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# Abundance of Bacterial and Fungal Populations in Selected Mangrove Sites in Cataan, Camiguin And Baliangao, Misamis Occidental

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**Abstract**

*A comparative study was conducted to determine if population density of bacteria and fungi varies between two geographically distant mangrove estuaries; a disturbed and relatively polluted mangrove in Cantaan, Camiguin and a protected and unpolluted mangrove in Baliangao, Misamis Occidental. Four transects in each site, each with five 1- square meter quadrats was established to determine the possible occurrence of zonation in the distribution of microorganisms across the mangrove ecosystem. Serial dilution and Standard Plate Count Technique were used to determine the colony-forming units (CFUs) of bacteria and fungi in a gram of soil.*

*Results showed that there were more CFUs of bacteria in Cantaan, Camiguin mangrove than in Baliangao site. Multiple regression analysis revealed that soil salinity and soil texture, among other abiotic factors, influence bacterial abundance in the mangrove sites. On the contrary, Baliangao mangrove had more fungi than in Cantaan estuary. Multiple regression analysis also showed that the amount of silt-detritus in the soil influences fungal populations. Bacterial and fungal populations exhibited clump pattern of dispersion. No zonation was observed in both bacterial and fungal populations in the two mangrove sites.*

*Key Words: Colony forming units, mangroves*

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## Introduction

Mangroves are highly productive ecosystem. They are characteristically more productive than either the ocean or the freshwater input (Odum, 1971). Mangrove soils have been shown to contain large amounts of organic matter that is built up from the land, freshwater and marine sources. It's soft and peaty nature harbors a multitude of microorganisms that may vary in kinds and number in different mangrove areas as affected by natural and anthropogenic factors.

These microorganisms in a mangrove community play a very important role in the dynamics of nutrient cycling, food chain and energy flow. Being a net heterotrophic community, the microbial community acts upon the detritus materials in the otherwise anoxic mangrove soil (Odum, 1971). Without these microbes, life in the mangrove ecosystem could not exist.

Microbial populations in soil usually fluctuate due to some soil conditions such as the amount and type of nutrients, availability of moisture, degree of aeration, temperature, pH, practices and occurrences such as floods and addition of manure and fertilizers (Alexander, 1977). It is, therefore, useful to determine and compare the microbial populations between mangrove areas to evaluate the relationship between the microorganisms and their abiotic environment.

Specific objectives set were as follows: (1) To know and compare the density of bacterial and fungal populations in Cantaan, Camiguin Island and Baliangao, Misamis Occidental mangrove estuaries using the Standard Plate Count Technique and (2) To know if zonation pattern exists on the bacterial and fungal populations along the intertidal zone.

## Methodology

### *Study Sites*

Two mangrove estuaries were chosen as the sampling sites (Figure 1), one in a relatively disturbed and polluted mangrove in Cantaan, Camiguin Island and protected mangrove in Baliangao, Misamis Occidental.

