Octa Journal of Environmental Research
International Peer-Reviewed Journal
Oct. Jour. Env. Res. Vol. 5(2):149-155
Available online http://www.sciencebeingjournal.com

Apr. – Jun., 2017
ISSN 2321 3655

Research Article

Octa Journal of Environmental Research
(Oct. Jour. Env. Res.) ISSN: 2321-3655
Journal Homepage: http://www.sciencebeingjournal.com

ENUMERATION OF SOIL BACTERIA IN THE SOIL OF THE TONS RIVER BANK FROM DIFFERENT ELEVATION

Parul B. Kotiyal* and Sudipto Bhowmik
Forest Soil and Land Reclamation Division, Forest Research Institute, Dehradun (Uttrakhand) India
Corresponding author's Email: **parulbhatt29@gmail.com**
Received: 15th Apr. 2017 **Revised**: 24th May 2017 **Accepted**: 30th Jun. 2017

Abstract: This study was performed to enumerate the number of bacteria per gram soil from the bank of river Tons in Dehradun district of state Uttarakhand, India form different elevations. The soil in this area is flooded with water in rainy season, while partially moist in winter and summer. The study was carried out to enumerate and tally the number of bacteria per gram against soil from elevation and relate the results with pH, organic carbon, available potassium (K), phosphorus (P) and nitrogen (N) of the collected soil samples. Factors changes like temperature, water holding capacity, soil properties, vegetation, slope, micronutrients, availability of sunlight, human activities were not included in the study. The study shows that the average number of bacteria per gm. of soil decreased with decrease in elevation. The pH, organic carbon, and potassium didn't show any particular trend of change according to elevation.
Keywords: Environmental quality; River bank; Soil bacteria.
Postal Address: Forest Soil and Land Reclamation Division, Forest Research Institute, Dehradun (Uttrakhand) India

INTRODUCTION

To understand the soil microbial ecology is an important step to study quality and health of soil. The microbial organisms of soil show their diversity according to several physico-chemical properties of soil as well as climatic conditions, vegetation and anthropogenic activities. They take important role in cycling nutrients, decomposing organic matters etc. The environment quality of water, soil and air is degraded increasingly. Therefore we need to raise the prevention of pollution by monitoring environmental quality. There were several monitoring methods on the environmental quality, especially biological method. Biological methods assess the presence of several species, such as plants, insects, fish, bacteria and viruses as environmental indicator.

Some species of bacteria have been used as indicators in monitoring environmental quality *e.g.*Coliform, *Escherichiacoli*, *Streptococcus* sp., *Pseudomonas* sp., *Vibrio* sp., *Clostridia* sp., *Bifid bacterium pseudolongum, Arcobacter* sp., *Thiobacillus* sp. and etc. The bacteria act as an indicator of household waste (human and animal feces, household waste and other), heavy metal pollution, crude oils and other pollution. According to Torsvik (1990) there are 200 identified bacterial genera and a single soil sample may contain over 4000 genetically distinct bacteria but only 1% is culturable. There are mainly two types of bacteria in soil, autochthonous (indigenous) and allocthonous (invaders or transients) (Coyne 1999). According to Hartman *et al*. (2008) found that pH was the best predictor of changes in soil bacterial communities. Soil bacteria can oidize compounds as phosphite, hypophosphite; aerobically (Admas

and Conrad, 1953), and anaerobically (Foster *et al.,* 1978). Ammonium oxidizers like *Nitrosomonus*, *Nitrosospira, Nitrosococcus, Nitrosolobus* and nitrite oxidizers like *Nictrobacter, Nitrospina,* and *Nitrococcus* lives in soil (Coyne, 1999). Bacteria like *Pseudomonus, Chromobacteria* and *Clostridium* take part in cellulose degradation (Coyne, 1999) and the abundance of bacteria like *Bacteroidetes Betaproeobacteria,* and *Acidobacteria* are strongly associated with estimated carbon availability (Lauber *et al.,* 2009). Yet there are many undiscovered species and relation between the soil bacteria and the environmental factors are subjects of research interests. This study of enumeration is basically done to find a relation between abundance of bacterial species in different elevation at different distance from the river water from the bank of Tons. The chosen sites temporarily remain under water in rainy season and remain partially moist in other times. The samples were collected in the month of August when the temperature of atmosphere is around 29.4-22.4^{0}C and the average rainfall is around 728.2 mm.

