ENUMERATION OF HETEROTROPHIC, IRON-PRECIPITATING BACTERIA IN THE SOIL SAMPLES COLLECTED FROM URBAN WASTE DUMPING SITES, MATARA DISTRICT, SRI LANKA

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Heterotrophic, iron-precipitating bacteria are capable of using organic radicals from soluble organic iron salts. This is widely applied in the removal of iron from organic solutions and during the decomposition of organic iron compounds, ionic iron is released. The current study was designed to enumerate heterotrophic iron-precipitating bacteria in soil samples, collected from urban waste dumping sites in eight locations, in the Matara district, Sri Lanka. Generally, the identified area was almost flat and there were no complex topographic features. Surface soil, separately sampled from three sampling sites at each location, were used as the test samples. The pH value of each collected sample was recorded. Each soil sample (1.00 g) was added to sterilized water (9.0 mL), followed by tenfold serial dilutions. For the enumeration of the total viable heterotrophic iron-precipitating bacteria, serially diluted samples were pour-plated with Ferric Ammonium Citrate Nitrate Agar. All soil samples were analysed in duplicated agar plate-based assays and the number of colonies was counted after incubation of the plates at room temperature for 2-3 days. Quantitative determinations were made based on colony-forming units per gram (CFU g^{-1}) of soil and expressed with 95% confidence interval limits. Further, the bacterial colony counts per gram of each soil sample were arranged in a completely randomized design and One-way analysis of variance was applied with Tukey's multiple comparison test. The results showed that the counts were significantly different among locations. The significantly highest counts were reported for the dumping sites at Walgama (pH 7.15) and Walpala (pH 6.90) areas and the relevant counts were recorded as 3.492×10^5 CFU g^{-1} and 3.442×10^5 CFU g^{-1}, respectively. The lowest count was recorded at the dumping site near Dikwella lagoon (pH 8.95). The study demonstrates the dispersion of heterotrophic, iron-precipitating bacteria in urban waste dumping sites, within the selected region, indicating that high counts were reported in near-neutral soil environments. The current findings would serve as a baseline for the further expansion of the research topic towards the application of these bacteria, for the removal of iron from accumulated organic waste.

Keywords: Heterotrophic, Iron-precipitating, Colony-forming units, One-way analysis of variance

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INTRODUCTION

Heterotrophic bacteria derive energy from organic compounds. In the fulfillment of their carbon requirement, heterotrophic iron-precipitating bacteria are capable of using the organic radicals of soluble organic iron salts (Harrison *et al.*, 1980). This is widely applied in the removal of iron from organic solutions. During the decomposition of organic iron compounds, ionic iron is released and free Fe^{3+} ions in an alkaline medium form water-insoluble, brownish-red, $Fe(OH)_3$ precipitate, which enables the easy detection of the colonies of heterotrophic iron-precipitating bacteria, in the agar plate-based assays.
The study was designed to enumerate heterotrophic iron-precipitating bacteria of the soil samples, in urban waste dumping sites located in Matara district and the overall results demonstrated the dispersion of counts of this bacterial group, in the selected locality.

METHODOLOGY

To enumerate heterotrophic iron-precipitating bacteria, soil samples were collected from eight locations in urban waste dumping sites in Matara district. The locations were almost flat and had no complex topographic features. Those were Dondra dumping site (5°55'55.35"N, 80°35'27.81"E), Weligama dumping site (5°58'38.56"N, 80°25'43.86"E), Thalalla dumping site (5°56'32.16"N, 80°37'0.24"E), Walpala dumping site (5°57'21.98"N, 80°32'52.84"E), Walgama dumping site (5°56'46.94"N, 80°30'47.82"E), Mirissa dumping site (5°56'53.74"N, 80°28'17.71"E), dumping site near Nilwala river basin (5°56'45.58"N, 80°32'54.09"E) and dumping site near Dikwella lagoon (5°58'18.03"N, 80°41'42.37"E).
Soil sampling was carried out as described in Ameh and Kawo (2017). Loose, unconsolidated, soil was used to attain composite samples. Surface soil, separately sampled from three sampling sites of each location, was used as the test samples. The pH value of each collected sample was recorded. The samples were carried in a sterilized container inside an ice pack to the laboratory. The soil samples were passed through a 4 mm sterilized sieve. Samples were placed at refrigerated conditions (4°C) until the preparation of initial suspension and decimal dilutions for microbial enumerations.
Preparation of initial suspension and decimal dilutions for microbial enumeration was carried out with few modifications to the method described in Ameh and Kawo (2017). Soil sample (1.00 g) was added to sterilized water (9.0 mL), followed by homogenization to obtain 10^{-1} dilution. Further, tenfold serial dilutions were made for colony counting. For the enumeration of total viable, heterotrophic, iron-precipitating bacteria, tenfold serially diluted samples were pour-plated with Ferric Ammonium Citrate Nitrate Agar. The plates were incubated at room temperature (28 ± 2 °C) for 2-3 days. All soil samples were analyzed in duplicates and the number of colonies were counted.
The number of colony-forming units per gram (CFU g^{-1}) of soil present in the each of the test sample was expressed for two successive dilutions with 95% confidence interval limits using the formula given in Lee *et al.* (2021);

