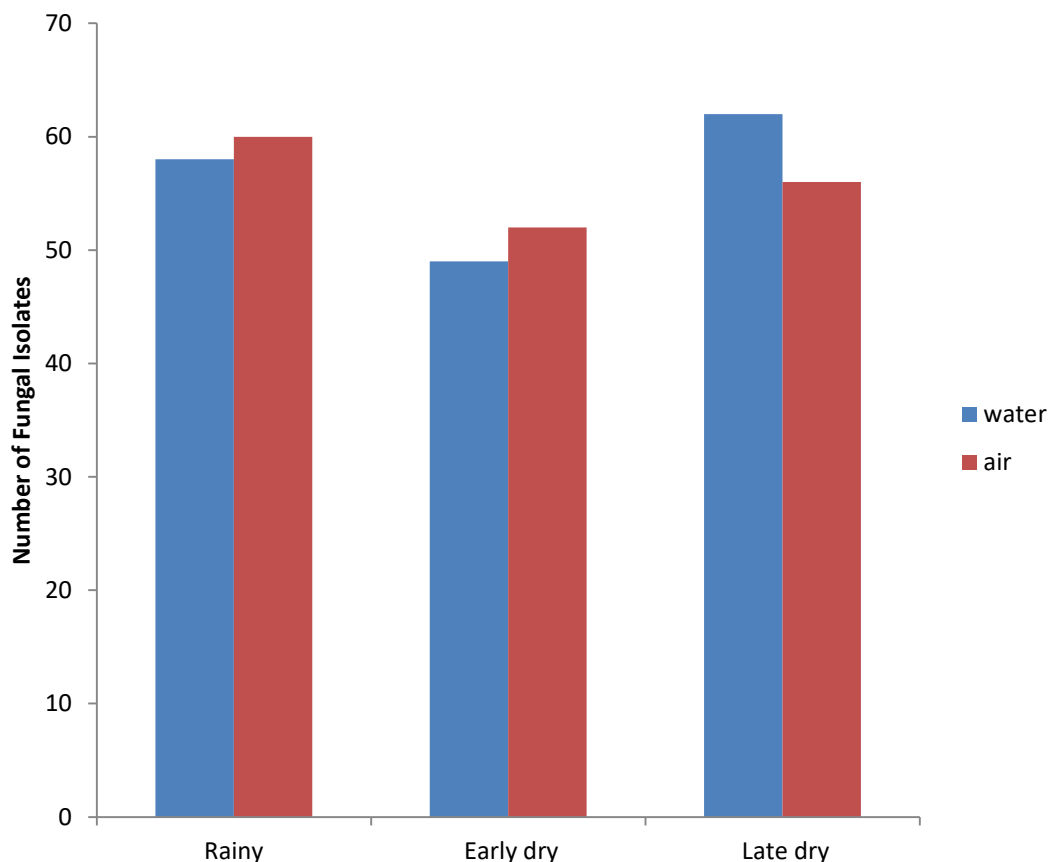


Figure 2: Frequency of Occurrence of Total Fungal Isolates

Fungi are more likely to be isolated from surface water (Hageskal *et al.*, 2006; Hageskal *et al.*, 2007) and this may be related to the larger amounts of organic matter in the surface water particularly as a lot of chicken waste is released into the water body of this study. From Figure 3, many of the fungi isolated from river water are known to be pathogenic, particularly *Aspergillus*. Warris *et al.* (2003) found that patients suffering from invasive aspergillosis were infected from either the air, water or both. The result shows that *A. flavus* has the highest percentage of occurrence 28% followed by *Trichoderma* 17%, *A. niger* 11%, *C. herbarum* 6.9%, *F. solani* 6.5%, *F. oxysporium* 6.2% also *S. fusca* 5.6%, *Penicilium italicum* (4.7%) *A. oryza* 4.4%, *S. candida* (2.9%), *B. westling* and *M. racemosus* each (2.6%) and the least isolates was *A. penicilloides* (1.8%). Figure 4 shows the percentage frequency of seven (7) fungi species detected in the Air, *A. flavus* has the highest frequency of (31.5 %), followed by *A. niger*(21.4%), *Trichoderma spp*(19.6%), *F. oxysporum* (12.5%), *S. candida*(6%), *M. racemosus*(5.3%) and *A. penicilloidea*(3.6%) of occurrence.

Conclusion

The result obtained on the investigation of microorganisms associated with the study area revealed that; it is detrimental to the community and populace

near or associating with the farm because medically important pathogenic microorganisms (bacteria and fungi) were found in both river and air, some of the bacteria obtained in high values are *Enterobacter*, *Proteus vulgaris*, *S. aureus*, *K. Pneumonia* and *E. coli*. The total heterotrophic bacterial counts in the three seasons were all above the WHO and EPA limit. Some of the Fungi found in high concentrations were *Aspergillus flavus*, *Trichoderma*, *A. niger*, *C. herbarum* all of these microorganisms can adversely affect human health once inhaled regularly and or ingested as the case may be, therefore the water and air quality are affected by the poultry operation. Seasons affect the growth and abundance of these microorganisms because the isolates and colony count significantly differ between the seasons with the rainy season having the higher number of bacterial isolates and higher colony count (cfu) .from the water whereas the late dry season has higher isolates from the air. Fungi isolates were higher in the late dry in the water sample but higher in the rainy season in the air. Air is also contaminated with harmful microorganisms emanating from chickens these emissions include odour, atmospheric visibility, and respiratory health issues. Therefore, Control of human activities, as well as safe disposal of excrement to prevent agricultural wastes from entering the water body, is the key to avoiding bacterial and fungal contamination of drinking

water and special attention should be given to creating awareness especially for rural communities to be sure of the water they use for domestic activities.

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