Vincy *et al* 2017 carried out a study with an objective to systematically examine the prevalence of indicator and pathogenic microorganisms and to compare the microbiological quality of the river water during the pre-monsoon and post-monsoon seasons. Water samples from 44 different sites during pre-monsoon and post-monsoon seasons were collected for the analysis. During the pre-monsoon period, the faecal coliform count ranged from 230 to 110,000 MPN/100 mL while there was a variation from 200 to 4600 MPN/100 mL during the post-monsoon period. When the faecal streptococci count was analyzed, it ranged from 140 to 110,000 MPN/100 mL during the pre-monsoon and 70 to 4600 MPN/100 mL during the post-monsoon seasons, respectively. In monitoring the environment quality, there are some parameters that are commonly used *i.e.* physics, chemistry and biology. Specifically, biological methods assess the presence of several species e.g. bacteria as indicators for environmental pollution. The review showed that Coliform, *E. coli*, *Streptococcus* sp. *Pseudomonas* sp. *Vibrio* sp., *Clostridia* sp., *Bifidobacterium pseudolongum*, *Arcobacter* sp., *Thiobacillus* sp., and others various of bacteria is effectively used as pollution indicators for detecting the fecal contamination, human activity waste, heavy metals, and crude oil (Sumampouw and Risjani 2014). The results of this bacteria assessment become the consideration in decision making of policy regards to the environmental quality.

EXPERIMENTAL

Collection of Samples: The sites were selected at the bank of Tons river at elevation 805-854 m. 566-577 m. and 603-618 m. at distances 1 to 10 m. from the flow of the water. The soil samples were collected and preserved at 4^{0}C in sterilized plastic bags. Part of the samples was air dried and sieved through 0.2 mm. mesh for analysis of the physico-chemical properties.

Analysis of Physicochemical Properties of Soil: The following processed were applied for each soil samples to estimate the physicochemical properties of soil.

For soil pH: 10 g of soil samples were taken in 100 mL beaker and 20 mL distilled water was added and stirred for 5 minutes. Then the beakers were kept aside for two hours. Then the samples were stirred again and pH was measured with pH-meter. Organic Carbon was estimated by taking 1gm soil sample in conical flask. 10 mL of 1N $K_2Cr_2O_7$ A.R. solution was added. Then 20 mL of concentrated H_2SO_4 was added. 200mL. of double distilled water was added to it and then kept aside for 5-10 minutes. Then 10 mL of orthophosphoric acid was added. 1mL diphenyl amine was added as indicator and titrated with 0.5N $FeSO_4$ solution until a light green colour appeared.

For Potassium: 5 g of soil sample was taken in flask. 25 mL of 1N ammonium acetate was added to it. Then the contents were filtered

through Whatman no 40. The filtrate was fed into the flame photometer and readings are recorded. Phosphorus was estimated by taking 5 g. Of soil, then 100 mL of Olsen's reagent and 1 spoon of carbon black C were added to make the extraction colourless. A blank was also run simultaneously. The contents were shaken for 30 minutes in a mechanical shaker. The contents were filtered through Whatman No 40 filter paper. 10 mL of filtrate was taken in 50mL volumetric flask and 10 mL of ammonium molybdate was added. After CO_2 evolution the neck of the flask was washed down and solution was distilled to 40 mL by adding 20 mL distilled water. 0.25mL (5 drops) of the 0.1M chlorostanmous acid was added. The contents were shaken properly and diluted it to a volume of 50 mL by adding 10 mL distilled water. The observance was recorded at 660 nm with the help of UV spectrophotometer.

Nitrogen: 5 g of soil sample was taken in digestion tube, 20 mL $KmNO_4$ and 50mL of distilled water was added and digestion tube was placed in distillation unit. Finally ammonia liberated during this process was collected in a conical flask containing few drops of mixed indicator and N/50 H_2SO_4. Then titrated with NaOH solution.