$$\frac{\sum c}{V\,[n_1 + 0.1n_2\,]d} \pm \frac{1.96\sqrt{\sum c}}{V\,[n_1 + 0.1n_2\,]d}$$

where, $\sum c$ = sum of the colonies counted on all the plates, n_1 = number of plates retained in the first dilution, n_2 = number of plates retained in the second dilution, d = dilution factor corresponding to the first dilution retained and V = volume of the inoculum used in each dish (mL).

Further, the number of colony-forming units per gram of soil, were arranged in completely randomized design. One-way analysis of variance (ANOVA) was applied with Tukey's multiple comparison test to determine the significant differences of bacterial counts among the locations. The data were analyzed using the software SPSS (IBM, Armonk, NY) and the results were considered significant, if the associated P-values<0.05.

RESULTS AND DISCUSSION

Ferric Ammonium Citrate Nitrate Agar is generally recommended for the isolation and enumeration of iron bacteria. The initial color of the medium was light amber and appeared as a slightly opalescent gel in the Petridishes. Upon the deposition of $Fe(OH)_3$ precipitate, the colonies appeared in brown to rust-red color.

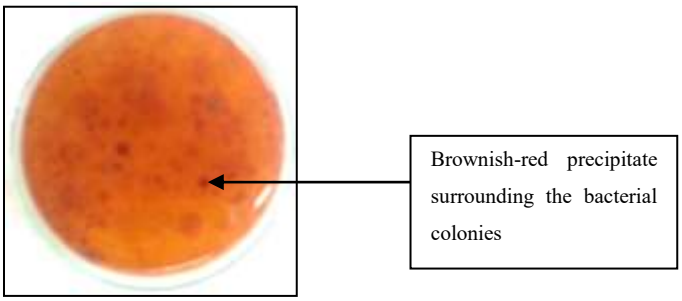

Figure 1. Formation of the brownish-red precipitate surrounding the bacterial colonies on Ferric Ammonium Citrate Nitrate Agar medium, after three-day incubation.

Quantitative determinations were made for the Petridishes with colony counts in the range 30-300. The bacterial colony counts fewer than 30 colonies were not statistically acceptable (too few may not be representative of the sample) and more than 300 colonies on a plate were likely to produce over-lapping colonies. The number of colony-forming units per gram of soil present in the each of the test sample was expressed for two successive dilutions for each sampling site.

Table 1. Bacterial counts of eight locations in urban waste dumping sites in Matara district.

Location	Site	Bacterial counts (CFU g^{-1}) with 95% confidence interval limits
Location 1 Dondra dumping site (pH 4.45)	Site 1	$(3.091\pm0.232)\times10^3$
	Site 2	$(3.332\pm0.241)\times10^3$
	Site 3	$(2.945\pm0.227)\times10^3$
Location 2 Weligama dumping site (pH 5.60)	Site 1	$(3.082\pm0.232)\times10^4$
	Site 2	$(2.968\pm0.228)\times10^4$
	Site 3	$(3.336\pm0.241)\times10^4$
Location 3 Thalalla dumping site (pH 6.05)	Site 1	$(3.168\pm0.235)\times10^4$
	Site 2	$(3.327\pm0.241)\times10^4$
	Site 3	$(3.614\pm0.251)\times10^4$
Location 4 Walpala dumping site (pH 6.90)	Site 1	$(3.418\pm0.244)\times10^5$
	Site 2	$(3.436\pm0.245)\times10^5$
	Site 3	$(3.473\pm0.246)\times10^5$

Location 5 Walgama dumping site (pH 7.15)	Site 1	$(3.477\pm0.246)\times10^5$
	Site 2	$(3.509\pm0.248)\times10^5$
	Site 3	$(3.491\pm0.247)\times10^5$
Location 6 Mirissa dumping site (pH 7.95)	Site 1	$(3.323\pm0.241)\times10^5$
	Site 2	$(3.355\pm0.242)\times10^5$
	Site 3	$(3.368\pm0.243)\times10^5$
Location 7 Dumping site near Nilwala river basin (pH 8.20)	Site 1	$(3.241\pm0.238)\times10^4$
	Site 2	$(3.373\pm0.243)\times10^4$
	Site 3	$(3.418\pm0.244)\times10^4$
Location 8 Dumping site near Dikwella lagoon (pH 8.95)	Site 1	$(3.045\pm0.231)\times10^3$
	Site 2	$(3.205\pm0.237)\times10^3$
	Site 3	$(2.655\pm0.215)\times10^3$

