ISOLATION AND IDENTIFICATION OF SOIL MICROORGANISMS FROM INDUSTRIAL BELT OF CUTTACK, ODISHA

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Abstract— Industrialization has led sharp increase in Man made activities and disturbances in the environment. Soil microorganisms act as indicators of soil health and are responsible for breakdown of harmful substances in the soil along with circulation of nutrients. The present study was carried out in the industrial region of Cuttack. The industrial belt has mostly small scale industries which release waste in the form of iron, drug residues and paint residues. The waste decreases the organic matter of the soil which in turn acts as indicator of soil fertility. The study was carried out to isolate and identify various microorganisms and study their role in soil . Different types of gram positive and negative bacteria were isolated and differentiated based on biochemical tests.

Index Terms— Gram positive bacteria, Gram negative bacteria, Bioindicator

1 INTRODUCTION

Soil is composed of broken rock particles that have been altered by weathering and erosion. Soil is altered from its parent material by lithosphere, hydrosphere, atmosphere, and biospheric interactions (Chesworth, 2008).
The biological activity is heavily concentrated in the topsoil upto 30 cm depth. In top soil, the biological components occupied a small fraction (<0.5%) of the total soil volume and make up less than 10% of the total organic matter in soil.

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These biological components consist of mainly soil organisms, especially microorganisms. Soil inhabits several diverse groups of microorganisms, but the most important amongst them are: bacteria, actinomycetes, fungi, algae and protozoa. Microorganisms are the key players in the cycling of nitrogen, sulphur, and phosphorus, and the decomposition of organic residues, thereby affecting nutrient and carbon cycling globally (Pankhurst et al, 1997). The energy is derived from the microbial decomposition of dead plant and animal organic matter. By this process, the organic residues are converted to biomass or mineralized to CO_2, H_2O, mineral nitrogen, phosphorus, and other nutrients. When microbes are grazed by other organisms such as protozoa and nematodes, mineral nutrients immobilized in microbial biomass are released . Microorganisms also play role in transformation and degradation of waste materials and synthetic organic compounds (Torstensson et al, 1998).

Microorganisms respond quickly to changes, hence they are rapidly adapted to environmental conditions. This adaptation allows them to discriminate soil health assessment, changes in microbial populations and activities. Thus they may act as an excellent indicator of change in soil health (Kennedy et al, 1995; Pankhurst et al, 1995).

In comparison to higher organisms, microorganisms respond quickly to environmental stress. Under some circumstances, changes in

microbial populations or activity can provide an early sign of soil improvement or an early warning of soil degradation (Pankhurst et al, 1995).

2 STUDY AREA

The study was carried out from September, 2011-October, 2012, in industrial area surrounding Jagatpur and Khapuria and one sample was collected from SCB Medical College Campus, Cuttack.

3 MATERIALS AND METHOD

Physico-chemical and microbiological tests were carried out of the collected samples. Temperature was recorded on the field. Samples were collected in zip lock plastic pouches and brought to the laboratory for experimental work. Standard methods were followed to conduct various tests (APHA 1992, 1998).Isolation of bacteria from soil samples was done by taking 1 gm of soil sample in a flask and 2 ml of sterile distilled water was added to it. The flask was kept on shaker (120 rpm) for 1 hour. Different dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) were prepared and 0.1 ml suspension was spread from each dilution tube to sterile N-agar plates. All plates were incubated at 28±2^{0}C for 24 hours. Different colonies were observed and three colonies of gram positive and gram negative bacteria were selected for identification. Isolation was followed by identification of selected microorganisms. Loopful growth of bacteria was inoculated into 5 ml sterile N-broth. All tubes were incubated at 28±2^{0}C for growth. HiBacillus Identification Kit (KB013) and HiAssorted biochemical Test Kit was used. The results were interpreted according to standards given in the interpretation chart.