Culture of bacteria and slide preparation: 10^{-5} dilution was made from 10 g of soil sample in 0.85% NaCl blank.15 mL nutrient agar was poured in each sterilized petridishes. For each dilution two petridishes were used. 1 mL of the aliquot was spread on the medium of petridish. Then the petridishes were incubated at 37°C for 48 hours in inverted position. After 48 hours the different colonies were counted with the help of colony counter. The number of bacteria per gram soil was calculated by using the following formula-

$$\text{Number of Bacteria (per gram)} = \frac{\text{Number of Colonies x Dilution Factor}}{\text{Dry Weight of Soil}}$$

Dilution Factor = 10

RESULTS AND DISCUSSION

The average pH in soils of different elevation is shown in figure 1. The lowest value was 7.9 in a sample from elevation 603-618 m, whiles the highest value 8.3 was in samples from all three elevations. The average percentage of organic carbon in soils of different elevation is shown in figure 2. The lowest value was 0.54% in a sample from elevation 603-618 m while the highest value 1.68 was in sample from elevation 566-577 m (Figure 2). The average percentage of nitrogen in soils of different elevation is shown in figure 3. The lowest value was 0.01176% in a sample from elevation 566-577 m while the highest value, 0.02240% was in sample from elevation 805-854 m. The average percentage of potassium in soils of different elevation is shown in figure 4. The lowest value was 0.002% in all samples from elevation 566-577 m, while the highest value, 0.004%, was in samples from elevation 603-618 m. The average percentage of phosphorus in soils of different elevation is shown in figure 5. The lowest value was 0.0010 % in all samples from elevation 603-618 m while the highest value, 0.0021 %, was in sample from elevation 603-618 m. The average number of bacteria in soils of different elevation is shown in figure 6. The lowest value was 570000 in a sample from elevation 566-577 m while the highest value, 1650000 was in sample from elevation 603-618 m (Figure 6).

Table 1. Physico-chemical properties of soil with respect to Elevation

Sample Elevation	Distance from water (m)	pH	O.C. %	N %	P %	K %	Number of bacteria
805-854 m	1	8.3	1.40	0.02240	0.0019	0.003	1410000
	5	8.2	1.36	0.02128	0.0017	0.003	1450000
	10	8.2	1.44	0.01568	0.0015	0.002	1370000
603-618 m	1	7.9	0.54	0.02128	0.0010	0.004	970000
	5	8.0	0.80	0.01232	0.0021	0.004	1650000

	10	8.3	1.28	0.01960	0.0016	0.003	1310000
566-577 m	1	8.3	0.90	0.01176	0.0016	0.002	570000
	5	8.3	1.16	0.01728	0.0014	0.002	1300000
	10	8.1	1.68	0.01400	0.0016	0.002	1260000