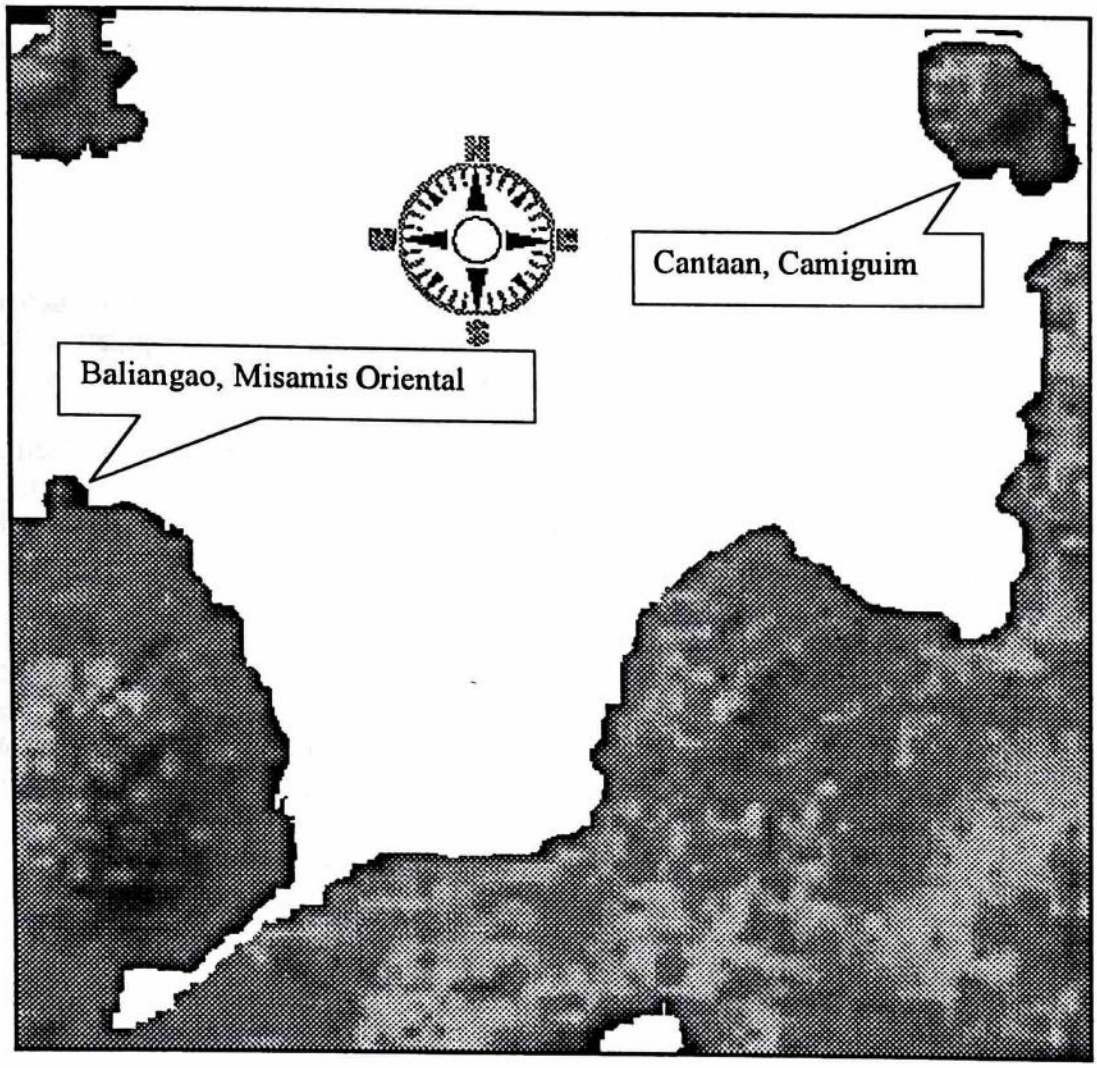



*Figure 1. Map showing the location of the two mangrove sites in Baliangao, Misamis Oriental and Cantaan, Camiguin*

**Transect Method**

Four 50-m transect lines were established perpendicular to the shore in each mangrove site extending from seaward to landward direction. These transects were considered as replicates. Five 1 x 1 $m^2$ quadrats were established landward at regular intervals (10 m apart) along each transect line.

## Collection of Soil Samples

A modified PVC pipe (17.8 long and 8 cm diameter) was used as a soil corer in the sediment collection. This was done by boring 5 cm. into the substratum at three points within each quadrat. These samples were mixed together thoroughly to represent a composite homogenized quadrat sample.

Samples were kept at low temperature inside a cooler and transported to the laboratory for microbial analysis.

### *Determination of Some Abiotic Parameters*

Some physical parameters were noted in each quadrat at the time of sampling such as: water temperature, soil temperature, water salinity, soil salinity, and air temperature. The amount of soil organic content was determined in the laboratory using loss-on-ignition method based on ash-free dry weight of the samples.

Sediment grain size composition was determined using the Modified Settling Method whereby percentage composition of grain sizes was determined from the total amount of soil sample in suspension. Grain size composition such as gravel (coral rubbles or pebbles), sand (coarse or fine) and silt was based on Wenworth Grade Scale (Brower, 1977).

### *Preparation and Sterilization of Culture Media*

The media were prepared and used in a manner specifically dictated by a particular experimental procedure. Marine Agar Medium Plus Nystatin (MAN) and Potato Dextrose Agar Plus Streptomycin (PDAS) were used to grow bacteria and fungi, respectively. Salt solution (0.85% saline solution) isotonic with the cell cytoplasm was used as diluents to prevent lysis or plasmolysis of microbial cells. Culture media and glasswares were subjected to routine sterilization at 15 psi at 121°C for 15-20 minutes.

### *Serial Dilution and Standard Pour-Plate Dilution Method*

Serial dilution of up to $10^6$ was prepared by adding one gram of soil sample into the diluents. One ml. of aliquot portions in triplicates were transferred to petri dishes and mixed with appropriate melted culture media. Plates were incubated for 48 hours for bacterial growth and one week for fungal growth.

## Data Collection

The growth of colonies was observed after incubation. The colonies in plates were counted and the number obtained multiplied by the reciprocal of the dilution factor to obtain the standard plate count per gram of soil. Dilution plates were selected so that the total number of colonies on a plate is 25-250. The colonies observed were identified into major groups such as bacteria or fungi.

## Data Analysis

Two-way Analysis of Variance (ANOVA) was used in the analysis of the experimental data and comparison of means. The dispersion pattern of both bacteria and fungi was computed using the modified Two-Term Local Quadrat Variance (TTLQV) and graphically shown using Microsoft Excel software (Ludwig & Reynolds, 1988). Multiple regression analysis was done to determine the possible influence of the abiotic factors on the abundance of microorganisms in the two selected mangrove sites (Ludwig & Reynolds, 1988).

## Results

*Bacteria*

Colony-forming units of bacteria was significantly higher in Camiguin mangrove site than in Baliangao mangrove (Table 1) as shown in the MANOVA results (Table 2).

