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ORIGINAL RESEARCH ARTICLE

# Effect of Bacterial Isolates on Reduction of Physico – Chemical Characteristics in Sugar Mill Effluent

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**ABSTRACT**

Environmental pollution has been recognized as one of the major problems of the modern world. Sugar industry is one of the most important agro based industries in India and is highly responsible for creating significant impact on rural economy in particular and countries economy in general. The sugar industry is playing an important role in the economic development of the Indian sub continent, but the effluents released produce a high degree of organic pollution in both aquatic and terrestrial ecosystems. They also alter the physico-chemical characteristics of the receiving aquatic bodies and affect aquatic flora and fauna. In the present study, the effect of bacterial isolates on the reduction of physico – chemical characteristics in sugar mill effluent was investigated. The sugar mill effluent was bioremediated by using the bacteria isolated from sugar mill effluent. Maximum reduction of physico – chemical characteristics was observed in the *Bacillus subtilis* followed by *Serratia marcescens*, *Enterobacter asburiae*, *Pseudomonas fluorescens*, *Bacillus weihenstephanensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Brevibacterium halotolerance* whereas *Proteus mirabilis* showed least reduction of physico - chemical characteristics in sugar mill effluent.

**Key words:** Sugar mill effluent, Physico-chemical properties, Bioremediation and Bacteria.

## 1. INTRODUCTION

Sugar industry is backbone of rural, agricultural and socioeconomic development in India. Many industries are directly or indirectly dependent on sugar industry which in turn is responsible for overall development of state in this context sugarcane production is of vital importance for its products and by-products. Disposal of industrial waste is the major cause of soil pollution. Number of chemical industries, mainly tannery, paper, textiles, atomic and electric power plants discharge pollutants. They content organic and inorganic as well as non biodegradable material touch toxic chemicals affect the soil parameters and there by the soil fertility.

Sugar factory effluent has an obnoxious odour and unpleasant colour when released into the environment without proper treatment. Farmers have been using these effluents for irrigation, and found that the growth, yield and soil health were reduced. Contaminants, such as chloride, sulphate, phosphate, magnesium and nitrate, are discharged with the effluent of various industries, which create a nuisance due to physical appearance, odour and taste. Such harmful water is injurious to plants, animals and human beings. The effects of various industrial effluents on seed germination, growth and yield of crop plants have captivated the attention of many workers [1, 2].

Diverse sugar industry effluents disposed of in soil and water cause major pollution problems. The sugar industry plays an important role in the economic development of India, but the effluents released produce a high degree of organic pollution in both aquatic and terrestrial ecosystems [3]. The effluents also alter the physico-chemical characteristics, and flora and fauna of receiving aquatic bodies. In addition, sugar factory effluent discharged in the environment poses a serious health hazard to the rural and semi-urban populations that use stream and river water for agriculture and domestic purposes. Fish mortality and damage to paddy crops due to sugar industry waste-waters entering agricultural land have been reported [4]. Sugar factory effluent that has not been treated properly has an unpleasant odor when released into the

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environment. Farmers using these effluents for irrigation to reduce water demand have found that plant growth and crop yield were reduced and soil health was compromised. Because sugar industry effluents are commonly used for irrigation, it is essential to determine how crops respond when exposed to industrial effluents.

In general, sugar mill effluents contain a significant concentration of suspended solids, dissolved solids, a high BOD, COD, considerable amount of chlorides, sulphates, nitrates, calcium, magnesium and sugar concentration. For mills that have an attached distillery, the numerous distillation stages produce a highly contaminated effluent, with BOD and COD concentration of about 40,000-100,000 mg/L, called sillage. In addition to that, heavy metals such as zinc, copper, magnesium, lead and iron were usually seemed to be present in the effluent [5].

Earlier several microbes were reported to take effective part in bioremediation of industrial wastes [6, 7]. The mechanism by which microorganisms act includes biosorption, intracellular accumulation and enzyme-catalyzed transformation [8]. On the basis of energetic requirements, biosorption seems to be the most common mechanism [9]. Furthermore, this is the only option where dead cells can be applied as bioremediation agent. Nevertheless, systems with living cells allow more effective bioremediation processes as they can self-replenish and remove metals *via* different mechanisms [10]. In the present study, the effect of bacterial isolates on the reduction of physico – chemical characteristics in sugar mill effluent was investigated.

