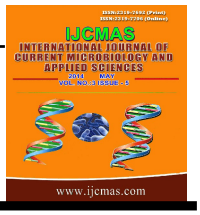
*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 3 Number 5 (2014) pp. 51-58**
**http://www.ijcmas.com**




**Original Research Article**

# Screening and physico - chemical characterization of textile effluent and their effect on *Vigna mungo* growth

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**K e y w o r d s**

Textile waste; *Vigna mungo*; *Bacillus spp;* biochemical test; Gram's staining.

**A B S T R A C T**

The present study was aimed with Textile waste (i.e., liquid and solid) samples were collected from Thirupur District. These samples were subjected to analyse the physico – chemical parameter includes Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Solids, Total Suspended Solid, Total Dissolved Solid as soon as the sample was brought to the laboratory. Estimation of Sulphur, Phosphate, Chloride, Nitrogen, Calcium, Copper, Iron, Manganese and Zinc content were assessed by titrimetric and turbidity method respectively. After incubation following strains of *Alcaligenes spp, Bacillus subtilis, B.pumilus, B.cereus, B.megaterium, B.licheniformis, B.alvei, B.macerans, B.maxima, E.aerogens, E.coli, Klebsiella pneumoniae, Micrococcus spp, Lactobacillus spp, Pseudomonas florescence, P.putida, Streptococcus spp, Staphylococcus spp, S. aureus* and *Serratia spp* were isolated and identified using staining and biochemical test. Total bacterial count was performed by colony counter. Among these species, *Bacillus spp* were effectively produces the protease enzyme. Here, the textile waste was influenced the growth of *Vigna mungo* in planting method.

## Introduction

Diversity has been estimated that our planet is about 4.6 billion years old. Fossilized remains of prokaryotic cells around 3.5 to 3.8 billion years old have been discovered in stromatolites and sedimentary rocks (Brown and Dooliltle 1997). Microbial diversity increased greatly as oxygen became more plentiful (Williams and Embley 1996). Microbial diversity offers an immense field of environment friendly options for mineralization of contaminants on their transformation into less harmful, non-hazardous compounds. In studying, the diversity of indigeneous microorganisms capable of degrading different pollutants because of their varied effects on the environment. It constitutes the most extraordinary reservoir of life in the biosphere. Microbial communities are

subjected to various perturbations, such as variation of pH, temperature, organic loading rates and toxicant level. The ecological studies on microbial communities may provide useful information on their capability of degradation of wastes by native microbes (Pelczar et al., 1986).

Liquid waste is the waterborne human, domestic and farm wastes. It may include industrial effluent, subsoil or surface waters. Human wastes include faecal materials. Domestic wastes include food wastes and wash water. Industrial water borne wastes are acid, oils, greases, animal and vegetable matter discharged by factories. Since liquid wastes from different sources accumulate in sewage, its chemical composition varies depending up on the sources. This also causes variation in the microbial flora (Grand and Long 1981). Solid waste disposal has been an issue which humans, since they began gathering together in large, permanent settlements. With the migration of people to urban settings, the volume of solid waste in a given area greatly increased. There are two basic sources of solid wastes: non municipal and municipal. Non municipal solid waste is the discarded solid material from industry, agriculture and mining oil. Some solid wastes are unsafe to the health and well – beings of humans. Generally, the most common waste product is paper (about 40% of the total) (Angenent et al., 2004). The main objectives of the study is to collect liquid and solid waste from textile industry in Thirupur Dt, Tamil Nadu, South India, to determine physico-chemical parameters of liquid and solid waste of textile mill, to isolate and identify the bacterial sp from textile waste using serial dilution method and plating technique, Gram's staining, motility and biochemical test, to determine the enzymatic activity of predominant organism i.e *Bacillus spp* because it is a major enzyme producer beside some of the gram negative organisms and to study the effect of textile waste (both liquid and solid waste) on V*igna mungo* growth by Pot culture experiment.

## Materials and Methods

### Sample collection and Physico- chemical characteristics of wastes (Qiang et al., 2010)

Textile effluents, sludge and dye contaminated soil samples from effluent sites were collected in sterile sampling carriers in the textile industry of Thirupur Dt, Tamil Nadu, South India. The textile mill solid and liquid dumping wastes collected at monthly interval. The physico - chemical characteristics of samples such as pH, temperature, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Estimation of Total Solids, Estimation of Total Dissolved Solid, Estimation of Total suspended solid, Estimation of Chloride , Sulphate, Nitrogen, Potassium and Phosphorous content were assessed by titrimetric and turbidity method respectively. Analysis of different metal ions in the effluent sample was determined by Atomic Absorbion Spectrophotometer (AAS) as per the standard method.

