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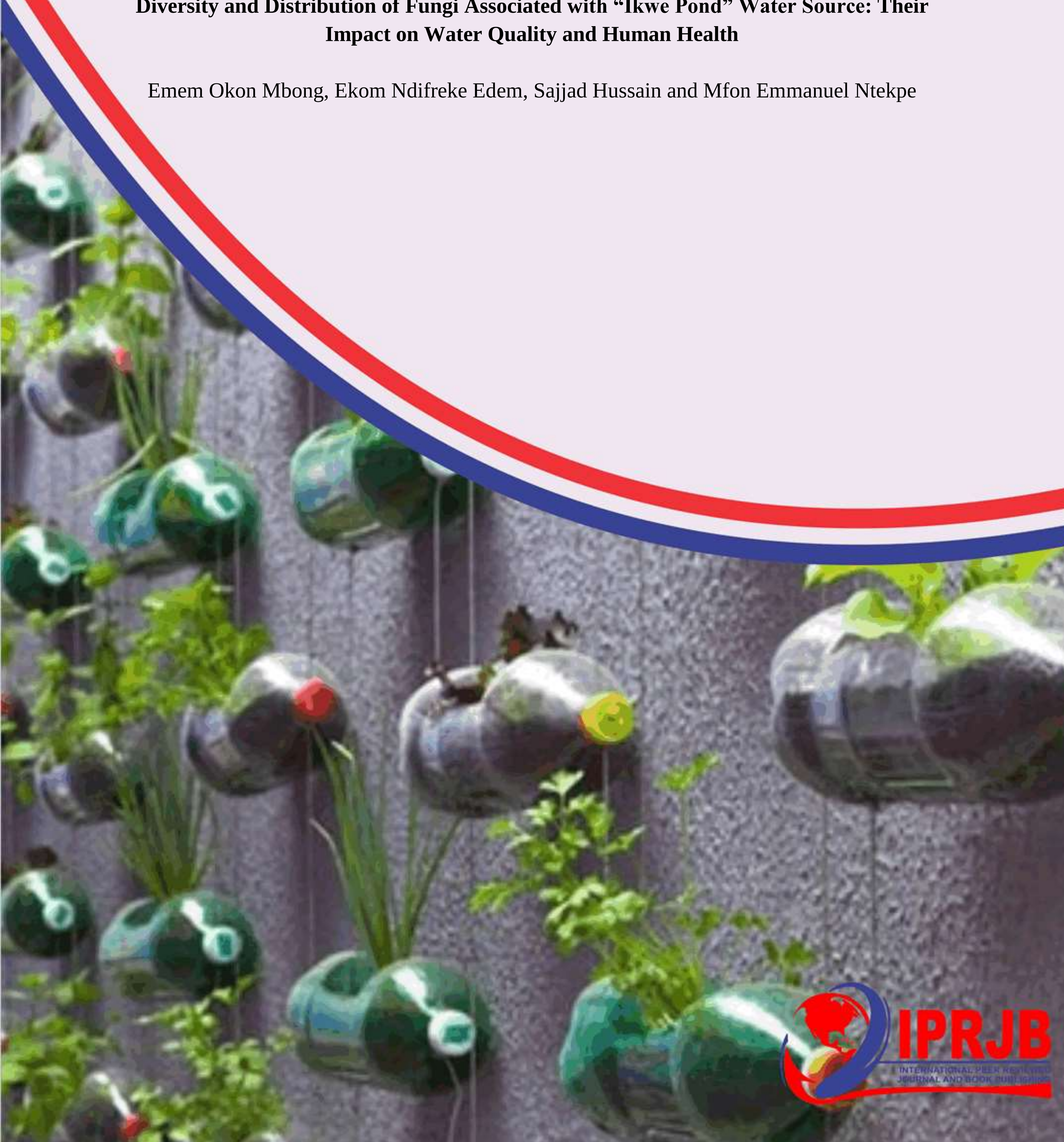

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Diversity and Distribution of Fungi Associated with "Ikwe Pond" Water Source: Their Impact on Water Quality and Human Health

[1]Emem Okon Mbong[1]*, Ekom Ndifreke Edem[2], Sajjad Hussain[3], Mfon Emmanuel Ntekpe[4]