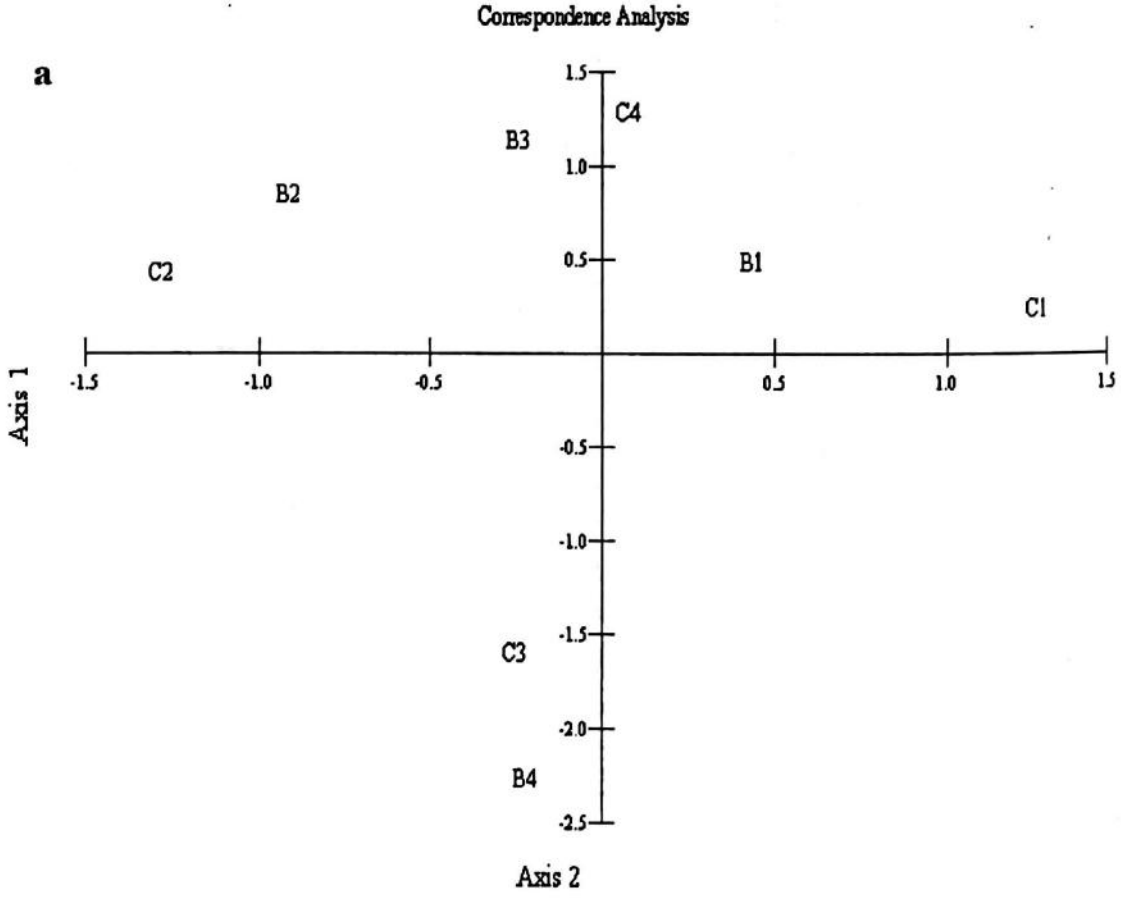
***Table 1.*** *Colony forming units(CFUs) of bacteria per gram of soil after 24 hours of incubation.*

| QUADRATS | TRANSECTS | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | Qtotal | Qmean |
| | | Baliangao | | | | |
| Q1 | 7000000 | 18000000 | 2000000 | 35000000 | 62000000 | 15500000 |
| Q2 | 20000000 | 19000000 | 21000000 | 3000000 | 63000000 | 15750000 |
| Q3 | 6000000 | 55000000 | 17000000 | 16000000 | 94000000 | 23500000 |
| Q4 | 13000000 | 15000000 | 16000000 | 22000000 | 66000000 | 16500000 |
| Q5 | 13000000 | 25000000 | 14000000 | 13000000 | 65000000 | 16250000 |
| Ttotal | **59000000** | **1.32E+08** | **70000000** | **89000000** | **350000000** | **87500000** |
| Tmean | **11800000.0** | **26400000** | **14000000** | **17800000** | | |
| Grand Total | | | | | **140000000** | |
| Grand Mean | | | | | | **35000000** |
| | | Camiguin Is. | | | | |
| Q1 | 158000000 | 30000000 | 79000000 | 13000000 | 280000000 | 70000000 |
| Q2 | 237000000 | 27000000 | 27000000 | 28000000 | 319000000 | 79750000 |
| Q3 | 31000000 | 146000000 | 44000000 | 17000000 | 238000000 | 59500000 |
| Q4 | 59000000 | 87000000 | 87000000 | 8000000 | 241000000 | 60250000 |
| Q5 | 79000000 | 106000000 | 53000000 | 38000000 | 276000000 | 69000000 |
| Ttotal | **564000000** | **3.96E+08** | **2.9E+08** | **1.04E+08** | **1.354E+09** | **3.39E+08** |
| Tmean | **112800000.0** | **79200000** | **58000000** | **20800000** | | |
| Grand Total | | | | | **270800000** | |
| Grand Mean | | | | | | **67700000** |

***Table 2.*** *Analysis of variance on the colony-forming units (CFUs) of bacteria after 24 hours of incubation.*

| S.V. | df | MS EFFECT | df ERROR | MS ERROR | F | P-LEVEL |
|---|---|---|---|---|---|---|
| site | 1 | 25200.4 | 30 | 2174.633 | 11.58834** | 0.001903 |
| zonation | 4 | 91.21 | 30 | 2174.633 | 0.04194 | 0.996476 |
| sitex zon | 4 | 229.34 | 30 | 2174.633 | 0.10546 | 0.97972 |

