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Domestic and Peri-Domestic Study Reveals the Presence of Probably *Acanthamoeba castellanii*

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Abstract

Acanthamoeba is free living amoeba consisting of many species that are naturally pathogenic and have been isolated from different environmental sources. The purpose of this study was to determine the prevalence and relative abundance of *Acanthamoeba species* in soil and water samples within the University of Jos environment and to create public health awareness on the dangers of this parasitic protozoan. The study was conducted in the eleven (11) Faculties of the University of Jos. Soil and water samples were collected from each of the faculties, cultured and morphologically identified for positive samples. In addition, quantitative data on occurrence were examined to help better understand the potential risk to the university community. The prevalence of *Acanthamoeba* based on the chi-square analysis indicates that there is a significant difference between the number of *Acanthamoeba* species in the soil and water samples collected ($P < 0.05$). Relative abundance of *Acanthamoeba* based on the Man-Whitney test indicates that there was no significant difference in the distribution of *Acanthamoeba* species in the water and soil samples between different faculties ($P > 0.05$). Morphological identification indicates the presence of probably *Acanthamoeba castellani*. The demonstration of the presence of *Acanthamoeba* species in soil and water sources calls for awareness among the clinical community, as cases of keratitis and granulomatous amoebic encephalitis might have never been recorded due to lack of expertise or unawareness amongst the clinical commu-

nity. An improved treatment of water supply and strict adherence to water act needs to be strictly encouraged.

Keywords

Acanthamoeba, *Acanthamoeba castellani*, Water, Soil, Jos-Nigeria

1. Introduction

Acanthamoeba is free living amoeba of the genus-amoeba and consists of many species that are naturally pathogenic [1]. They could be found in both natural and man-made environment and have been isolated from soil and water habitats (fresh water, seawater, surface water, swimming pools, mineral water, streams, bottled water, pond water, stagnant water, salt water lakes, river water, distilled water bottles, beaches). They have also been isolated from dust, muds, factory discharges, air-conditioners, cooling towers as well ventilating ducts, hospital units (dialysis and surgical), contact lens solutions, water-air inter-face, sewage, compost, sediments, soil, vegetables, contact lenses and their cases. In addition, atmosphere, human nasal cavities, pharyngeal swabs, lungs tissues, skin lesions, corneal biopsies, cerebrospinal fluid (CSF) and brain necropsies among other environments throughout the world indicate the ubiquitous nature of these organisms [2]-[8]. Although there is no evidence that it is transmitted by ingestion. A number of Amphizoic (ability to live both free in nature and as pathogens in a host) amoeba species which includes *Acanthamoeba* species, *Balamuthia* species and *Naegleria* species, occasionally invade hosts skin, eye and central nervous system to cause infections such as cutaneous infections, keratitis, and granulomatous amoebic encephalitis (GAE), respectively; brain, pregnant women, diabetic patients, AIDs and cancer patients [2] [4] [5] [9] [10]. Furthermore, Acanthamoeba functions as reservoir of diverse microorganisms of humans and acts as vehicles for the spread of pathogens between the environment and humans but can still complete their life cycles without entering a human or animal host in its natural environment [11] [12]. Several *Acanthamoeba* species such as *A. castellani*, *A. polyphaga*, *A. rhysodes* and *A. hatchetti* have been documented as causative agents of keratitis and other human infections [5] [13] [14] [15]. The evidence of the anti-Acanthamoeba antibodies realized in majority of healthy individuals is an indication of common exposure to these pathogens [16] [17] [18] [19]. Although the overall isolation is time consuming, their recognition at their genus level is by their polygonal cysts, whereas their accurate species determination and pathogenic potential involve molecular analysis (PCR, RFLP-PCR or DNA sequencing) and several behavioral tests (osmo-tolerance, temperature tolerance and cytotoxicity assays) which can require axenic and clonal amoeba cultures [13]. In the laboratory, Agar culture is the mainstay for laboratory detection of Acanthamoeba from clinical and environmental samples. Due to the risk of free-living amoeba in endangering human

health, effective actions can be done to eliminate or control this organism, the purpose of this study was to determine the prevalence and relative abundance of *Acanthamoeba species i*n soil and water samples within the University of Jos environment and to create public health awareness on the dangers of this parasitic protozoan within the University of Jos environment.