4 RESULTS AND DISCUSSION-

Microorganisms Isolated from the soil samples

	SOIL SAMPLES	
1	**Trimurti (Aluminium Industry)**	
	Gram positive Bacilli	**Gram negative**
	Bacillus subtilis	*Pseudomonas alcaligenes*
	B. thuringensis	*Aeromonas caviae*
	B. coagulans	*E. Vulneris*
2.	**Phyto Drug Industry**	
	B. thuringensis	*Shigella flexneri*
	Bacillus subtilis	*P. monteilii*
	Bacillus subtilis	*E. vulneris*
3.	**Oro Drug Industry**	
	B. cereus	*Shigella sonnei*

	B. thuringensis	*Serratia plymuthica*
	B. subtilis	*Shigella boydii*
4.	**SCB Hospital Waste**	
	B. thuringensis	*Shigella flexneri*
	B. coagulans	*P. aeruginosa*
	B. cereus	*P. alcaligenes*
5.	**Konark Cement Industry**	
	B. cereus	*P. aeruginosa*
	B. subtilis	*E. vulneris*
	B. thuringensis	*P. putida*
6.	**Mahindra (Aluminium Industry)**	
	B. thuringensis	*Vibrio mimicus*
	B. thuringensis	*Aeromonas caviae*
	B. subtilis	*P. aeruginosa*
7.	**Bubuna Drug Industry**	
	B. cereus	*P. putida*
	B. thuringensis	*Aeromonas hydrophila*
	B. coagulans	*Cedecea neteri*

Table 1: List of the Isolated microorganisms (Gram positive and Gram negative)