**highly significant



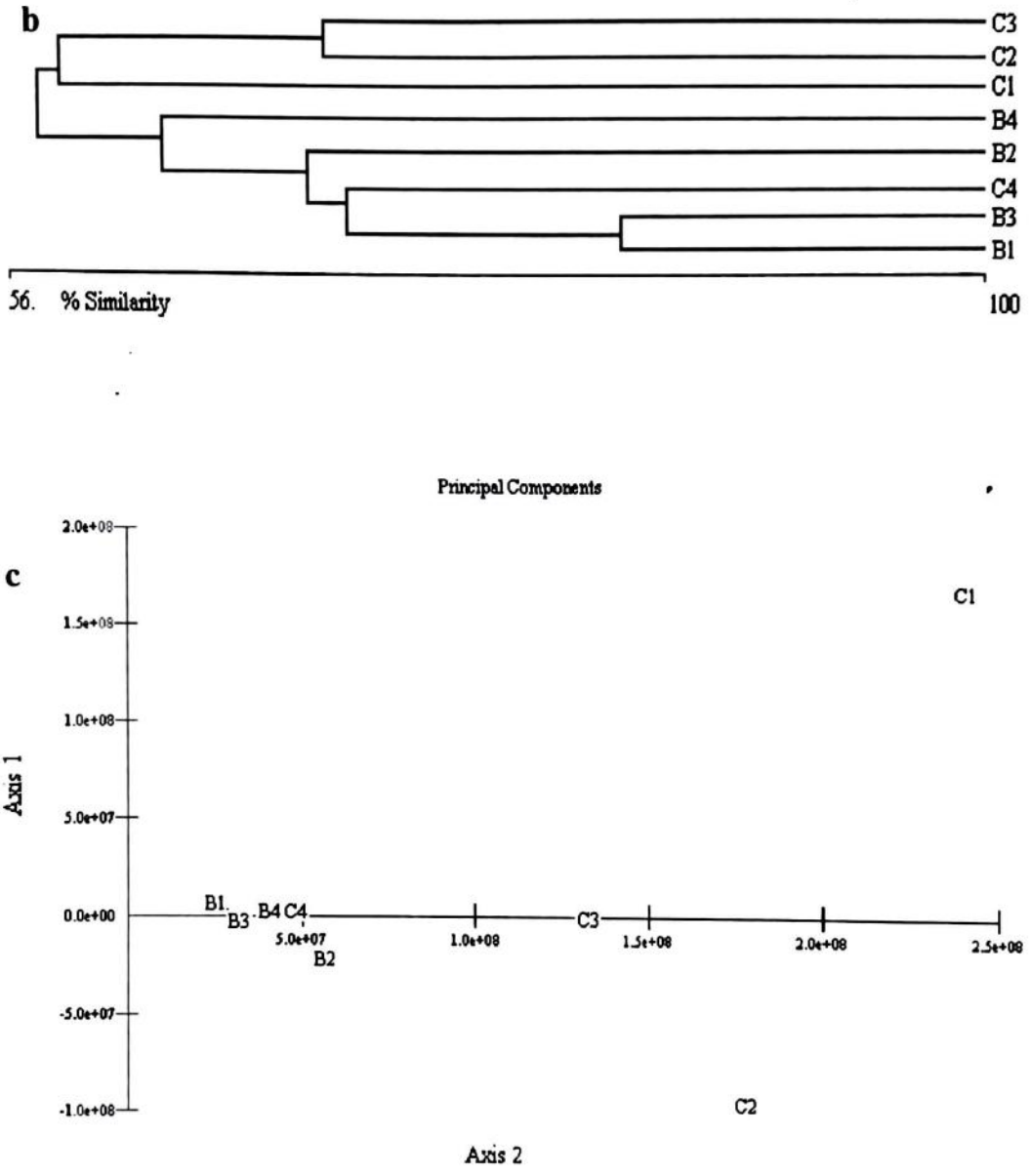



***Figure 1.*** *Dendrogram (a) correspondence (b) and Principal component maps showing the relationships among bacterial populations from four transect areas in in Camiguin and Baliangao, Misamis Occidental (C!-C4 =Camiguin, B1-B4 = Baliamgao).*

Multiple regression analysis (Table 3) showed that bacterial abundance was lower in the more muddy soils of Baliangao mangrove (Figure 2). The result showed that for every unit increase in the amount of clay and silt-detritus, there is corresponding decrease in the CFU-bacteria by a factor of 3.648 and 0.547, respectively.