2. Materials and Methods

Study Area: The study was conducted in the eleven (11) Faculties of the University of Jos, Plateau State, Nigeria. University of Jos is located in Jos North Local Government Area of Plateau State Nigeria. It is located at longitude 9.9499˚N and at latitude 8.8897˚E. A Global Positioning System was used in the marking of each collection site (Figure 1).

Sample collection

Soil sample: Two 200 g soil samples were collected from two Departments in each Faculty of the University of Jos, using a hand trowel to scoop soil from the surface (6 inch depth) and measured with the wooden rule. With the aid of a cup, each 200 g soil sample was transferred to a sterile plastic bag/plastic bucket. They were carefully labelled and then taken to the laboratory for experimental processing.

Water sample: A cup full of tap water samples were also obtained from different Faculties and then taken to the laboratory for further analysis.

Culturing media of the *Acanthamoeba*

Compounding of Nutrient Agar: This was prepared according to the methods of Isenberg and Garcia [18]. The various chemical/solvents/weights

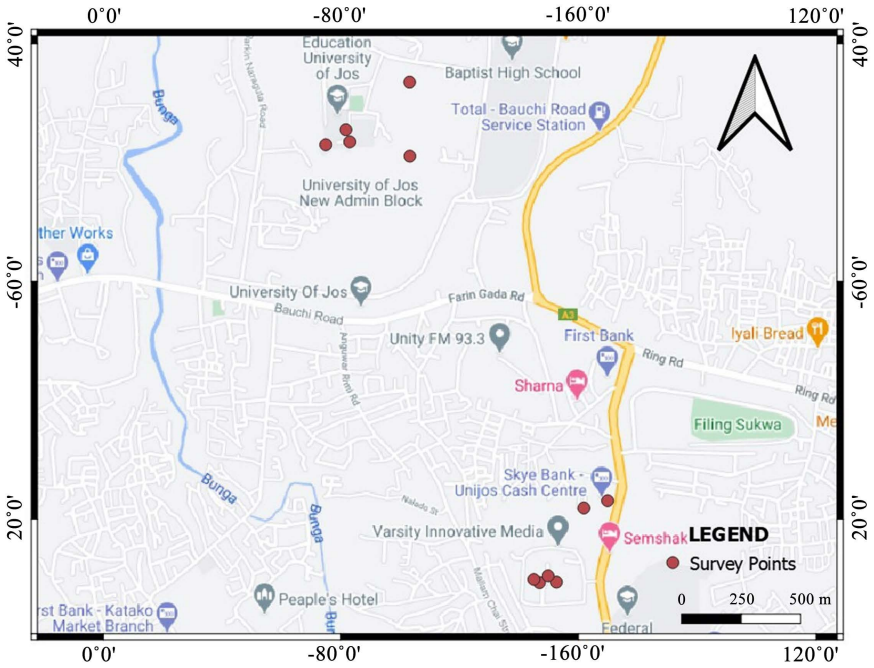


Figure 1. Map of the University of Jos and adjourning areas with brown points indicating study areas.

needed for the compounding are as shown in Table 1. Non-Nutrient Agar and Page's saline contain buffers to provide amoebae with a neutral environment. Neff's Amoeba Saline (AS) [19] 10 mL of each stock solution (10×) were added to 1litre of deionized water, mixed, sterilized by filtration and aliquoted into needed volumes.

Preparation of Non-nutrient Agar plates (ø90 × 15 mm): Acanthamoeba have been reported to be easily grown on non-nutrient agar plates seeded with *Escherichia coli* or *Klebsiella pneumoniae* [20] [21]. A smear or streak of a suitable bacterial food organism such as *Escherichia coli* or *Klebsiella pneumoniae* was made over the agar surface; plates were sealed with tapes, inverted and incubated in boxes lined with wet paper towels or incubator to maintain humidity. Acanthamoeba was observed to migrate across the plate using bacteria as a food source. Over proliferation of bacteria was prevented by the non-nutrient agar. With incubation at 32˚C, the migration tracks of the amoebae were usually easily visible within 48 hours, but occasionally longer incubation (up to two weeks) is needed [22].