## 2. MATERIALS AND METHODS

### 2.1. Collection of sugar mill effluent sample

The Sugar mill effluent to be bioremediated was collected from the outlet of Mooungilthuraipattu, Villupuram district, Tamil Nadu, India. Before sampling the effluent, the polythene container was cleaned thoroughly using distilled water. Immediately after the effluent sampling, the effluent sample was taken to the laboratory and stored at room temperature in the laboratory for further analysis using standard methods.

### 2.2. Analysis physico-chemical characteristics of sugar mill effluent

The collected sugar mill effluent was assessed for various physico - chemical parameters like colour, odour, temperature, pH, Electrical conductivity, Turbidity, etc. Physico-chemical properties such as total suspended solids (TSS), total dissolved solids (TDS), total hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), chloride, calcium, magnesium, sulphate, nitrogen, phosphorous, sodium, potassium and heavy metals (Iron, Zinc, Copper, Lead and Manganese) were measured using standard methods [11].

### 2.3. Bacterial isolates used

The bacterial isolates used for the bioremediation of sugar mill effluent were, *Proteus mirabilis*, *Bacillus subtilis*, *Serratia marcescens*, *Enterobacter asburiae*, *Pseudomonas fluorescens*, *Bacillus weihenstephanensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Brevibacterium halotolerance*).

### 2.4. Bioremediation of sugar mill effluent by bacterial isolates

The sugar mill effluent was bioremediated by using the bacterial isolates. The suspension of 24 hrs fresh cultures of bacterial isolates was used for the bioremediation studies. The bacterial suspensions were prepared in saline solution (0.89% sodium chloride). A loopful of culture was inoculated in the saline (100 ml) and incubated at 37°C for 24 hours [12]. After that, the bacterial suspensions (100ml/L) were inoculated into the sugar mill effluent and incubated at room temperature. The physico – chemical characteristics of the bacterially bioremediated effluent was analyzed by using standard methods [11].

## 3. RESULTS AND DISCUSSION

Sugar industry is one of the most important agro based industries in India and is highly responsible for creating significant impact on rural economy in particular and countries economy in general. Sugar industries rank second among the agro based industries in India. Sugar industry is seasonal in nature and operates only for 120 to 200 days in a year. A significant large amount of waste is generated during the manufacture of sugar and contains a high amount of production load particularly in items of suspended solids, organic matters, effluent, sludge, pressmud and bagasse.

In the present study, physico – chemical characteristics of the collected sugar mill effluent was analyzed and the results were showed in (**Table 1**). The sugar mill effluent was acidic nature with brown colour and emitted unpleasant smell. The temperature of collected sugar mill effluent was 33°C and the EC was 2.58 $dSm^{-1}$. The TSS and TDS present in collected sugar mill effluent were 479 and 1540 mg/L respectively. It also showed high value of BOD (1090 mg/L) and COD (3260 mg/L). High amount of calcium (393 mg/L), magnesium (299 mg/L), chloride (377 mg/L), sodium (106 mg/L), potassium (125 mg/L), sulphate (430 mg/L), nitrogen (1300 mg/L), phosphorous (6.17 mg/L) and the toxic heavy metals ($Fe^{2+}$- 17.00 mg/ L, $Zn^{2+}$- 0.79 mg/L, $Pb^{2+}$- 0.52 mg/L, $Cu^{2+}$- 0.346 mg/L and $Mn^{2+}$- 0.095 mg/L) were recorded in the collected sugar mill effluent sample.