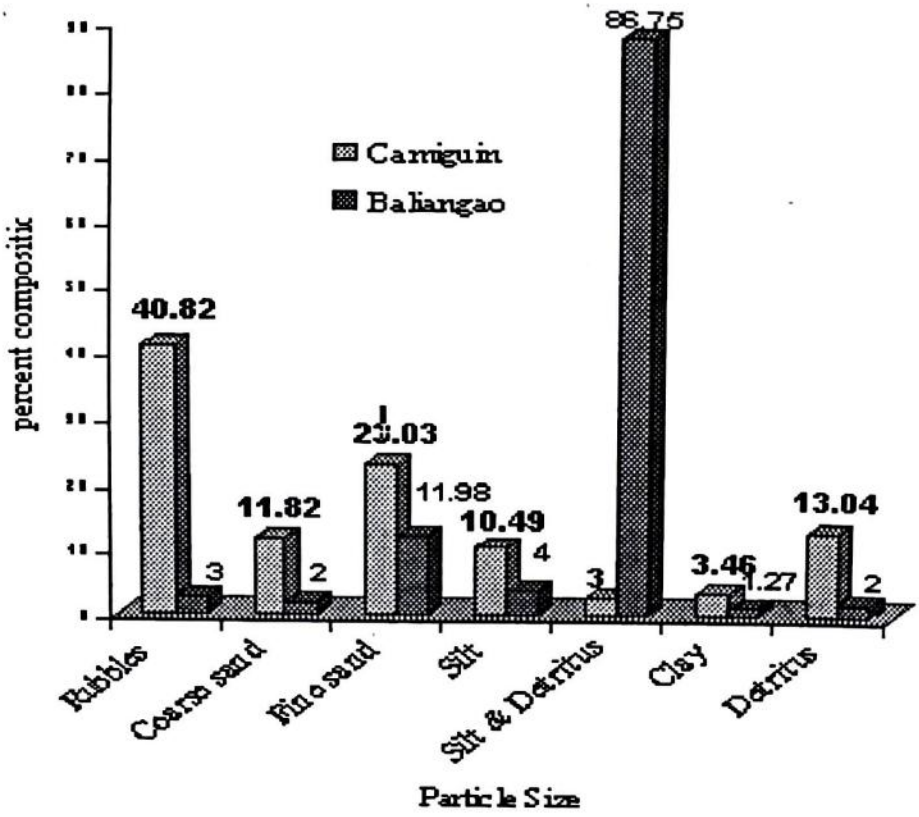



***Figure 2.*** *Graph showing comparison between mangrove sites of the soil particle size using modified settling method.*

***Table 3.*** *Multiple regression analysis to show the significant influence of soil salinity and soil texture on the CFUs of bacteria.*

| PARAMETER | ESTIMATES | STD. ERROR | T STAT | P VALUE |
|---|---|---|---|---|
| constant | -391.425 | 472.457 | -0.828 | 0.414 |
| air temp | -6.2 | 3.987 | -1.555 | 0.13 |
| clay | -3.647 | 1.926 | -1.894 | 0.068* |
| fine sand | -0.325 | 0.44 | -0.739 | 0.466 |
| Org content | -1.868 | 5.512 | -0.339 | 0.737 |
| Silt detritus | -0.547 | 0.287 | -1.908 | 0.066* |
| soil salinity | 11.769 | 6.261 | 1.879 | 0.069* |
| soil temp | -0.156 | 3.426 | -0.046 | 0.964 |
| water salinity | 3.793 | 8.062 | 0.47 | 0.642 |
| water temp | 1.547 | 1.971 | 0.785 | 0.439 |

* significant

On the other hand, the range of soil salinity (39.76-41.2 ppt) in Camiguin mangrove favors bacterial growth by a factor of 11.769. This was revealed in the higher bacterial abundance in Camiguin with higher soil salinity (Table 4). The Durbin-Watson (DW) statistic test shows that there is probably not any serious autocorrelation in the residuals.

***Table 4.*** *Summary of the abiotic factors taken during sampling.*

| | CAMIGUIN | BALIANGAO |
|---|---|---|
| Soil Salinity | 40.34+ 0.72 | 38.02 + 2.81 |
| Water Salinity | 39.48 + 0.88 | 38.94 + 1.25 |
| Soil Temperature | 27.6 + 0.35 | 34.07 + 2.81 |
| Water Temperature | 27.98 + 0.035 | 38.08 + 4.35 |
| Air Temperature | 27.94 + 0.38 | 31.22 + 1.79 |

The spatial pattern of bacterial population in Baliangao and Camiguin showed a degree of clumping (Figure 3) at regular pattern. However, differences in clump size were evident.

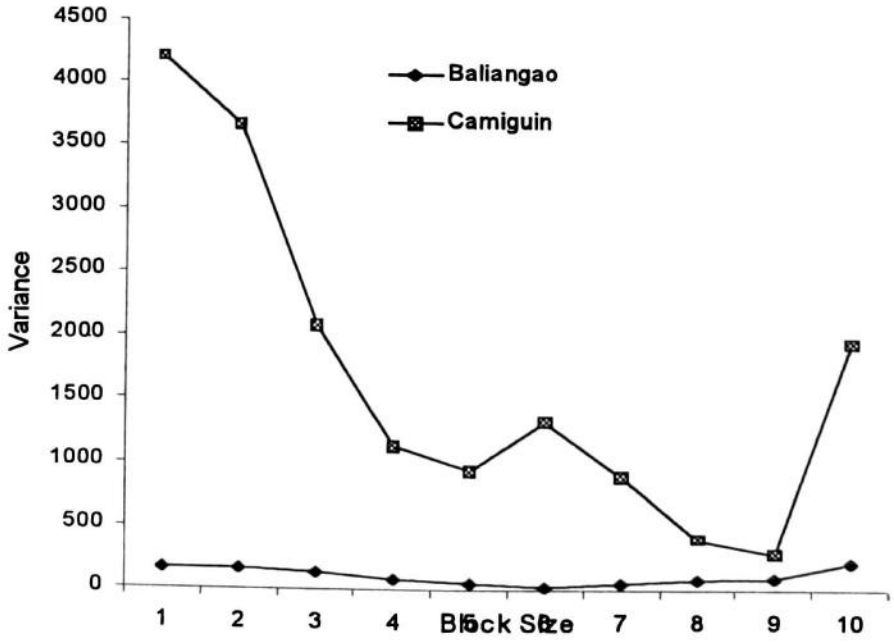



***Figure 3.*** *Graph showing the clump dispersion pattern of bacteria using two-term local quadrat variance (TTLQV).*

**Fungi**

The number of CFUs of fungi, on the other hand, was significantly higher in Baliangao than in Camiguin site (Table 5) as revealed in the MANOVA results (Table 6).