Procedures for Preparation of Non-nutrient Agar, Environmental and Laboratory samples.

Preparation of Non-Nutrient Agar *E. coli* plates: Non-Nutrient Agar plates were placed in a 35˚C - 37˚C incubator for approximately 30 minutes (to dry the plates). While plates were being incubated, a heavy suspension (equivalent to a 4.0 McFarland turbidity standard or higher) of *E. coli* (ATCC® 11775 is recommended, but any *E. coli* strain may be used) in a tube of Page's Saline was prepared. To each of the plate's surface, a 2 - 3 pipette drops of the suspension

Table 1. Non-Nutrient agar and page's saline contain buffers to provide amebae with a neutral environment.

Non-Nutrient Agar Stocks (10×) Weight/pH/Temp (grams per 1 litre. deionized H_2O)	
Sodium Phosphate (Na_2HPO_4)	0.0142 gm
Potassium Phosphate (KH_2PO_4)	0.0136 gm
Sodium Chloride (NaCl)	0.012 gm
Magnesium Sulfate ($MgSO_4$)	0.0004 gm
Calcium Chloride ($CaCl_2$)	0.0004 gm
Agar	15.0 gm
Final pH	6.8 ± 0.2 at 25˚C
Page's Saline	
Sodium Phosphate (Na_2HPO_4)	0.0142 gm
Potassium Phosphate (KH_2PO_4)	0.0136 gm
Sodium Chloride (NaCl)	0.012 gm
Magnesium Sulfate ($MgSO_4$)	0.0004 gm
Calcium Chloride ($CaCl_2$)	0.0004 gm
Final pH	6.8 ± 0.2 at 25˚C

were added and then spread using an L-shaped spreader. The plates were then stored at 2˚C - 8˚C and thereafter used for testing within 1 week of inoculation.

Preparation of Soil samples: About 1 - 2 grams of soil samples from each Faculty were suspended in a tube of Page's Saline. Thereafter, to each plate were added a Pipetted 2 - 3 drops of suspension of two previously Non-Nutrient Agar *E. coli* plates. This was not spread [23] [24].

Preparation of Water samples: With the aid of a syringe, 10 ml of water sample were dispensed into a Falcon centrifuge tube and centrifuged for 10 minutes at 250 ×g. A pipet was then used to remove supernatant and the re-suspended sediment using 0.5 ml of Page's saline. Subsequently, a pipet (2 - 3 drops) of suspension was added onto the middle of the plate surface of prepared Non-nutrient Agar *E. coli* plate. This was also not spread [20] [25] [26].

Incubation: One of each plate (inoculated media side down) was incubated overnight at 30˚C - 32˚C and 35˚C - 37˚C, allowing excess fluid to evaporate. After initial incubation, Non-Nutrient Agar plates were sealed with paraffin strip and allowed for continued incubation.

Examination of Plates: Beginning on the second to seventh day of incubation, the surface of the agar was examined using a microscope with 10× objective (it is not necessary to remove sealing tape from plates). The observation at one end of each visible feeding track was a confirmation of the presence of amoeba. But where no tracks were visible, a continued daily observation was employed for up to a period of ten (10) days before reporting the samples as negative for amoeba. For the identification of isolates and evaluation of Free-living Acanthamoeba, a loop was used to scrape up to ten individual clones of samples on the agar where amoeba are located, and a wet mount was made, viewed under oil-immersion using phase microscopy and observed for cyst forms and trophozoite with acanthapodia (spine-like pseudopods) on their surface; this is characteristic of *Acanthamoeba* species [27] [28] [29] [30]. Extract from cultures indicates Amoeba cysts [31], and is identified as belonging to one of the cyst morphological clusters (*Acanthamoeba species* I to III) as recognized by Pussard and Pons [32].