Serial dilutions were performed by using the collected liquid and solid samples to isolate the bacterial species. The samples were diluted with tube containing 9ml of sterile distilled water and mixed thoroughly to make a 1:10 dillution ($10^{-1}$). Then 1 ml of diluted sample was transferred to the next tube and serially diluted into the series of test tubes having

9 ml of sterile distilled water. 0.1ml of serially diluted sample was taken from $10^{-4}$ to $10^{-7}$ dilution and was spreaded over the nutrient agar plates were incubated at 37°C for 24 hours. After incubation colonies were observed on the plates (Booth 2006). After incubation isolated bacterial *spp* were identified by Gram staining, motility test and biochemical test. The most common method of enumerating the total microbial cells is the direct counting of cell suspension in a counting chamber of known volume using a microscope.

**Screening for Enzymatic activity (Pandobedrinana et al., 2011)**

The single colony observed on the nutrient agar plate and inoculated into Skim Milk Agar plates (Peptone from casein 5.0 g/l, Beef extract- 2.5 g/l, Skim milk powder- 1.0g/l, Glucose- 1.0 g/l, Agar- agar-10.5 g/l, pH 8) and incubated at 37$^{o}$C for 24 hrs. After incubation, a clear zone of Skim milk hydrolysis indicates the presence of protease production organisms.

**Pot Culture Experiment (Parvathi, 1985)**

The seedlings of *Vigna mungo* were transplanted in five pots of equal size 20 cm in height and 6 cm in dm. Garden soil was used as the culture medium. The pots were provided with water facilities. There were 5 treatments resulting from combination of Raw Liquid Waste, Raw Solid Waste, Treated Liquid Waste, Treated Solid Waste and Control. The pots were maintained in the open shade at the temperature of 27ºC - 30ºC. After 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ days of growth 5 plants per pot were removed from all samples and studied for the following morphological parameters. They were, height of the plant (in cm), number of leaves (per plant), number of roots (per plant), shoot length (in cm), root length (in cm), root nodules (per plant).

## Results and Discussion

The present study was aimed to investigate the bacterial diversity from Thirupur district in Tamil Nadu, South India at monthly variations (March 2013 – August 2013).The pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Estimation of Total Solids, Estimation of Total Dissolved Solid, Estimation of Total suspended solid, temperature, macronutrients (Chloride, Sulphur, Nitrogen, Calcium and Phosphate) and micronutrients (Iron, Copper, Zinc and Manganese) were analysed and compared with seasonal variation of Thirupur District (Table-1).

Samples were used for the isolation of Bacterial species using serial dilution and plating methods. Serially diluted sample was poured into the nutrient medium showed the number of bacterial species. These colonies were identified by gram staining using Bergey's Manual of determinative bacteriology.

Bacterial isolates such as of *Alcaligenes spp, Bacillus subtilis, B.pumilus, B.cereus, B.megaterium, B.licheniformis, B.alvei, B.macerans, B.maxima, E.aerogens, E.coli, Klebsiella pneumonia, Micrococcus spp, Lactobacillus spp, Pseudomonas florescence, P.putida, Streptococcus spp, Staphylococcus spp, S. aureus*and *Serratia spp* were isolated and identified in textile liquid and solid waste of Thirupur Dictrict (Table – 2).

*Bacillus* species were dominantly present in textile effluent then other bacterial isolates. The textile waste (i.e., liquid and

solid) containing several types of bacterial species. They were identified by Biochemical test includes Indole, MR, VP, Citrate, Catalase and Urease and Gram's staining technique (Table – 3). Total bacterial population were analysed by using colony counter. In April month maximum growth of bacterial population were identified.

The protease producing *Bacillus spp* were identified with the help of the zone formation in the Skim Milk Agar medium. The zone was formed due to the proteolytic activity of the organisms which cleaves protein molecules in the Skim Milk Agar Medium

**Pot culture Experiment**

The effect of Textile waste (i.e., liquid and solid waste) on the growth of *Vigna mungo* was studied and compared with control. After seed inoculation, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day to observe the morphological characteristics and photochemical analysis was carried out. After $28^{th}$ day of seedling, the maximum height, number of leaves, number of leaves, shoots length, root length and root nodules were noted in T3 and $T_4$ compared with other treatment include $T_1$, $T_2$ and Control (Table - 4).