*Table 5. Colony forming units(CFUs) of fungi per gram of soil after one week of incubation.*

| QUADRATS | TRANSECTS | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | Qtotal | Qmean |
| | | | Baliangao | | | |
| Q1 | 24000000 | 27000000 | 42000000 | 19000000 | **112000000** | **28000000** |
| Q2 | 27000000 | 19000000 | 31000000 | 32000000 | **109000000** | **27250000** |
| Q3 | 34000000 | 25000000 | 42000000 | 24000000 | **125000000** | **31250000** |
| Q4 | 36000000 | 27000000 | 31000000 | 59000000 | **153000000** | **38250000** |
| Q5 | 30000000 | 17000000 | 35000000 | 33000000 | **115000000** | **28750000** |
| Ttotal | **151000000** | **1.15E+08** | **1.81E+08** | **1.67E+08** | **614000000** | **1.54E+08** |
| Tmean | **30200000.0** | **23000000** | **36200000** | **33400000** | | |
| Grand Total | | | | | **245600000** | |
| Grand Mean | | | | | | **61400000** |
| | | | Camiguin | | | |
| Q1 | 9000000 | 23000000 | 2000000 | 11000000 | **45000000** | **11250000** |
| Q2 | 34000000 | 8000000 | 4000000 | 6000000 | **52000000** | **13000000** |
| Q3 | 11000000 | 28000000 | 6000000 | 2000000 | **47000000** | **11750000** |
| Q4 | 20000000 | 15000000 | 11000000 | 3000000 | **49000000** | **12250000** |
| Q5 | 10000000 | 31000000 | 2000000 | 2000000 | **45000000** | **48250000** |
| Ttotal | **84000000** | **1.05E+08** | **25000000** | **24000000** | | |
| Tmean | **16800000.0** | **21000000** | **5000000** | **4800000** | | |
| Grand Total | | | | | **47600000** | |
| Grand Mean | | | | | | **19300000** |

*Table 6. Analysis of variance (ANOVA) to show the highly significant variation of sites on the colony-forming units of fungi.*

of sites on the colony-forming units of fungi.

| Effect | df | MS Effect | df Error | MS Error | F | p-level |
|---|---|---|---|---|---|---|
| site | 1 | 3534.4 | 30 | 112 | 31.5571** | 0.000004 |
| zonation | 4 | 43.037 | 30 | 112 | 0.3842 | 0.81812 |
| sitex zon | 4 | 39.463 | 30 | 112 | 0.3523 | 0.840316 |