Statistical analysis: Chi-square test was used to test significant difference between the number of *Acanthamoeba species* in the soil sample compared to the water sample collected. The Man-Whitney test was used to test the difference in the distribution of *Acanthamoeba species* in the water and soil sample between different faculties. In each instance P-value of 0.05 was used to test the significance of the analysis.

3. Results

Identification of Isolates: Analysis: The clonal isolation of *Acanthamoeba* species using Non-nutrient agar with each plate seeded *with Escherichia coli allowed* a rapid and efficient isolation of *Acanthamoeba* cyst, as well as their hatching and trophozoites multiplication was achieved, as clonal growth was

observed from 48 hrs of incubation. The data of the presence of *Acanthamoeba castellanii* across 11 different Faculties, including the various water and soil samples collected in each faculty are summarized in Table 2, and their growth capacity at an incubator temperature, 37˚C. Plates were discarded after 15 days of observation. Summary of the distribution and abundance of *Acanthamoeba* colonies across various faculties are as shown on a clustered bar chart in Figure 2.

Prevalence of Acanthamoeba: Based on the chi-square test or analysis, there was significant difference between the number of *Acanthamoeba species* in the soil sample compared to the water sample collected ($P < 0.05$) (Table 1).

Relative abundance of Acanthamoeba: Based on the Man-Whitney test, there was no significant difference in the distribution of *Acanthamoeba species* in the water and soil sample between different faculties ($P > 0.05$). U-calculated = 16.5; U-tabulated = 12; Therefore, U-calculated > U-tabulated. Thus, reject Null hypothesis (Table 1). The clustered bar chart showed a distribution of

Table 2. Sampled areas and origin of colonies of *Acanthamoeba* species collected from the University of Jos, Nigeria.

Area sampled	Origin/No. of colonies		Total (%)	Characterization of colonies	Prevalence in soil/water (χ^2)	P-value	Man-Whitney test (Concentration of colonies in Faculties)	P-value
	Water (%)	Soil (%)						
Faculty of Agriculture	2 (1.85)	3 (2.78)	5 (4.63)	*A. castellannii*				
Faculty of Medical Sciences	1 (0.93)	4 (3.70)	5 (4.63)	*A. castellannii*				
Faculty of Arts	2 (1.85)	4 (3.70)	6 (5.56)	*A. castellannii*				
Faculty of Natural Sciences	7 (6.48)	12 (12.04)	19 (18.52)	*A. castellannii*				
Faculty of Education	5 (4.63)	11 (10.19)	16 (14.82)	*A. castellannii*				
Faculty of Engineering	4 (3.70)	5 (4.63)	9 (9.33)	*A. castellannii*	**7.6**	**0.0058**	**16.5**	**0.243**
Faculty of Law	3 (2.78)	6 (5.56)	9 (8.33)	*A. castellannii*				
Faculty of Pharm. Sciences	3 (2.78)	3 (2.78)	6 (5.56)	*A. castellannii*				
Faculty of Veterinary Medicine	6 (5.56)	4 (3.70)	10 (9.52)	*A. castellannii*				
Faculty of Social Sciences	8 (7.41)	4 (3.70)	12 (11.11)	*A. castellannii*				
Faculty of Environmental Sciences	4 (3.70)	6 (5.56)	10 (9.52)	*A. castellannii*				
TOTAL	**45 (41.67)**	**63 (58.3**	**108 (100)**					

U-calculated = 16.5; U-tabulated = 12; Therefore, U-calculated > U-tabulated. Thus, reject Null hypothesis.

Acanthamoeba species in soil and water sample of which the colonal isolation marks its peak in the Natural Science Faculty under the soil sample collected. While the colonal count in water sample was highest under the Social Sciences Faculty (Figure 2). Pictorial representation of plates of various samples of both water and soil collected from the eleven Faculties are as shown on Figures 3-13.