Similar findings which were recorded in the present study was also reported by Lakshmi and Sundaramoorthy [13]; Rathore *et al*. [14]; Thamizhiniyan *et al*. [15] and Borale and Patil [16]. The variations in physico- chemical properties may be due to the processes involved, raw materials used and chemicals used in the sugar mill. The acidic nature of sugar factory effluent may be due to the release of hydrochloric acid during the process of extraction of sugar from sugar cane as reported by Balagopal *et al*. [17]. The presence of high BOD clearly indicated the high quality of biological oxidizable organic matter. The high content of BOD causes oxygen depletion, where as presence of high level of total suspended solids and total dissolved solids might be due to the insoluble organic and inorganic matter present in the effluent [18]. The increased COD levels in the effluent could be due to the presence of high amount of suspended solids.

The changes in colour and odour of the bacterially treated sugar mill effluent were analyzed in the present study. The untreated raw effluent was brown in colour and emitted unpleasant odour. The sugar mill effluent treated with *Bacillus subtilis*, *Serratia marcescens*, *Enterobacter asburiae*, *Pseudomonas fluorescens* and *Bacillus weihenstephanensis* was colourless and odorless. Light brown colour and unpleasant odour was observed in the sugar mill effluent treated with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. No colour and odour reduction was observed in *Brevibacterium halotolerance* and *Proteus mirabilis* treated sugar mill effluent. The effluent treated with *Brevibacterium halotolerance* and *Proteus mirabilis* was brown in colour and emitted unpleasant odour. Colour is very important factor for the aquatic life for making food from sun-rays. This photosynthesis activity reduced due to dark colouration is affecting other properties (**Table 2**).

The water temperatures plays on important role in influencing abundance the water discharged from industries which has generally higher temperature affect the land adversely discharging not effluents also cause loss of heat energy. Temperature is basically important for its effect on certain chemical & biological radiations taking place in water for organism & inhabiting aquatic media. The rise in temperature accelerated the chemical reaction in oxygen. Beruch *et al*. [19] recorded the temperature 18% degree of the Gelabil in Assam. Kannan and Rajasekaran [20] recorded the value of temperature of printing effluent 28.80°C. pH is one of the important biotic factor that serves as an index for pollution. The wide narration in the pH value of effluent can affect the rate of biological reaction and survival of various microorganisms. The presence or absence of various ionic special can have direct relation with pH of the effluent. Senthil kumar *et al*. [21] observed the pH of sugar mill effluent was in between 6.0 to 7.6. Matkar [22] reported that the pH of sugar mill effluent was 4.5. In the present study, temperature, pH and electrical conductivity (EC) of the untreated raw effluent were 36°C, 4.64 and 2.58 $dSm^{-1}$. EC is the measure of the ability of an aqueous solution to convey an electric current. This ability depends upon the presence of ions, their total concentration, mobility, valence and temperature. The bacterial isolate *Bacillus subtilis* showed best reduction in temperature and electrical conductivity (32°C and 2.27 $dSm^{-1}$) followed by *Serratia marcescens*, *Enterobacter asburiae*, *Pseudomonas fluorescens*, *Bacillus weihenstephanensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Brevibacterium halotolerance* whereas *Proteus mirabilis* showed the lowest reduction in temperature and electrical conductivity (**Fig 1**). The results indicate the high level of the pH (4.64) was recorded in sugar mill effluent and fall within the permissible limits. The pH in the effluent is towards the higher value indicating the acidity conditions and thus will have an adverse effect on the soil permeability and soil microflora [23]. The acidic nature of sugar mill effluent was highly reduced by the bacterial isolate *Bacillus subtilis* (pH – 6.5).

Total Dissolved Solids (TDS) refers to all dissolved materials present in the water. Combined sugar mill effluents generally do not have a TDS measure high enough to have an adverse environmental impact. Discharge of water with a high TDS level would have adverse impact on aquatic life, render the receiving water unfit for drinking, reduce crop yields if used for irrigation, and exacerbate corrosion in water systems [24]. Total suspended solids (TSS) reduce light penetration and, as a result, plant production in the

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receiving water body by increasing turbidity and can also clog fish gills [25, 26]. In the present study, TSS and TDS was highly reduced by *Bacillus subtilis* (TSS – 330 mg/L; TDS – 775 mg/L) followed by *Serratia marcescens*, *Enterobacter asburiae*, *Pseudomonas fluorescens*, *Bacillus weihenstephanensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Brevibacterium halotolerance*. The bacteria *Proteus mirabilis* (TSS – 470 mg/L; TDS – 1503 mg/L) showed the lowest reduction of TSS and TDS (**Fig 2**). Thorat *et al*. [27] the industrial effluent waste and observed TDS 2850 mg/L. Rao *et al*. [28] studied industrial effluent and recorded TDS which range from 8700 mg/L to 10000 mg/L. Avasan Maruthi *et al*. [29] reported the TSS of sugar mill effluent as 220 to 790mg/L.