** highly significant

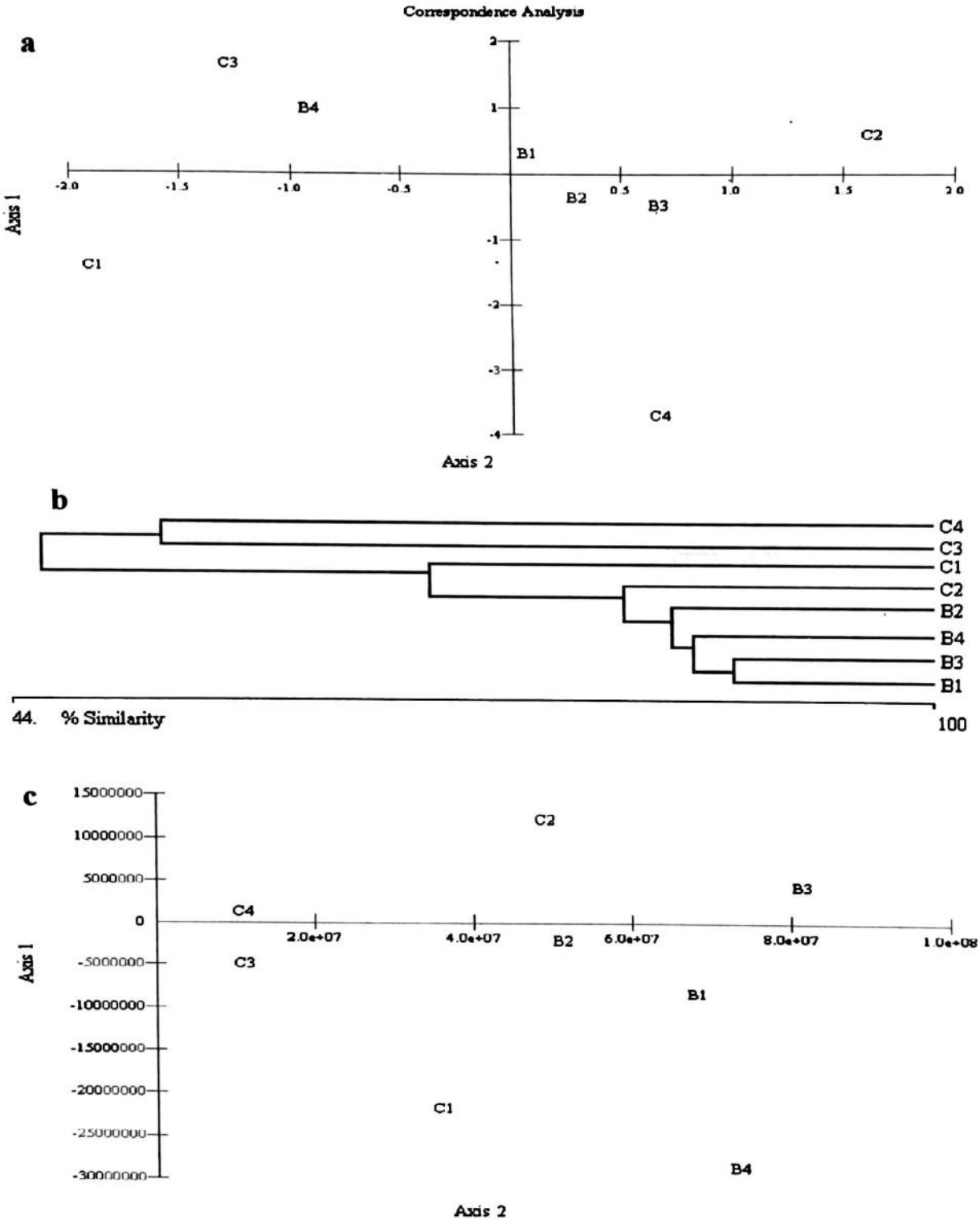



*Fig. Dendrogram (a) correspondence (b) and Principal component maps showing the relationships among fungal populations from different transects in Camiguin and Baliangao, Misamis Occidental.*

Multiple regression (Table 7) models shows that the amount of silt detritus causes an increase in CFU-fungi by a factor of 0.186. The Durbin-Watson (DW) statistic shows that there is probably not any serious autocorrelation in the residuals.

*Table 7. Multiple regression analysis to show the significant influence of soil salinity and soil texture on the CFUs of fungi.*

| PARAMETER | ESTIMATES | STD. ERROR | T STAT | P VALUE |
|---|---|---|---|---|
| constant | 52.268 | 101.844 | 0.513 | 0.612 |
| airtemp | -0.627 | 0.859 | -0.73 | 0.471 |
| clay | 0.425 | 0.415 | 1.024 | 0.314 |
| finesand | -0.126 | 0.095 | -1.328 | 0.194 |
| orgcon | 1.197 | 1.188 | 1.007 | 0.322 |
| silt detritus | 0.186 | 0.062 | 3.011 | 0.005* |
| soil salinity | 0.218 | 1.349 | 0.161 | 0.873 |
| soil temp | 0.992 | 0.738 | 1.342 | 0.189 |
| water salinity | -1.669 | 1.738 | -0.961 | 0.344 |
| water temp | 0.005 | 0.425 | 0.012 | 0.99 |

* significant

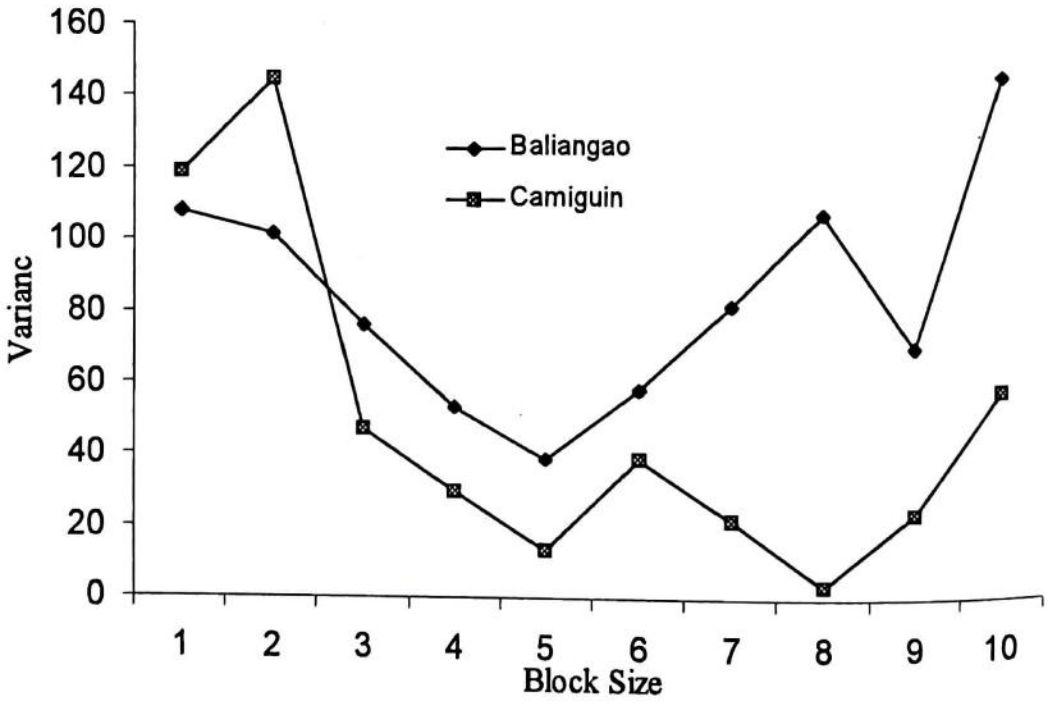



*Figure 4. Graph showing the clump dispersion pattern of fungi using two-term local quadrat variance (TTLQV).*