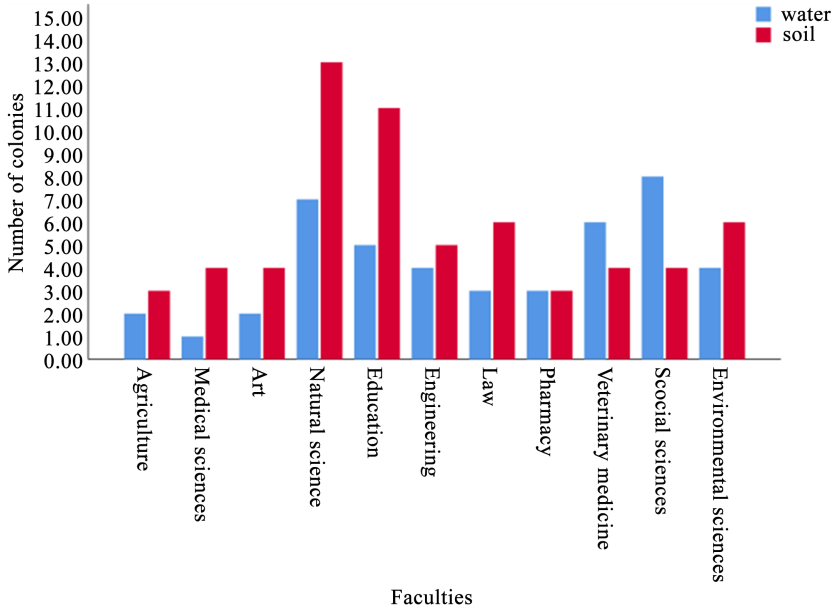
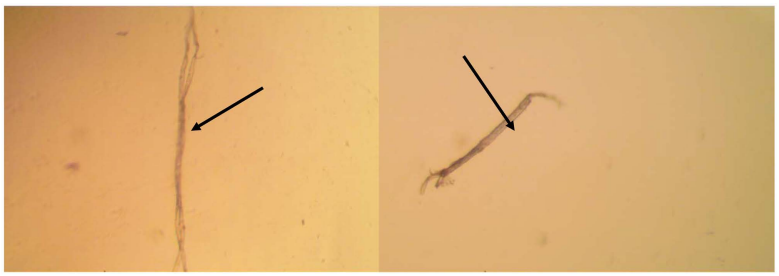


Figure 2. Clustered bar chart showing distribution of *Acanthamoeba* colonies across faculties.

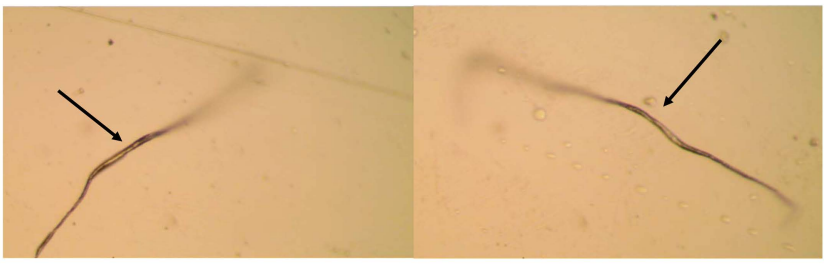


(a) (b)

Figure 3. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of agriculture.

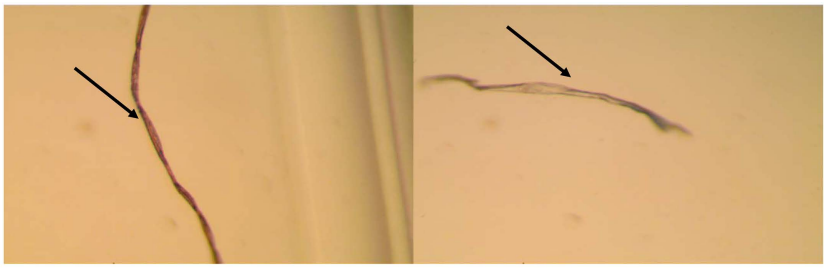
(a) (b)

Figure 4. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of medical sciences.