Our finding similar to the (Arun Prasad and Bhaskara Rao 2010) dye decolorizing isolates, *Bacillus* sp., *Klebsiella sp. Salmonella* sp. and *Pseudomonas* sp. were isolated from the textile effluent samples collected from Elampillai, Tamil Nadu. Different parameters such as various carbon source, nitrogen source, temperature, pH and inoculum size were optimized for decolorization of Orange 3R by using bacterial isolates. *Pseudomonas* sp. and *Bacillus* sp. showed maximum dye decolorization of 89% at the end of under optimum condition. But the *Bacillus* sp. was found to be more efficient in dye decolorization. All parameters studied in this paper were found to be effective for all isolates. The results reported here warrant further investigation to establish the usefulness of these isolates for bioremediation and biodegradation application such as waste water treatment. High decolorization extent and conditions show the potential for this bacterial strain to be used in the biological treatment of dyeing mill effluents.

Our finding similar to (Forster and Wase 1987) effluent quality depends not only on the treatment process in the aeration tank, but also on the separation of the flocs from the treated effluent by a final sedimentation tank. The latter was dependent on flocs settle ability which was primarily affected by the density of the flocs, as in the case of sludge bulking caused by filamentous bacteria such as *B.licheniformis* which interferes with the performance of many sewage works both locally and overseas. Another operational problem commonly encountered was bacterial foaming which was caused by the excessive growth of filamentous bacteria such as *K.pneumoniae*. The control measures for these problems will be discussed.

In the present investigation, it is found that the total bacterial diversity and physiological grouping in the textile liquid and solid waste disposal in textile mill of Thirupur. Our results of this findings and literature suggest a great potential for bacteria to be used to remove color from dye waste. This observation has established that the bacteria are adaptive in nature and can degrade contaminants.

**Table-1; Physico - Chemical Parameter of Textile Waste**

| Physico - Chemical Parameter of textile waste | March | | April | | May | | June | | July | | August | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Liquid | Solid | Liquid | Solid | Liquid | Solid | Liquid | Solid | Liquid | Solid | Liquid | Solid |
| pH | 7.4 | 6.9 | 6.4 | 5.8 | 7.2 | 6.1 | 6.8 | 7.2 | 7.9 | 7.1 | 7.2 | 8.1 |
| Temperature (0°) | 33 | 34 | 38 | 31 | 32 | 29 | 28 | 30.4 | 31.2 | 30 | 32.6 | 39 |
| TS (mg/l) | 926 | 811 | 710 | 789 | 826 | 812 | 820 | 856 | 799 | 898 | 739 | 792 |
| TDS (mg/l) | 5875 | 2715 | 4915 | 2613 | 5813 | 1912 | 5118 | 2317 | 4135 | 3115 | 5995 | 3175 |
| TSS (mg/l) | 1995 | 890 | 995 | 1170 | 1100 | 1110 | 710 | 958 | 815 | 920 | 850 | 795 |
| Chloride (mg/l) | 36 | 33 | 32 | 40 | 41 | 43 | 45 | 40 | 46 | 39 | 33 | 34 |
| Sulphur (mg/l) | 24 | 32 | 31 | 31 | 29 | 25 | 19 | 17 | 18 | 31 | 35 | 29 |
| Nitrogen (mg/l) | 0.1 | 0.2 | 0.6 | 0.5 | 0.4 | 0.3 | 0.7 | 0.10 | 0.7 | 0.8 | 0.9 | 0.8 |
| Calcium (mg/l) | 0.16 | 0.17 | 0.11 | 0.18 | 0.68 | 0.71 | 1.72 | 0.92 | 0.71 | 0.81 | 0.91 | 0.31 |
| Phosphate (mg/l) | 0.22 | 0.28 | 0.11 | 0.48 | 0.33 | 0.31 | 0.30 | 0.23 | 0.12 | 0.13 | 0.71 | 0.69 |
| Copper (ppm) | 0.22 | 0.28 | 0.33 | 0.24 | 0.25 | 0.12 | 0.18 | 0.17 | 0.16 | 0.11 | 0.19 | 0.90 |
| Manganese( ppm) | 0.43 | 0.38 | 0.49 | 0.31 | 0.33 | 0.58 | 0.71 | 0.89 | 0.91 | 0.56 | 0.67 | 0.79 |
| Iron (ppm) | 0.21 | 0.11 | 0.09 | 0.14 | 0.31 | 0.37 | 0.41 | 0.59 | 0.61 | 0.72 | 0.54 | 0.23 |
| Zinc (ppm) | 0.31 | 0.23 | 0.38 | 0.81 | 0.33 | 0.39 | 0.37 | 0.53 | 0.38 | 0.41 | 0.43 | 0.33 |
| BOD (mg/l) | 435 | 398 | 415 | 318 | 491 | 382 | 475 | 351 | 485 | 389 | 400 | 415 |
| COD (mg/l) | 890 | 564 | 650 | 863 | 950 | 750 | 917 | 812 | 913 | 893 | 814 | 918 |