Reduction BOD and COD of bacterially treated effluent was studied in the present research. The BOD and COD were highly reduced by *Bacillus subtilis* (BOD – 588 mg/L; COD – 1323 mg/L) followed by *Serratia marcescens*, *Enterobacter asburiae*, *Pseudomonas fluorescens*, *Bacillus weihenstephanensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Brevibacterium halotolerance*. The bacteria *Proteus mirabilis* (BOD – 1082 mg/L; COD – 3205 mg/L) showed the lowest reduction of BOD and COD (**Fig 3**). Senthil kumar *et al*. [21] observed the BOD of sugar mill effluent in 635 mg/lit to 950 mg/lit range. Trivedi *et al*. [30] observed the effluent of a sugar mill from different unit BOD value of mixed effluent ranged between 320 mg/L to 720 mg/L and final effluent 80 mg/L to 640 mg/L. He also observed the COD value as 2400 mg/L.

Reduction in Hardness and Oil & grease of bacterially treated effluent was assessed. The Hardness and Oil & grease were highly reduced by *Bacillus subtilis* (Hardness – 32 mg $CaCO_3$/L; Oil & grease – 14 mg/L) followed by *Serratia marcescens*, *Enterobacter asburiae*, *Pseudomonas fluorescens*, *Bacillus weihenstephanensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Brevibacterium halotolerance*. The bacteria *Proteus mirabilis* (Hardness – 274 mg $CaCO_3$/L; Oil & grease – 18 mg/L) showed the lowest reduction of Hardness and Oil & grease (**Fig 4**).

Chlorides are generally present in natural water. The presence of chloride in natural water attributed to dissolution of salt deposits discharge of effluents from chemical industries oil well operations, sewage discharges initiation drainage, contamination from refuse leachates, and sea water intrusion in coastal area. Matkar [31] observed that the effluent from sugar industry was having 450 mg/lit and 455 mg/lit of chloride in untreated effluent and the treated effluent contains 156 mg/lit and 162 mg/L of chloride. Reduction of calcium, magnesium, chloride, sodium, potassium, sulphate, nitrogen and phosphorous concentration in bacterially treated sugar mill effluent was analyzed. The contents were highly reduced by *Bacillus subtilis* followed by *Serratia marcescens*, *Enterobacter asburiae*, *Pseudomonas fluorescens*, *Bacillus weihenstephanensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Brevibacterium halotolerance* whereas *Proteus mirabilis* showed the lowest reduction (**Fig 5, 6 & 7**).

Sulphate may enter natural water through weathering of deposits. Effluent from certain industries also may be major sources of sulphate to be receiving water. Suphur itself has never been limiting factor in aquatic system, the normal levels of sulphates are more than adequate to meet plants needs, odours conditions are easily greater when water is over loaded with organic waste to the point that oxygen is removed, that $SO_4$ as electron acceptor is often used for the breakdown of organic matter and produced $H_2S$ and rotten egg smell. Manal [32] observed sulphate in sugar industry effluent that was 550 mg/ lit and 555 mg/lit in untreated and treated effluent showed 256 mg/lit and 262 mg/lit. Senthil kumar *et al*. [21] observed sulphate range between 200 mg/lit to 93 mg/lit according to flowing distance from effluent discharging unit to 5 km long.