[1]Department of Environmental Biology, Heritage Polytechnic, Ikot Udoata, Eket, Nigeria

[2]Department of Medical Microbiology, University of Uyo Teaching Hospital, Uyo, Nigeria

[3]Department of Microbiology, Harbin Medical University, Harbin, China

[4]Department of Microbiology, Heritage Polytechnic, Ikot Udoata, Eket, Nigeria

Abstract

Purpose: Fungal infections are becoming more and more important because of increasing numbers of immunosuppressed patients. Nonetheless, waterborne fungi are associated with variety of health related conditions. Fungi in the aquatic ecosystem perform essential functions in biogeochemical cycles. However, knowledge of fungal communities associated to a particular aquatic ecosystem is important. In the present study, we seek to investigate the fungal distribution and diversity in Ikwe Pond, located in Eket.

Methodology: A total of thirty different water points were sampled using 30 thoroughly washed 0.50L sterile plastic containers and cultured on SDA plates with 1ml of dilution factors 10^{-3} and 10^{-6} used as inoculums. Six fungal species namely *Candida tropicalis*, *Aspergillus niger*. *Cryptococcus* sp, *Absidia* sp, *Geotricium* sp, were isolated from the 30 sampling points.

Findings: Upstream recorded a total of 27 spores, midstream had 14 spores while downstream recorded 8 spores. Also the colony count revealed that upstream had the highest number of fungal spores count. Clearly, the upstream (59.3%) and the midstream (78.6%) sampling points were dominated by *Aspergillus niger* while the downstream water column was dominated by *Candida tropicalis* (50%).

Unique Contribution to Theory, Practice and Policy: In many countries, there has been little focus on the occurrence of fungi in water which can lead to severe health related issues but with monitoring on the frequency and distribution of fungi in our waters, this perceived health related complications can be checked.

Keywords: *Fungi, Ikwe pond, Upstream, Aquatic, Ecosystem, Health*

INTRODUCTION

Fungi are not only numerous they are also very diverse and ubiquitous among all ecosystems on earth, including aquatic environments. As decomposers, pathogens, and mutualistic symbionts with plants and animals, fungi play a major role in ecosystem processes including nutrient cycling, bioconversions, and energy flows. Fungi are globally distributed, but different species have distinctive geographical distributions that depend on hosts and climate. Fungal communities are being affected by global change, including climate change, land use change, pollution, pesticides and fertilisers, and movement of biota. The aquatic ecosystem comprise of highly diverse fungal groups including ascomycetes, basidiomycetes, chytridiomycetes, and zygomycetes (Shearer *et al*., 2007). Aquatic ecosystems, however, remain frequently overlooked as fungal habitats, although fungi potentially hold important roles for organic matter cycling and food web dynamics (Grossart *et al*., 2019).

There are more than 600 species of fungi found in the aquatic ecosystem at different regions (Wong *et al*., 1997). Geographical occurrence of aquatic fungi is significantly different between countries, for example, about 548 ascomycetes have been reported from freshwater habitats, mostly from Europe, North America, and South-East Asia (Raja *et al*., 2009).

Aquatic fungi play an essential role in ecosystems, which has been thoroughly reviewed by Krauss *et al*., (2011). Aquatic environments harbor many taxonomically unknown fungi, for example, a new fungal phylum Cryptomycota was recently discovered from the sediment in a pond (Jones *et al*., 2011). Aquatic fungi have various ecological roles: some are major decomposers of organic matters in marine ecosystems; some are parasites, pathogens, and mutualists with other marine organisms (e.g., algae and animals) (Hyde *et al*., 1998); some involve in denitrification processes in marine sediments (Cathrine *et al*., 2009; Jebaraj *et al*., 2010).