**Table.2** Morphological and Cultural characterization of Bacterial isolates

| S.No | Isolated organisms | Gram's staining | Shape | Motility |
|---|---|---|---|---|
| 1 | *Alcaligenes spp* | - | Rod | Motile |
| 2 | *Bacillus subtilis* | + | Rod | Motile |
| 3 | *B.pumilus* | + | Rod | Motile |
| 4 | *B.cereus* | + | Rod | Motile |
| 5 | *B.megaterium* | + | Rod | Motile |
| 6 | *B.licheniformis* | + | Rod | Motile |
| 7 | *B.alvei* | + | Rod | Motile |
| 8 | *B.macerans* | + | Rod | Motile |
| 9 | *B.maxima* | + | Rod | Motile |
| 10 | *E.aerogenes* | - | Rod | Non-Motile |
| 11 | *E.coli* | + | Rod | Motile |
| 12 | *K.pneumoniae* | + | Rod | Motile |
| 13 | *Micrococcus sp* | + | Spherical | Non-Motile |
| 14 | *Lactobacillus sp* | + | Rod | Non-Motile |
| 15 | *P. fluroscence* | - | Rod | Non-Motile |
| 16 | *P.putida* | - | Rod | Non-Motile |
| 17 | *Streptococcus sp* | + | Cocci | Non-Motile |
| 18 | *Staphylococcus sp* | + | Cocci | Non-Motile |
| 19 | *S. aureus* | + | Cocci | Non-Motile |
| 20 | *Serratia spp* | - | Rod | Motile |

**Table.3** Biochemical Characterization of Isolated Bacterial isolates from Liquid and Solid Waste

| S.No | Isolated organisms | Indole | MR | VP | Citrate | TSI | Urease | Catalase |
|---|---|---|---|---|---|---|---|---|
| 1 | *Alcaligenes spp* | - | + | - | + | A/A | - | + |
| 2 | *Bacillus.subtilis* | - | + | - | - | A/A | - | + |
| 3 | *B.pumilus* | - | + | - | + | K/A | + | - |
| 4 | *B.cereus* | - | + | - | - | A/A | - | + |
| 5 | *B.megaterium* | - | + | - | - | A/A | - | + |
| 6 | *B.licheniformis* | - | + | + | + | A/A | - | + |
| 7 | *B.alvei* | - | - | + | - | A/A | + | + |
| 8 | *B.macerans* | - | + | + | - | A/A | + | - |
| 9 | *B.maxima* | + | + | - | - | K/A | - | + |
| 10 | *E.aerogenes* | - | + | + | + | A/A | + | - |
| 11 | *E.coli* | + | + | - | - | K/A | - | + |
| 12 | *K.pneumoniae* | - | - | + | + | A/A | - | + |
| 13 | *Micrococcus sp* | - | + | - | + | K/A | + | - |
| 14 | *Lactobacillus sp* | - | - | + | + | A/A | + | - |
| 15 | *P. fluroscence* | - | - | + | + | A/A | - | + |
| 16 | *P.putida* | - | - | - | + | A/A | - | + |
| 17 | *Streptococcus sp* | - | + | + | - | A/A | + | - |
| 18 | *Staphylococcus sp* | - | + | - | - | K/A | - | + |
| 19 | *S. aureus* | - | + | - | - | K/A | - | + |
| 20 | *Serratia spp* | - | - | + | + | A/A | - | + |