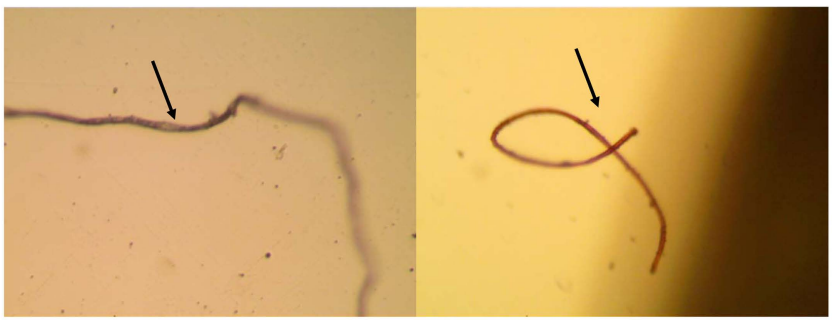
(a) (b)

Figure 5. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of arts.



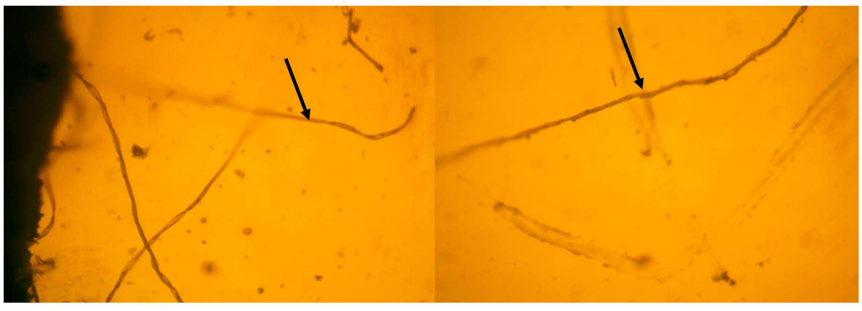
(a) (b)

Figure 6. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of education.



(a) (b)

Figure 7. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of engineering.

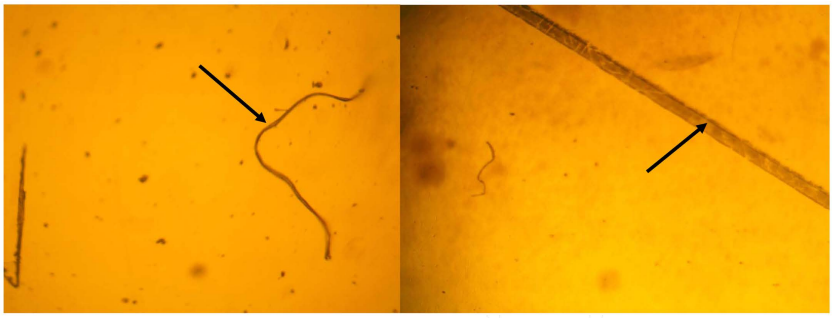


(a) (b)

Figure 8. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of law.

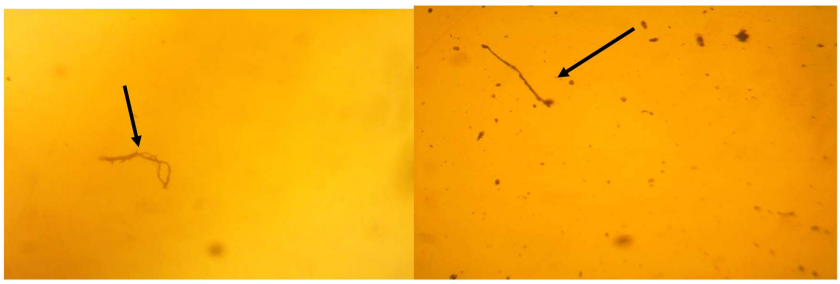
(a) (b)

Figure 9. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of pharmaceutical sciences.



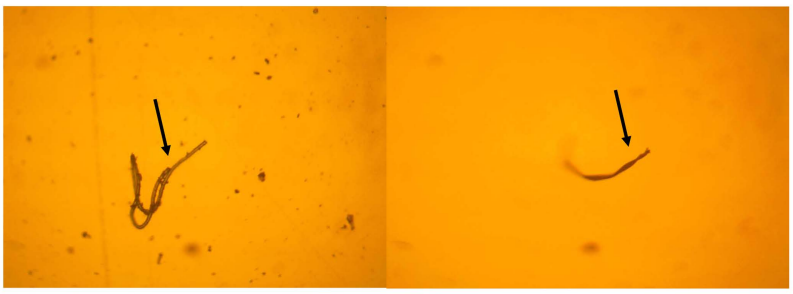
(a) (b)

Figure 10. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of natural sciences.