Water, even when treated, has occasionally been associated with harmful infectious disease outbreaks, mostly caused by human and animal enteric pathogens (Bozzuto *et al.* 2010). Pathogenic microorganisms like bacteria, viruses and parasites are well known microbial water contaminants (Szewzyk *et al.* 2009). On the contrary, fungi have not been widely considered when discussing waterborne pathogens, but are now regarded as an emerging chronic water quality problem (Yamaguchi *et al.*, 2007; Sonigo *et al.*, 2011; Ashbolt, 2015). However, humans may be directly or indirectly exposed to fungi and their toxins either through ingestion of contaminated water and seafood (fish, prawns, Spirulina, etc.), or by ingestion of plant products (vegetables and fruit) irrigated using contaminated water (Magwaza *et al*., 2017). Some water sources are contaminated with pathogenic aquatic fungal species causing public health related concerns, hence, it very pertinent to investigate the occurrence and diversity of fungi in aquatic systems, especially concerning their role in water quality and human health (Eissa *et al*., 2013). Therefore this study investigated the occurrence and distribution of fungi in Ikwe pond to highlight the extent of their diversity and uniqueness.

MATERIALS AND METHOD

Ikwe Pond is located in the remote part of Onna Local Government Area of Akwa Ibom State, Nigeria. Onna is situated in the South-Western part of Akwa Ibom State and lies between latitude 4.10 and 4.43 north of equator and longitudes 7.47 and 7.57 east of the Greenwich meridian. The the area lies between the rainforest belt of southern Nigeria with annual rainfall exceeding 2000mm, at temperature of 22 – 35 C, relative humidity of 60 – 90 % with distinct wet and dry seasons. The area is highly marked with intensive onshore offshore oil and gas exploration and exploitation.

Collection of Water Samples

Water was obtained at thirty different points using 30 thoroughly washed 0.50L sterile plastic containers. The plastic bottles were well covered and preserved in an ice box and transferred immediately to the laboratory for analysis.

Fungal Analysis

Glass wares (bottles and test tubes) were washed with detergent and allowed to dry and then autoclaved at 121^{o}C for 15minutes to achieve sterilization. The work bench was disinfected using 70% alcohol. Sample was brought to the laboratory for the serial dilution and the sample of each point was analyzed. Test tube racks were set with six test tubes inside for each sample. Serial dilution was done for each sample with 9ml of sterile water pipette into test tube labeled 10^{-1}. 1ml was pipette from test tube 10^{-1} into 10^{-2}, and further to 10^{-6}. Sixty SDA plates as duplicates for each sample were used to inoculate 1ml each of $10^{-3}$and 10^{-6}dilutions for each sample. Controls were also made for each sample using 1ml from sample 1-30 each.

Culture

Sixty sterile petri dishes were set to be used; the prepared SDA was poured into each of the sample plate and allowed to set. 1ml of the serial dilution for sample 1- 30labeled 10^{-3} and 10^{-6} were each aseptically dispensed onto the 60 SDA plates respectively. A glass spreader (Hockey stick) was used to spread the sample around the surface of the media. The plates were wrapped, properly labeled and kept under room temperature for five (5) days for proper identification of fungi.

Sub-Planting

All positive plates were sub-planted on fresh SDA plates. The sub-culture was carried out to purify the fungi isolates. During the sub-culture, an inoculating loop flamed in a bursen-burner was used to pick the colony and smeared on the agar plate. This was further incubated at room temperature for 7 days. Fungal colonies were isolated upon formation, stained with lactophenol and observed under the microscope. Fungi so observed were identified using appropriate taxonomic guides (Watanabe, 1994; Doggett, 2014).

Wet Mounting

A sterile microscopic slide was used and a dropped of lacto phenol cotton blue was placed on a slide and a pure colony from a sub-planting plate was picked mixed properly and cover with cover slip and was then viewed under the microscope using low and high magnification.

RESULTS

The total fungal count revealed that lkwe pond records a high number of fungal spores (49 spores) as observed from the thirty sampling points (Table 1). In this result upstream recorded a total of 27 spores, midstream had 14 spores while downstream recorded 8 spores respectively. Also the colony count revealed that upstream had the highest number of fungal spores count (Table 1). Table 2 reveals the distribution of fungal isolates in different sampling points within the pond. Clearly, the upstream (59.3%) and the midstream (78.6%) sampling points were dominated by *Aspergillus niger* while the downstream water column was dominated by *Candida tropicalis* (50%).