**Note :** (+) – Positive (-) – Negative (A/A) – Acid / Alkaline (K/A) – Acid/ Butt

**Table.4** Morphological parameters of *Vigna mungo* seedlings after treatment
Treated values are represented as Mean ± Standard deviation

| S. No | Morphological Parameters | 7th day | | | | | 14th day | | | | | 21st day | | | | | 28th day | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ | $T_4$ | C | $T_1$ | $T_2$ | $T_3$ | $T_4$ | C | $T_1$ | $T_2$ | $T_3$ | $T_4$ | C | $T_1$ | $T_2$ | $T_3$ | $T_4$ | C |
| 1 | Height of the Plant (in cm) | 1.2 ± 1.3 | 1.1 ± 1.2 | 1.9 ± 2.0 | 2.0 ± 2.1 | 1.1 ± 1.2 | 1.8 ± 1.9 | 1.7 ± 1.8 | 2.4 ± 2.3 | 2.6 ± 2.7 | 1.5 ± 1.6 | 2.0 ± 2.1 | 1.9 ± 2.0 | 2.6 ± 2.7 | 2.7 ± 2.8 | 1.8 ± 1.9 | 2.4 ± 2.5 | 2.1 ± 2.2 | 3.2 ± 3.3 | 3.0 ± 3.1 | 2.5 ± 2.6 |
| 2 | Number of leaves (per plant) | 4 ± 5 | 3 ± 4 | 6 ± 7 | 7 ± 8 | 4 ± 5 | 5 ± 6 | 5 ± 6 | 9 ± 8 | 10 ± 11 | 4 ± 5 | 8 ± 9 | 8 ± 9 | 15 ± 16 | 16 ± 17 | 7 ± 8 | 9 ± 10 | 10 ± 11 | 18 ± 19 | 17 ± 18 | 11 ± 12 |
| 3 | Number of roots (per plant) | 2 ± 3 | 1 ± 2 | 3 ± 4 | 4 ± 5 | 3 ± 4 | 4 ± 5 | 3 ± 4 | 7 ± 8 | 8 ± 9 | 3 ± 4 | 5 ± 6 | 6 ± 7 | 9 ± 10 | 8 ± 9 | 5 ± 6 | 6 ± 7 | 7 ± 8 | 11 ± 12 | 15 ± 16 | 10 ± 11 |
| 4 | Shoot length (in cm) | 0.2 ± 0.3 | 0.1 ± 0.1 | 0.4 ± 0.5 | 0.4 ± 0.5 | 0.2 ± 0.3 | 0.3 ± 0.4 | 0.2 ± 0.3 | 0.7 ± 0.8 | 0.8 ± 0.9 | 0.4 ± 0.5 | 0.5 ± 0.6 | 0.4 ± 0.5 | 0.8 ± 0.9 | 0.7 ± 0.8 | 0.5 ± 0.6 | 0.6 ± 0.7 | 0.7 ± 0.9 | 0.9 ± 0.8 | 0.7 ± 0.8 | 0.5 ± 0.6 |
| 5 | Root length (in cm ) | 0.1 ± 0.2 | 0.2 ± 0.3 | 0.3 ± 0.4 | 0.6 ± 0.7 | 0.4 ± 0.5 | 0.3 ± 0.4 | 0.4 ± 0.5 | 0.6 ± 0.7 | 0.7 ± 0.7 | 0.5 ± 0.6 | 0.4 ± 0.5 | 0.6 ± 0.7 | 0.8 ± 0.9 | 0.9 ± 1.0 | 0.5 ± 0.6 | 0.5 ± 0.6 | 0.7 ± 0.8 | 0.8 ± 0.9 | 0.9 ± 0.10 | 0.6 ± 0.7 |
| 6 | Root nodules (per plant) | 2 ± 2 | 4 ± 5 | 5 ± 6 | 6 ± 7 | 4 ± 5 | 3 ± 4 | 5 ± 6 | 7 ± 8 | 8 ± 9 | 6 ± 7 | 5 ± 6 | 8 ± 9 | 7 ± 8 | 7 ± 8 | 5 ± 6 | 6 ± 7 | 9 ± 10 | 9 ± 10 | 10 ± 11 | 7 ± 8 |

**Note: $T_1$ – Raw Liquid Waste ,$T_2$ – Raw Solid Waste, $T_3$ – Treated Liquid Waste, $T_4$ – Treated Solid Waste, C - control**

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