(a) (b)

Figure 11. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of veterinary medicine.



(a) (b)

Figure 12. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of social sciences.

(a) (b)

Figure 13. Colonies of *Acanthamoeba* species identified from soil (a) and water (b) from the faculty of environmental sciences.

4. Discussion

In most climes where environmental problems have been reported as contributory factors to public health challenge with attendant morbidity and mortality in the population, an encompassing high sensitive diagnostic method with precisions of higher specificity are important in order to reduce unnecessary treatment with presumptive drugs and to improve on accurate diagnosis of other pyretic illnesses. The results obtained from the study clearly indicated that there were high chances that presumptive diagnosis of people could be higher when compared with conventional Acanthamoeba diagnostic methods, implying that there could be high chances of treating people who are actually not sick of pyretic illnesses, a false-positive phenomenon. This agreed with the reports of WHO on malaria [33] [34], that presumptive treatments based on axillary temperature continued to remain a great challenge and therefore recommended that confirmation of parasites in body fluids for all suspected cases and treatments should only be applied to clinical feeling when parasitological diagnosis that requires a sensitivity of more than 90% are not available.

The results obtained from the study revealed the prevalence of *Acanthamoeba castellani* in both soil and tap-water. The isolation from both domestic tap water outlet and peri-domestic soil environment in all the Faculties of the University of Jos demonstrates that these are significant sources of this organism. It has been noted in an earlier study, that while there exists a relationship between Acanthamoeba in water and keratitis, the role of tap-water is not evidently implicit. Although a study by Kilvington *et al.* [35], suggests that municipal supplies which may have become contaminated enhanced the risk of presumed *Acanthamoeba* keratitis. Additional information on dose needed for infection and quantitative data on occurrence in drinking water supplies would help to better understand the potential risks to contact lens wearers and the general public [36]. Pathogenic *Acanthmoeba species* present in water and soil which are directly consumed or indirectly consumed sources by humans also indicated that they may serve as a transmission vehicle of waterborne pathogenic bacteria that might grow in the cytoplasm of the protozoa [37] [38], the growth of these organisms within the protozoa may provide protection from disinfectants and enhance their ability to cause disease which is an additional potential threat to humans. Providing an unsuitable habitat could potentially reduce these risks by

low organic matter and disinfectant residues would be expected to minimize the number of bacteria upon which this amoeba feeds, thus reducing the population.

Relative abundance of Acanthamoeba in various Faculties: Based on Man-Whitney test, there was no significant difference in the distribution of *Acanthamoeba species* in the water and soil sample between different Faculties ($P > 0.05$). The detection of *Acanthamoeba* species from both 11 water samples and 11 soil samples at various sites, indicated that these sites might trap the cyst or trophozoites of this protozoa, and by proliferation at the various area. The eleven (11) of each cultured samples of water and soil samples, all showed positive plates which were observed using a microscope of ×10 objective to examine the colony found with each colony being counted per plate. Furthermore, out of the 22 cultured plates of both soil and water samples, higher distribution of *Acanthamoeba* species was detected in the soil sample collected, which might be due to the environmental condition *i.e.* during dry season when the samples were collected and in which the work was carried out [39]. There should also be regular inspection of tap water, soil, air conditioners, fresh water, swimming pools, contact lens solution [5] [8] [40]. Given the large size of the trophozoite and cyst they would be easily removed by filtration in a water treatment plant [41].

5. Conclusion

This research has demonstrated the presence of *Acanthamoeba* species in the soil and water sources collected from University of Jos. Hence, this research calls for awareness among the clinical community, as cases of keratitis and granulomatous amoebic encephalitis might have never been recorded due to lack of expertise or unawareness amongst the clinical community. An improved treatment of water supply and strict adherence to water act needs to be strictly adhered to.

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Ethical Considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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