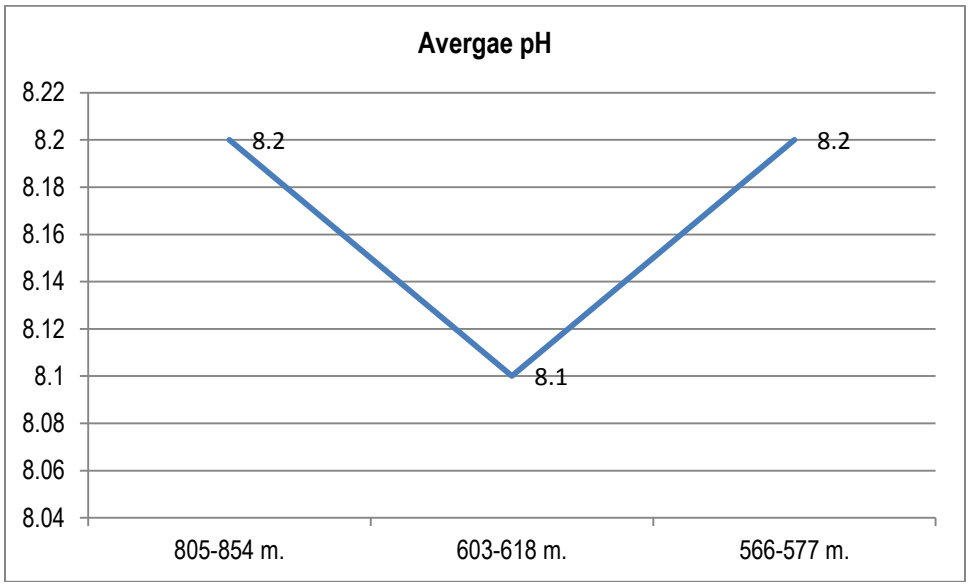

Figure 1. Average pH with Respect to Elevation

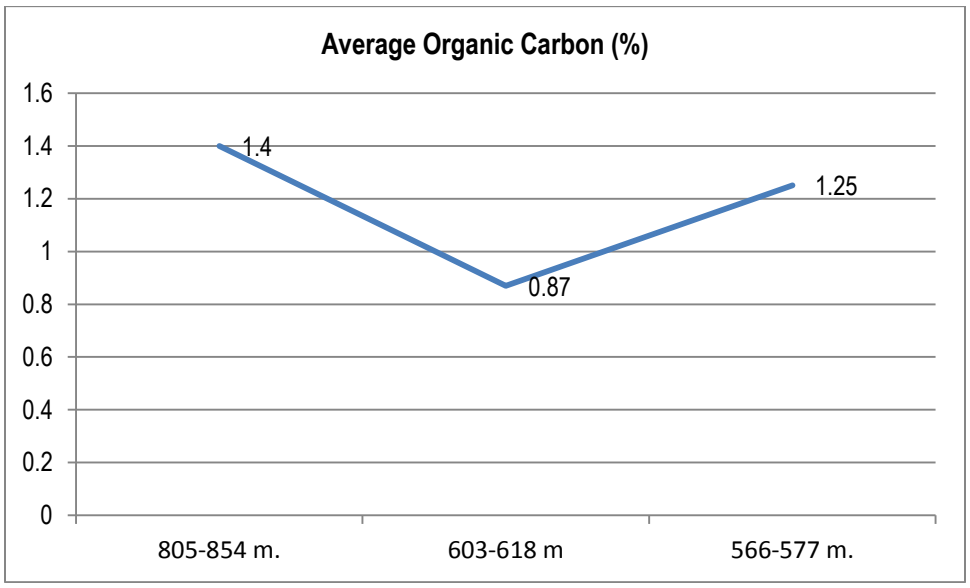

Figure 2. Average OC % with Respect to Elevation

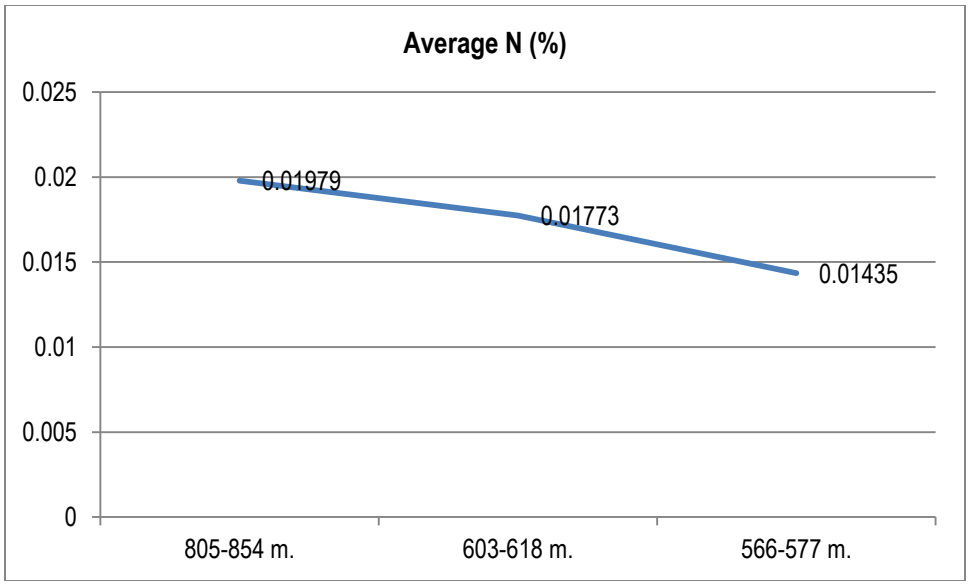

Figure 3. Average N% with respect to elevation

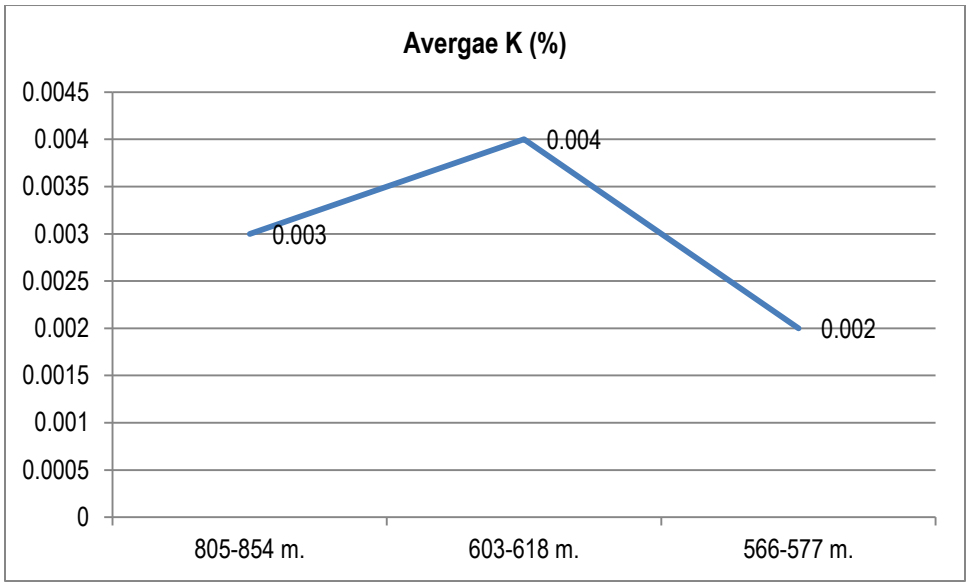

Figure 4. Average K% with Respect to Elevation

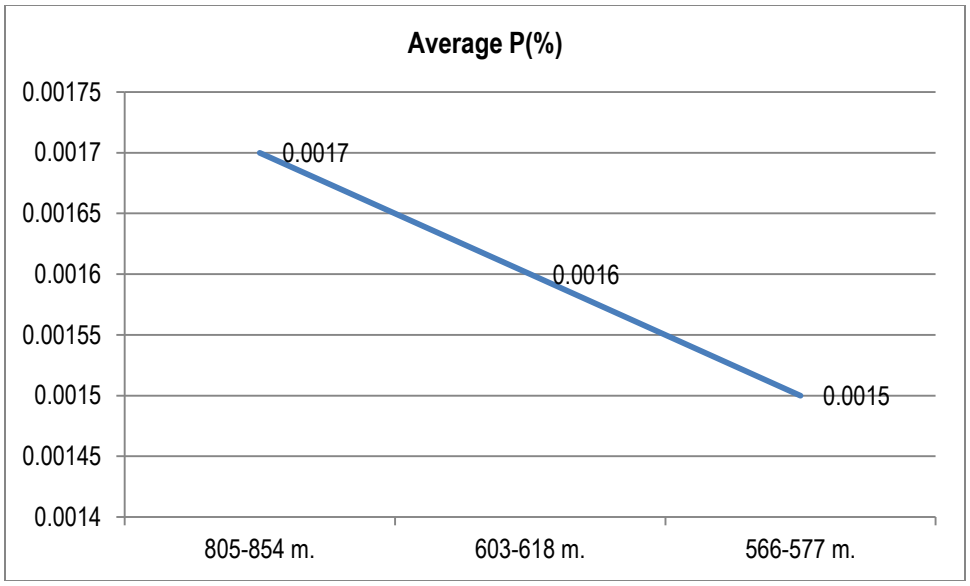

Figure 5. Average P% with respect to Elevation

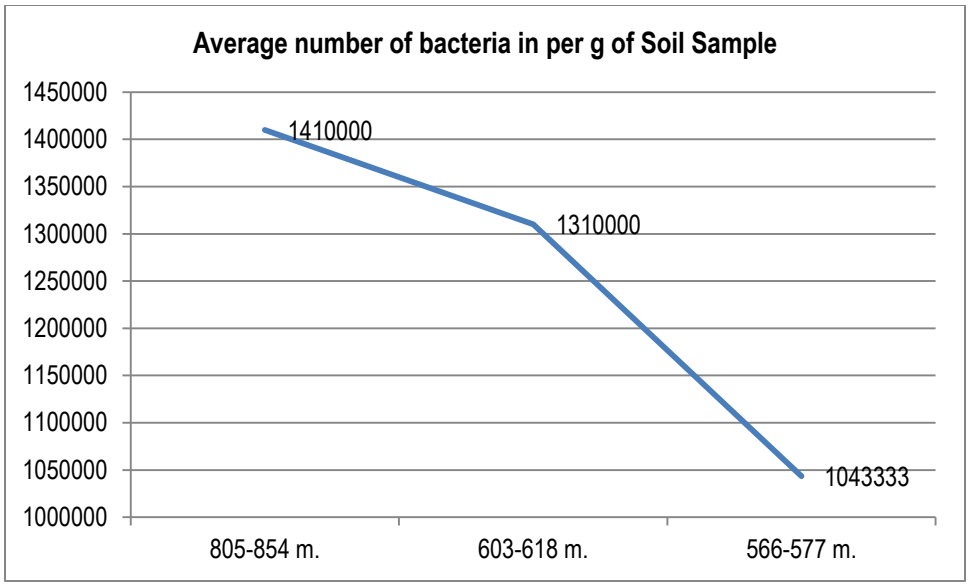

Figure 6. Average Number of bacteria per gram of soil with respect to elevation

The study shows that the average number of bacteria per gm. Of soil decreased according to decrease of elevation. The pH, organic carbon, and potassium are not showing any particular trend of change according to elevation. Average percentage of nitrogen and phosphorus is showing to decrease according to elevation. But the average number of bacteria cannot be directly associated with nitrogen or phosphorus, since in some samples with higher percentage of elevation, nitrogen or phosphorus are showing lower number of bacteria than samples with lower value of them. Hence the study did not come out with a particular relation with soil physicochemical properties with number of bacteria. Hence further studies on species specific relationship with elevation and physicochemical properties of can be done to understand the soil microbial ecosystem of this region.