Culture Plates of Isolated Microorganisms

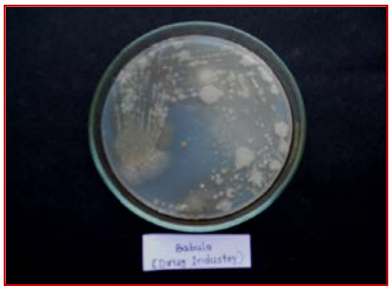

Plate 1.1: Bubuna drug Industry Culture Plate

Plate 1.4: Trimurti Industry culture plate

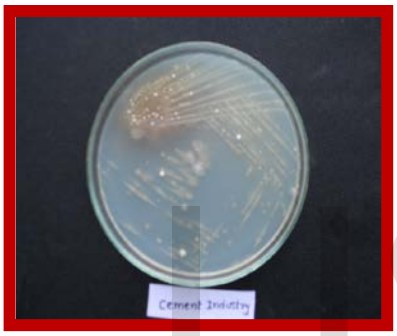

Plate 1.2: Konark Cement Industry Culture Plate

Plate 1.5: Oro drug Industry Culture plate

Plate 1.3: SCB Hospital waste culture plate

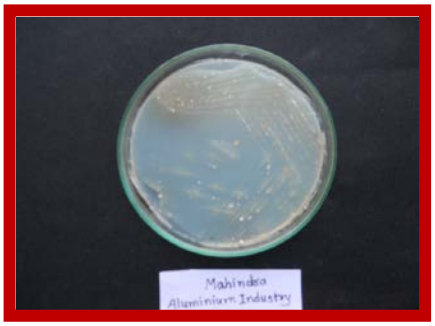

Plate 1.6: Mahindra Aluminium Industry Culture plate

Biochemical Tests for Gram positive & Gram negative Organisms

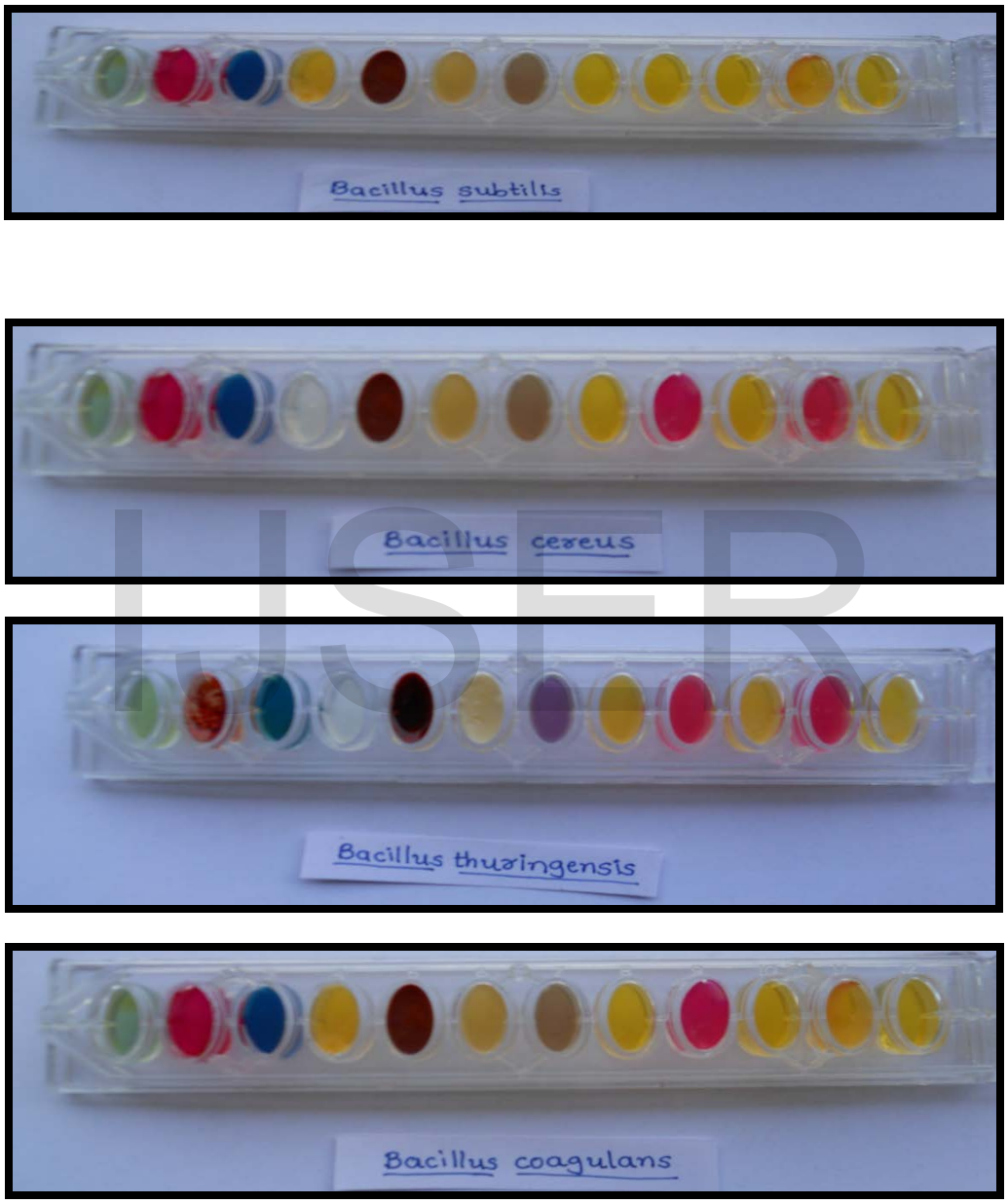

Plate 1.7: Biochemical tests for Gram positive organisms