## Discussion

The abundance of the bacterial and fungal populations in Baliangao and Camiguin mangrove estuaries are reflections of many environmental forces acting on these micro-inhabitants (Pelczar, 1987). So, in Camiguin mangrove site where bacterial population density is higher, fungal population is lesser. On the contrary, Baliangao mangrove site with higher fungal population has lower bacterial population density. The above results also raise the possibility of antibiosis by fungi so that in Baliangao mangrove site with higher CFUs of fungi, bacterial abundance was low.

### Bacterial Populations

The mangrove community in Camiguin Island was found to have higher CFUs of bacteria than in Baliangao. This confirms a study that claimed that higher bacterial population than fungal population is expected in sandy substrate than muddy substrate with other factors in ideal conditions (Tortora, 1992 as cited by Sanchez, 1998). This study also supports the vital role of oxygen or proper aeration to bacterial proliferation in soil.

The two mangrove sites differ largely in their soil type based on particle size. Camiguin has predominantly rocky to sandy substratum with little amount of silt and clay. Baliangao, on the other hand, has a very muddy substratum in all quadrats primarily consisting of silt with detritus materials with little amount of fine sand and clay.

This poorly drained area in Baliangao mangrove lowers bacterial community than the relatively well-drained site such as in Camiguin estuary. The resulting minimal oxygen or its absence makes the organic carbon to be incompletely metabolized leaving intermediary substances to accumulate and bacterial count to be less (Alexander, 1977). This humic and anoxic soil conditions that are both unfavorable for bacterial growth were features observed in Baliangao estuary with higher silt content in soil. In addition, the higher CFUs of bacteria in Camiguin, despite the more amount of clay than in Baliangao, could be attributed to the compensating effect of coral rubbles and pebbles to clay that increases the pore spaces of the soil and hence, aeration. The clay particles instead serve as adsorption surfaces for both the nutrients and the bacteria themselves.

Villejo (1993) noted that bacterial population in a mangrove site grows better in a higher salinity such as 39.76-41.2 ppt. in Camiguin area than the lower salinity (33.35-40.6 ppt) in Baliangao estuary. However, soil salinity may not be the primary or sole reason for the lower bacterial count in Baliangao site although it could be a contributory factor.

The difference in bacterial density between the two sites may also be attributed to the presence of more organic wastes (garbages) in Camiguin estuary. These wastes include those of allochthonous (humans and animals) as well as from autochthonous (decayed vegetation) sources. The presence of these pollutants may also account for the aggregate pattern of bacteria in the soil, whereby bacteria tend to attach on the surfaces of these decomposing materials. There is also rich colonization of bacteria on the fecal pellets produced by meiobenthos and macrobenthos (Giere, 1993).

There is no single factor, however, that could be used to explain the difference in bacterial density. Looking at the Camiguin mangrove as a whole, no zonation pattern was observed. The clump pattern of bacterial distribution was displayed in the entire site.

**Fungal Populations**

Contrary to bacterial population, there was higher number of colony-forming units of fungi in Baliangao than in Camiguin mangrove. Multiple regression analysis showed that the amount of silt-detritus influences fungal density. That is, the greater amount of silt-detritus in Baliangao results to favored growth of fungi rather than bacteria. This could be attributed to the effect of more silt to make the soil more anoxic. This anoxic condition hampers the decomposition rate, allowing plant debris to accumulate in partially decomposed state such as detritus, humus and other organic acids. With this, the soil in Baliangao tends to become acidic favoring growth and dominance of fungi instead of bacteria (Atlas & Bartha, 1990).

The higher fungal count in Baliangao could also be due to the possible occurrence of mycorrhizal association with the dense vegetation in the site. Fungi that grow attached to the mangrove roots avail more of organic nutrients and in return provide increased surface area for the absorption of sap to the plant roots. It has been said, "mycorrhizal condition is the rule among plants, not the exception" (Sylvia, 1996).

The lack of zonation of fungal population could be attributed to the monotonous nature or the spatial homogeneity in the two mangrove sites. There might be existence of microclimatic differences in the site that governs the clump nature of microbial distribution. Clumping suggests that individuals are aggregated in more favorable parts of the habitat (Ludwig & Reynolds, 1988). Clumping, however, is seemingly of regular pattern although the size and intensity of clumping varies. There are clumps that are distinct and some are not well defined.

## Conclusions

This study on the abundance of bacteria and fungi in Baliangao and Camiguin mangrove sites showed the following results: (1) Bacterial abundance was higher in the disturbed and relatively polluted mangrove area in Camiguin Island than in Baliangao; (2) Fungal density was higher in the protected mangrove site in Baliangao; (3) Multiple Regression Analysis revealed that these differences in microbial density could be attributed to soil salinity and amount of silt-detritus; (4) There was no zonation observed in both bacterial and fungal populations in the two mangrove sites; and (5) The bacterial and fungal populations in both sites exhibited clump pattern of dispersion of slightly regular pattern but of varying intensity and magnitude.

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