CONCLUSION

There were several monitoring methods on the environmental quality, especially biological

method. Biological methods assess the presence of several species, such as plants, insects, fish, bacteria and viruses as environmental indicator. Some species of bacteria have been used as indicators in monitoring environmental quality. This study of enumeration is basically done to find a relation between abundance of bacterial species at different elevation at different distance from the river water from the bank of Tons. The chosen sites temporarily remain under water in rainy season and remain partially moist in other times. The samples were collected in the month of August when the temperature of atmosphere is around 29.4-22.4^{0}C and the average rainfall is around 728.2 mm The study shows that the average number of bacteria per gm. of soil decreased with decrease in elevation. Bacterial population is also a measure to monitor environmental quality. Low number of bacteria means that the conditions of that particular environment is not conducive for organism growth and there is some degradation in environmental quality.

REFERENCES

Adams F. and Conrad J.P. (1953). Transition of phosphite to phosphate in soils. *Soil Sci*. 75:361-371

Coyne M.S. (1999). Soil Microbiology: an exploratory approach. Delmar Publishers, Albany, NY.

Foster T.L., Winans L. and Helms S.J.S. (1978). Anaerobic utilization of phosphite and hypophosphite by *Bacillus sp. Applied Environmental Microbiology*: 35:937-944

Hartman W.H., Richardson C., Vilgalys R., and Bruland G.L. (2008). Environmental and anthropogenic controls over bacterial communities in wetland soils. *Proc. NAtl. Acad. Sci*. USA 105: 17842-17847.

Lauber C. L., Hamandy M., Knight R. and Fierer N. (2009). Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale, *Applied and Environmental Microbiology,* pp.5111-5120.

Sumampouw O., Jufri and Risjani Y. (2014). Bacteria as indicators of environmental pollution: review. *International journal of ecosystem*. 6: 251-258

Torsvik V., J. Goksoyr and F.I. Daae (1990). High diversity in DNA of soil bacteria. *Applied and Environmental Microbiology* 56: 782-87.

Vincy M.V., Brilliant R., Kumar P. (2017). Prevalence of indicator and pathogenic bacteria in a tropical river of Western Ghats, India, *Applied Water Science* 2:833–844

Sources of Support: None.
Conflict of interest: None. Declared.