Reduction of heavy metals (zinc, iron, copper, lead and manganese) concentration in bacterially treated sugar mill effluent was determined. The heavy metals content were highly reduced by *Bacillus subtilis* (Zinc – 0.55 mg/L; Iron – 14.7 mg/L; Copper – 0.223 mg/L; Lead – 0.30 mg/L and Manganese – 0.058 mg/L) followed by *Serratia marcescens*, *Enterobacter asburiae*, *Pseudomonas fluorescens*, *Bacillus weihenstephanensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Brevibacterium halotolerance*. The *Proteus mirabilis* (Zinc – 0.78 mg/L; Iron – 16.9 mg/L; Copper – 0.345 mg/L; Lead – 0.50 mg/L and Manganese – 0.093 mg/L) showed the lowest reduction of heavy metals (**Fig 8**).

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**Table 1: Physico-chemical properties of collected sugar mill effluent**

| S. No | Parameters | Values | Standard by TNPCB (2009) |
|---|---|---|---|
| 1 | Colour | Brown | Colourless |
| 2 | Odour | Unpleasant | Odourless |
| 3 | Temperature | 36.0 | 40.0 |
| 4 | pH | 4.64 | 5.5 - 9 |
| 5 | Electrical conductivity | 2.58 | - |
| 6 | Total suspended solids (mg/L) | 479 | 200 |
| 7 | Total dissolved solids (mg/L) | 1540 | 200 |
| 8 | Total hardness (mg $CaCO_3$/L) | 278 | 250 |
| 9 | BOD (mg/L) | 1090 | 30 |
| 10 | COD (mg/L) | 3260 | 250 |
| 11 | Oil and grease (mg/L) | 19 | - |
| 12 | Calcium (mg/L) | 393 | 200 |
| 13 | Magnesium (mg/L) | 299 | 100 |
| 14 | Chloride (mg/L) | 377 | 600 |
| 15 | Sodium (mg/L) | 106 | - |
| 16 | Potassium (mg/L) | 125 | - |
| 17 | Sulphate (mg/L) | 430 | 12 |
| 18 | Nitrogen (mg/L) | 1300 | 600 |
| 19 | Phosphorous (mg/L) | 6.17 | 10 |
| 20 | Zinc (mg/L) | 0.79 | 0.01 |
| 21 | Iron (mg/L) | 17.00 | 1 |
| 22 | Copper (mg/L) | 0.346 | 0.01 |
| 23 | Lead (mg/L) | 0.52 | 0.05 |
| 24 | Manganese (mg/L) | 0.095 | 0.01 |

**TNPCB- Tamil Nadu pollution board*

**Table 2: Screening of bacterial isolates based on changes in colour and odour**

| S. No | Bacteria | Colour | Odour |
|---|---|---|---|
| 1 | *Bacillus subtilis* | Colourless | Odourless |
| 2 | *Serratia marcescens* | Colourless | Odourless |
| 3 | *Enterobacter asburiae* | Colourless | Odourless |
| 4 | *Pseudomonas fluorescens* | Colourless | Odourless |
| 5 | *Bacillus weihenstephanensis* | Colourless | Odourless |
| 6 | *Pseudomonas aeruginosa* | Light brown colour | Unpleasant |
| 7 | *Staphylococcus aureus* | Light brown colour | Unpleasant |
| 8 | *Escherichia coli* | Light brown colour | Unpleasant |
| 9 | *Brevibacterium halotolerance* | Brown colour | Unpleasant |
| 10 | *Proteus mirabilis* | Brown colour | Unpleasant |