Table 1: Total Fungal Spores Count for Ikwe Brown Water Pond

Sample point	No of spores counted	Cfu/ml
Upstream	27 (90%)	27×10^3cfu/ml
Midstream	14 (46.7%)	14×10^3cfu/ml
Downstream	8 (26.7%)	8×10^3cfu/ml

Table 2: Percentage Distribution of Fungal Isolates in Ikwe Brown Water Pond

Isolates	Upstream	Midstream	Downstream
Candida tropicalis	-	1 (7.1%)	4 (50.0%)
Cryptococcus neoformans	-	-	1 (12.5%)
Aspergillus niger	16 (59.3%)	11 (78.6%)	2 (25.0%)
Geotricium sp	4 (14.8%)	2 (14.3%)	1 (12.5%)
Absidia sp	8 (29.6%)	-	-

Table 3 reflects the morphological and cultural characteristics of fungal isolates indicating the colony color, type of soma, nature of hyphae, special vegetative structure, asexual spore, special productive structure, conidial head and vesicle shapes of all the probable organisms obtained from Atabonginlet stream. From the table, the total number of 6 fungal species was identified. The notable genera include: *Candida*, *Cryptococcus*, *Aspergillus*, *Geotricium* and *Absidia*. From the result in Table 4, it is recorded that midstream had the highest value for dominance value (1.478), downstream records highest Shannon diversity (0.686) while upstream records (0.543) for Simpson diversity.

Table 3: Morphological and Cultural Characteristics of Fungal Isolates in Ikwe Brown Water Pond

Colony color	Type of soma	Nature of hyphae	Special vegetative structure	Asexual spore	Special productive structure	Conidial head	Vesicle shape	Probable organism
Creamy	Filamentous	Pseudohyphae	Budding	Blastoconidia	Blastospores	Round	Oval	*Candida tropicalis*
Cream-brownish	Filamentous	Absent of hyphae	Budding	Conidia	Basidium	Smooth edged	Oval	*Cryptococcus neoformans*
Dark brown	Filamentous	Hyaline septate	Foot cell absent	Spherical arthoconidia	Conidiospores	Radiate	Biserate	*Aspergillusniger*
Whitish	Filamentous	Non-septate	Branched mycelium	sporangiospores	Sporangium	-	Oval	*Absidiaspp*
Whitish	Filamentous	Coarse	Arial mycelium	Rectangle conidia	Arthrospores	Rounded ends	Cartwheel shape	*Geotriciumspp*

Table 4: Fungal Diversity Associated with Ikwe Brown Water Pond

Sample point	Upstream	Midstream	Downstream
Shannon diversity(HI)	0.648	0.67	0.686
Simpson diversity (DI)	0.543	0.522	0.507
Dominance values (DV)	0.457	1.478	0.493

DISCUSSION

Humans are directly or indirectly exposed to fungal contaminations through fungal toxins either through ingestion of contaminated water and seafood (fish, prawns etc), or by ingestion of plant products (fruits, vegetables etc) (Magwaza *et al*., 2017), which may result in various diseases and infections. Farmers normally use water from this pond for watering crops. In a study by Sakshi *et al.*, (2015), it was confirmed that most water bodies are contaminated by micro-organisms and this consequently affects the quality of water, its utilization and the health of the consumers. In line with the WHO guidelines, water may be reputed as being fit and safe for human consumption if it cannot cause any significant health hazard when consumed being that its physical, chemical and biological criteria WHO, 1997). Nevertheless, the evidence obtained from Ikwe pond indicated the presence of five (5) fungal species. These includes; *Candida tropicalis, Cryptococcus neoformans, Aspergillus niger, Geotricium* spp*, Absidia* spp *and Geotricium* spp. The fungi diversity of this pond compares with reports by Sakshi and Alka, (2015) while studying the diversity of fungal species in sewage water of Durg District. Also the species isolated in this study corresponds with those listed by Saju, (2011). Intrestingly, the diversity and distribution of the mycobiota as noted in this result is patchy. This irregularity is a reflection of the variety of processes and activities perpetuated in different sections of the pond. Some of these activities include bathing, laundering, swimming, washing of motor cycles etc.