Pseudomonas alcaligenes


Pseudomonas monteilli


Pseudomonas aeruginosa


Pseudomonas putida

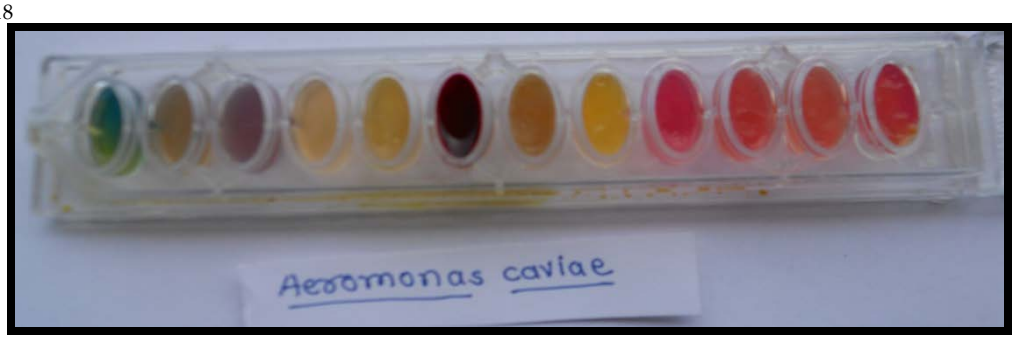

Plate 1.8: Biochemical tests for Gram negative organisms

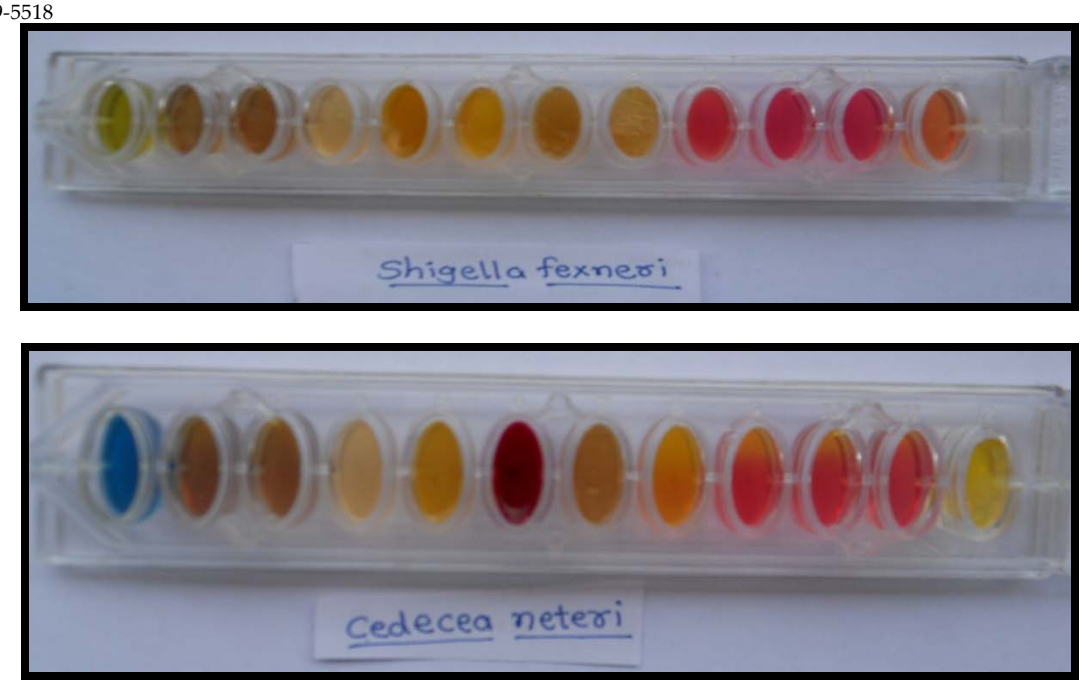

Plate 1.9: Biochemical tests for Gram negative organisms

Plate 2: Biochemical tests for Gram negative organisms

Sr. No.	Test	*Bacillus subtilis*	*Bacillus thuringensis*	*Bacillus coagulans*	*Bacillus cereus*
1	Malonate	-	NR	-	-
2	Voges Proskauer's	+	+	D	+
3	Citrate	+	+	-	+
4	ONPG	+	-	D	-
5	Nitrate reduction	+	+	-	D
6	Catalase	+	+	+	+
7	Arginine	-	+	D	D