**Fig 1: Changes in temperature, pH and EC of bioremediated sugar mill effluent**

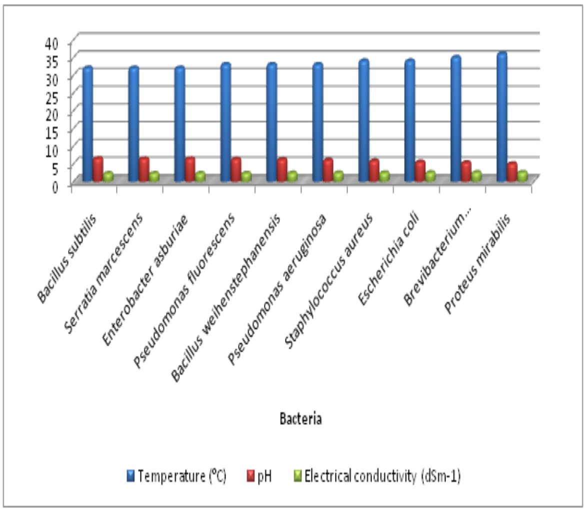


**Fig 2: Reduction in TSS and TDS in bioremediated sugar mill effluent**

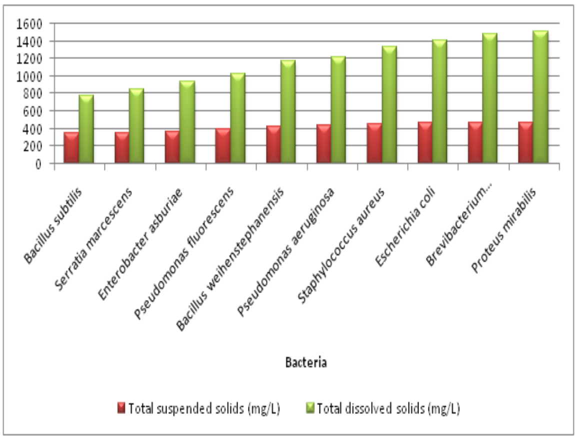


**Fig 3: Reduction of BOD and COD in bioremediated sugar mill effluent**

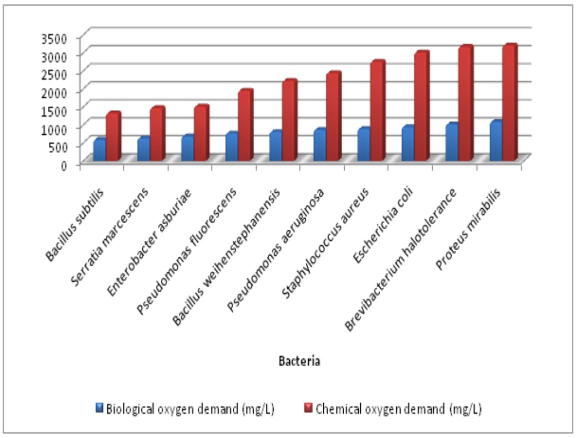


**Fig 4: Reduction of Hardness and Oil & grease content in bioremediated sugar mill effluent**

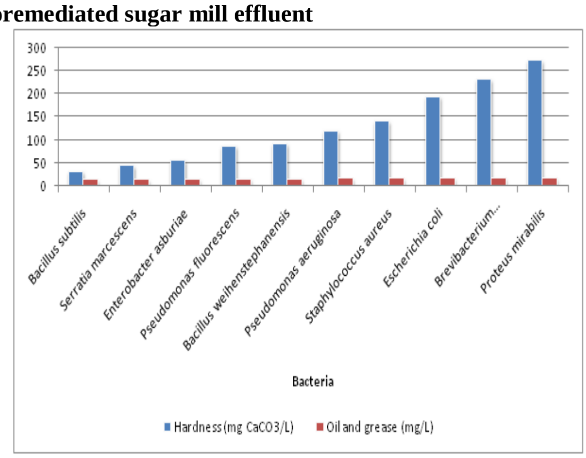


**Fig 5: Screening of bacterial isolates based on reduction of Calcium, Magnesium and Chloride concentration in bioremediated sugar mill effluent**

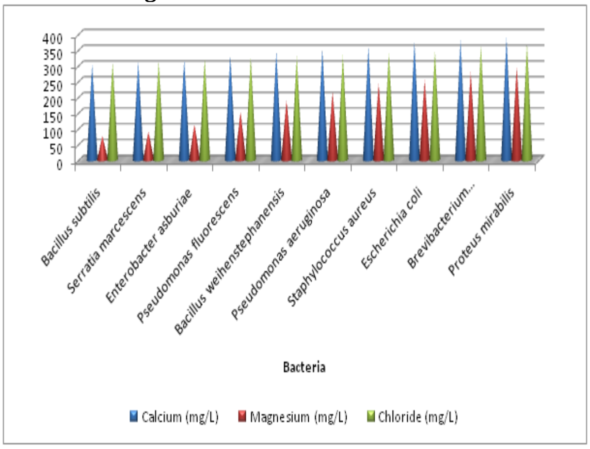

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**Fig 6: Reduction of Sodium, Potassium and Sulphate concentration in bioremediated sugar mill effluent**