This investigation detected *Aspergillus niger* as the most commonly encountered species in the pond. This is consistent with the observations made by earlier researchers including Avanitidou *et al*., (2000), Gunhild *et al*., (2006) and Okpako *et al*., (2009). These researchers concluded that the genus *Aspergillus* is most commonly isolated genera in aquatic systems. This is concurrent with our result. *Aspergillus* spp produces toxic organic compounds in seafood which cause allergies, asthma and various other infections (Pereira *et al*., 2010). However, the presence of these microbes in the pond water provokes thoughtful concern since it is a major source of water supply readily available for several low income households in Ikwe community for their domestic uses.

It is known that members of the dominant genus, *Aspergillus* have been implicated in the production of strongly potent and toxic hepatocarcinogenic compounds generally known as afflatoxins (Bennett and Klich, 2003). Specifically, *Aspergillus niger* is associated with common allergy and may trigger opportunistic infections in hospital immunized patients (De

Hoog *et al.*, 2000). Again in a related research, Parveen *et al*., (2011) named Aspergillus as one of the most commonly isolated genera in water having the highest contribution to microbial contamination in the aquatic system. *Aspergillus niger* has again been noted as the causative agent of Aspergillosis in which the fungus infects the lungs and spreads to other organs, producing abscesses and necrotic lesions (Okpako *et al*., 2009).

The occurrence of *Cryptococcus neoformans* is well noticed and may infer that the water body is rich in decaying organic materials of plant origin. This agrees with the findings of Cogliathi *et al*., (2016) who reported that massive colonies of this organism were isolated from tree barks, hollows and fissures on tree trunks as well as decaying wood in the Mediterranean basin. *Cryptococcus neoformans* is a sporadic or opportunistic yeast organism which is responsible for Cryptococcosis, a fungal infection acquired through the inhalation of the dehydrated cells or spores of the fungus capable of penetrating the pulmonary alveoli and spreading through into the blood stream resulting in Pneumonia, soft tissue infections and *Cryptococcal meningitis* (Kwong-Chung *et al.,* 2014).

Also the presence of *Absidia* species go a way to confirm the presence of organic load in the water column since this species have been associated with decaying plant matter (Saju, 2011). This may explain the brown coloration of the water. Hay (2014), *Absidia* constitute one of the genera which may cause aggressive pulmonary and paranasal infection among predisposed groups such as diabetics, immune compromised individuals. Notably, the isolation of *Geotrichum* sp and *Candida tropicalis* from the pond confirms the reports of other researchers while studying shrimp cultured pond (Ochoa *et al.,* 2015) and other domestic water sources (Ayanbimkpe, 2012). Ochoa *et al.,* (2015) confirmed that *Geotrichum* species is a cosmopolitan yeast-like fungus found in soil, water and air. Specifically the presence of *Candida* species and *Geotrichum* species in the water renders it a risk factor to unsuspecting consumers who may not boil the water before drinking or bathing.

Conclusion

In summary, the morphological examination of various type of spores producing fungi are present in lkwe pond water, during the period present investigation showed a total of 6 species of fungi which were *Aspergillus niger, Candida tropicalis, Cryptocococus* spp, *Absidia* spp and *Geotricium* spp. The diversity indices within the ikwe brown water pond were lower than those reported in other natural waters. The frequency of fungal occurrence was found to be harmful contributing to water contamination which may be lead to outbreak of serious health problems to human and animals population.

The disposal of untreated effluent and other human activities into freshwater ecosystems is a common phenomenon. This can impact microbial diversity of the aquatic environments, consequently affecting the ecosystem functioning and health of the aquatic system (Abdel-Raheem and Shearer, 2002). However, the zeal of this research is anchored by the availability of limited literature on microbiological diversity associated with Akwa Ibom indigenous water bodies especially Ikwe pond as part of the growing corpus of literature on limnology in this era of intensified global conservation efforts.

The aim of this study was to identify the fungal diversity associated with Ikwe brown water pond.

Recommendations

It's is Recommended that:

1. Examination of water quality on regular basis to avoid destructive effects on human health.
2. Disposal of domestic and agricultural waste into water bodies should be discouraged.
3. Adequate awareness programme should be organised in order to control the water pollution which may lead to disease outbreak.

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