8	Sucrose	+	D	D	D
9	Mannitol	+	-	-	-
10	Glucose	+	+	+	+
11	Arabinose	+	-	D	-
12	Trehalose	+	+	+	+

Table 2: Gram Positive Organisms (Result entry data sheet)

\+ = Positive (more than 90%)

\- = Negative

NR = Not Reported

D = Delayed reaction

Test	***Pseudomonas alcaligenes***	***Pseudomonas monteilii***	***Pseudomonas aeruginosa***	***Pseudomonas putida***	***Aeromonas caviae***	***Aeromonas hydrophila***	***Escherichia vulneris***	***Shigella flexneri***	***Shigella sonnei***	***Shigella Boydii***	***Serratia plymuthica***	***Vibrio mimicus***	***Cedecea***
Citrate utilization test	V	+	+	+	V	V	-	-	-	-	V	+	+
Lysine	-	-	-	-	-	V	V	-	-	-	-	+	-
Ornithine	-	-	-	-	-	-	-	-	+	-	-	+	-
Urease	-	-	V	V	-	-	-	-	-	-	-	-	-
TDA	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate Reduction	V	-	+	-	+	+	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	+	-	-	-	-	-	-	-

Production													
Glucose	-	+	+	+	+	+	+	+	+	+	+	+	+
Adonitol	ND	ND	ND	ND	-	-	-	-	-	-	-	-	-
Lactose	-	-	-	V	V	V	V	-	-	-	V	V	V
Arabinose	ND	ND	-	V	+	+	+	V	+	V	+	-	-
Sorbitol	ND	ND	-	ND	-	-	-	V	-	V	V	-	+

Table 3: Gram Negative Organisms (Result entry data sheet)

\+ = Positive (more than 90%)

\- = Negative (more than 90%)

V = 11-89% Positive

ND = No data available

5. CONCLUSION

Soil Microorganisms

Different strains of bacteria have been isolated from sampling sites. The group consisted of both gram positive as well as gram negative bacteria. Soil microbes are sensitive to heavy metals than plants or animals. In trace amounts, many metals like Cu, Mn, Fe, and Zn play a vital role in the metabolism of microbial cells. The distribution of microorganisms in soil is affected by the presence of many heavy metals and contaminants like Cd, Hg and Pb. Soil bacteria are responsible for availability of nitrogen to plants. Our environment has been polluted by anthropogenic activities and many hazardous substances are degraded by soil microorganisms. The soil sample was collected from industrially polluted area where the soil is being contaminated by the release of residues from drug , automobile spare part and paint manufacturing industries. Yet the soil shows the presence of both gram positive and gram negative bacteria.

References

1. APHA, (1992 and 1998). Standard methods for the examination of water and waste water, AWWA and WPCF, 20th edition.

2. Chesworth (2008). *Encyclopedia of soil science*. Dordrecht, Netherlands: Springer, xxiv, ISBN 1-4020-3994-8.

3. Kennedy, A. C. and Papendick, R. I. (1995). Microbial characteristics of soil quality. Journal of soil and water conservation May-June:243-248.
4. Pankhurst, C. E., Doube, B. M., and Gupta, V. V. S. R. (1997). Biological indicators of soil health: Synthesis. In: Biological Indicators of Soil Health. CAB International, pp. 419-435.
5. Pankhurst, C. E., Hawke, B. G., McDonald, H. J., Kirkby, C. A., Buckerfield, J. C., Michelsen, P., O'Brien, K. A., Gupta, V. V. S. R., and Doube, B. M. (1995). Evaluation of soil biological properties as potential bioindicators of soil health. Australian Journal of Experimental Agriculture 35:1015-1028.
6. Torstensson, L., Pell, M., and Stenberg, B. (1998). Need of a strategy for evaluation of arable soil quality. Ambio 27:4-8.