The normality of the data were checked and Bartlett's test has shown the homogeneity of the variances among eight locations (P-value=0.08). One way analysis of variance test showed that the bacterial counts were significantly different among the locations (P<0.05). Further Tukey's multiple comparison test was applied to compare the bacterial counts of each location.

Table 2. The results of Tukey's multiple comparison test for the mean bacterial counts of eight locations.

Location	Mean bacterial counts (CFU g^{-1})
Dondra dumping site (pH 4.45)	$3.123\times10^{3\,d}$
Weligama dumping site (pH 5.60)	$3.129\times10^{4\,c}$
Thalalla dumping site (pH 6.05)	$3.370\times10^{4\,c}$
Walpala dumping site (pH 6.90)	$3.442\times10^{5\,a}$
Walgama dumping site (pH 7.15)	$3.492\times10^{5\,a}$
Mirissa dumping site (pH 7.95)	$3.348\times10^{5\,b}$
Dumping site near Nilwala river basin (pH 8.20)	$3.344\times10^{4\,c}$
Dumping site near Dikwella lagoon (pH 8.95)	$2.968\times10^{3\,d}$

Means that share a common superscript letter in the column are not significantly different (P>0.05) and means that do not share superscript letters are significantly different (P<0.05).

Diagrammatic illustrations on the relationship between the pH values of the soil samples and the bacterial counts, were indicated in Figure 2. Results were expressed in logarithmic form for the convenience of interpretation of bacterial counts.

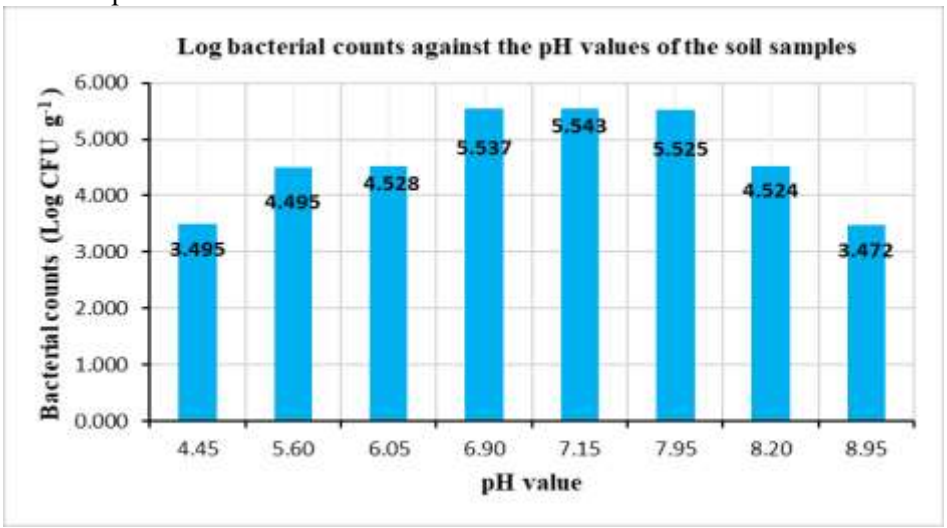

Figure 2. Results expressed on log basis for the bacterial counts against the pH values of soil samples.

High counts of heterotrophic, iron-precipitating bacteria were reported in the soil environments with near neutral pH values. The lowest count (3.472 Log CFU g^{-1}) and the second lowest (3.495 Log CFU g^{-1}) were obtained for an alkaline (pH 8.95) soil sample and an acidic (pH 4.45) soil sample, respectively.

CONCLUSIONS/RECOMMENDATIONS

The study demonstrated the dispersion of heterotrophic, iron-precipitating bacteria in urban waste dumping sites, within the selected locations in Matara district and in all the tested soil samples reasonably high counts of heterotrophic iron-precipitating bacteria were detected. The significantly highest counts were reported for the dumping sites at Walgama (pH 7.15) and Walpala (pH 6.90), indicating that high counts of heterotrophic, iron-precipitating bacteria were reported in the soil environments with near neutral pH values. The findings would serve as a baseline for the future expansion of the study, in the application of these bacteria, for the removal of iron from accumulated organic waste.

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