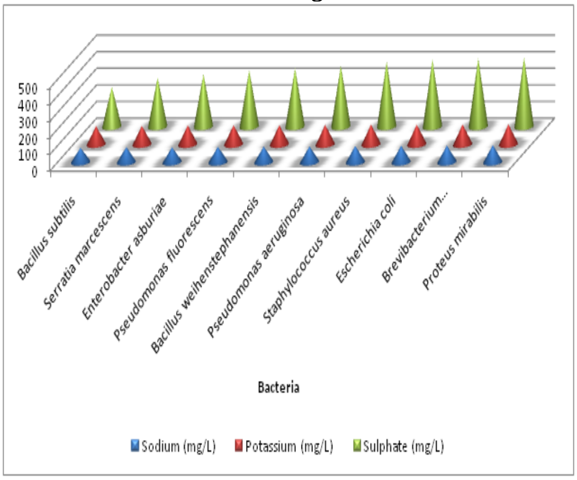



**Fig 7: Reduction of Nitrogen and Phosphorous concentration in bioremediated sugar mill effluent**

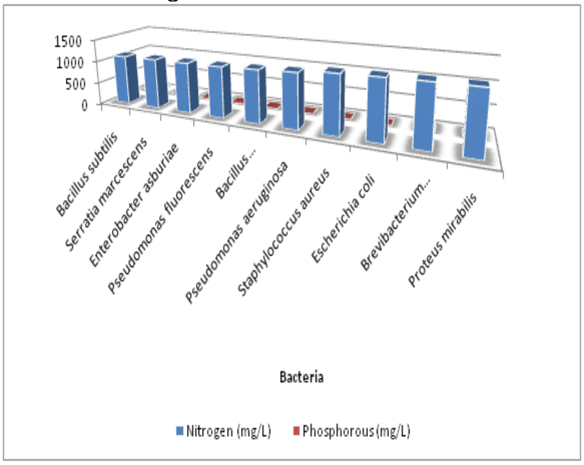
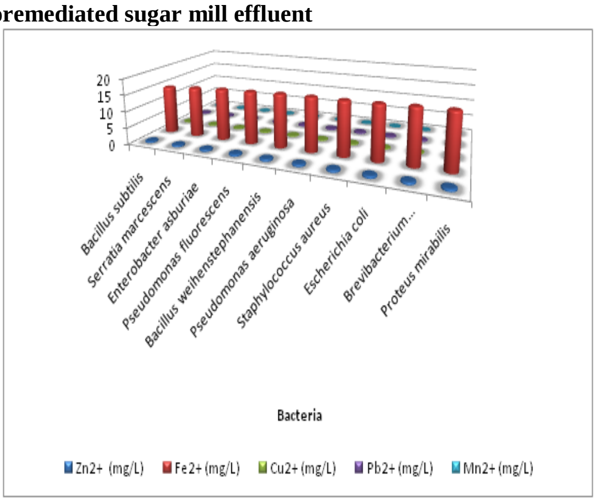



**Fig 8: Reduction of Heavy metals concentration in bioremediated sugar mill effluent**




## 4. CONCLUSION

This study concluded that physico-chemical parameters were relatively high in the sugar factory effluent and severely affected the environment and water bodies. The sugar industry effluent which is untreated highly toxic to plants and it is not permissible for irrigation. The bacterial isolates used for the bioremediation of sugar mill effluent and showed good reduction in physico – chemical properties after three months of treatment. Among the ten bacterial isolates used, three bacterial isolates *viz*., Bacillus subtilis, Serratia marcescens and Enterobacter asburiae showed best reduction of physico – chemical characteristics in sugar mill effluent. Biotreatment offers easy, effective, economical and ecofriendly techniques and utilization of bacterial isolates can be applied for fine tuning of sugar mill effluent treatment. The treated effluents of industry are not highly polluted and can be used for irrigation purpose.

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