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Impact of Sugar Mill Effluent to Environment and Bioremediation: A Review

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Abstract: Industrial pollution has been continues to be a major factor causing the degradation of the environment around us, affecting the water we use, the air we breathe and the soil we live on. The exponential increase in industrialization is not only consuming large areas of agriculture lands, but simultaneously causing serious environmental degradation as well as to soil. Water originating from various industries is finding their place in agriculture. The challenge is to properly incorporate the disposal of the wastes in a controlled management programme so that the applied industrial solid wastes do not contribute any problem of pollution to soil, soil microbes and environment. The present review was focused on impact of sugar mill effluent to environment and bioremediation. The present review deals with the following topics: Impact of sugar mill effluent to environment, Physico-chemical and biological characteristics of sugar mill effluent, Bioremediation of various industrial effluent and toxic substances by microbial isolates and Recycling of microbially remediated sugar mill effluent for agricultural use.

Key words: Sugar mill effluent · Pollution · Physico-chemical characteristics and Bioremediation

INTRODUCTION

Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and dwindling supply has made the treatment and reuse of industrial effluents an attractive option. The problem of environmental pollution on account of essential industrial growth is practical terms, the problem of disposal of industrial water, whether solid, liquid or gaseous. All the three types of wastes have the potentially of ultimately polluting water [1, 2]. Use of industrial effluent and sewage sludge on agricultural land has become a common practice in India as a result of which these toxic metals can be transferred and concentrated into plant tissues from the soil. These metals have damaging effects on plants themselves and may become a health hazard to man and animals. Above certain concentrations and over a narrow range, the heavy metals turn into toxins [3].

India is an agriculture based country and a major user of water resource for irrigation [4]. But, there is a great demand in water for irrigation while gallons and gallons of effluents are let out into water sources as untreated. The most important effluent discharging industries are sugar mills, thermal power plants, paper mills, textiles, distilleries, fertilizer units, electroplating plants, tannery industries, sago factories, oil refineries, pesticide and herbicide industries. These industrial effluents containing heavy metals pose a serious threat to the ecosystem [5]. Use of these industrial effluents and sewage sludge for agriculture have become a common practice in India as a result of which these toxic metals get transferred and accumulated into plant tissues from soil [6].

Sugar Industry: 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 [7].

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Among the various industries, sugar mill is one of the largest and most important agro based industries in India. It plays a major role in creating rural economy of the country as a whole. The sugar mill operates for 3 to 6 months per year. A considerable amount of waste water is released during crushing of sugar cane. These waste waters are disposed into nearby water bodies and they are being used for irrigation. The discharge of this effluent into water bodies or on soil is causing a serious problem of water pollution resulting in severe damage to the flora and fauna and environmental degradation [8]. Besides, the heavy metals are non-biodegradable and persist for longer periods in aquatic as well as terrestrial environments thus they can exert detrimental effect on human health and environment due to the toxicity of heavy metals [9].

Sugar industry, the most important among agro based industries contributing significantly to rural economy in particular and national economy in general generates huge amount of waste during the manufacture of sugar. The most important crop from which sugar can be produced in commercial quantity are sugarcane. India is a largest sugar producing country [10]. These sugar industries are playing an important role in the economic development of the India but the effluents released produce a high degree of organic pollution in both aquatic and terrestrial ecosystems. They alter the Physico- chemical characteristics of the receiving aquatic bodies and affect aquatic flora and fauna [11].

Sugar factory effluent produces obnoxious odour and unpleasant color when released into the environment without proper treatment. Farmers have been using these effluents for irrigation, found that the growth, yield and soil health were reduced [12]. The life in effluent is highly diverse and consists of interacting population of microorganisms and effluent fauna and their activities affect physical, chemical and biological characteristics of effluent. Some potential fungal strains such as *Penicillium pinophilum*, *Alternaria gaisen*, *Aspergillus flavus, Fusarium monolifome, A.niger* were isolated from sugarcane industrial effluent [13].

The sugar mill wastes contains a high amount of production load particularly, suspended solids, organic matters, pressmud and several air pollutants [14]. Wastewater from sugar mills with its high Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Dissolved Solids (TDS) rapidly depletes available oxygen when discharged into water bodies and have adverse impact on aquatic life so much so rendering the receiving water unfit for drinking and domestic purposes, reducing crop yields if used for irrigation and exacerbating corrosion in water systems and pipe [15].

Impact of Sugar Mill Effluent to the Environment: Sugar industry is the backbone of rural, agricultural and socio - economic 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 such toxic chemicals affect the soil parameters and there by the soil fertility [16].

Advances in science and technology and the industrial revolution have enabled humans to plot resources. Though industrialization contributes to economical development, most important natural resources like water and soil are commonly polluted with by-products, waste materials and non-utilized parent chemical compounds from the industries which in-turn ultimately affect the agricultural production and food security [17]. All types of revolutions fulfill the needs of growing population on one side and on other side it caused all kinds of pollution which altered the physico-chemical and biological characteristics of the environment [18]. Thus, environmental pollution has become an important global phenomenon, which has demanded attention from all over the countries.

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 [19].

Diverse sugar industry effluents disposed 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 [20]. The effluents also alter the physico-chemical characteristics, flora and fauna of receiving aquatic bodies. In addition, sugar factory effluent discharged in the environment posses 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 [21]. Sugar factory effluent that has not been treated properly has an unpleasant odor when released into the 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 and it is essential to determine how crops respond when exposed to industrial effluents.

It has been already reported that an average of 30,000 - 40,000 litres of effluent was generated per tons of sugar processed [22]. The sugar mill effluent, as it leaves the premises, has a relatively clear appearance. However, after stagnating for sometime it turns black and starts emitting foul odour [23]. 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, various heavy metals were found in the effluent [24].

During earlier days treating effluents were achieved by ion exchange, chemical oxidation and chemical precipitation [25]. For advanced purification, different physico-chemical methods such as active carbon adsorption, ion exchange and reverse osmosis are used. Each of these methods has its own merits and demerits. Chemical precipitation and electrochemical treatments are ineffective, especially when metal ion concentration in aqueous solutions is lower than 50 mg/L. Moreover, such treatments produce large amount of sludge to be treated with great difficulties. Ion exchange, membrane technologies and activated carbon adsorption processes are extremely expensive [26].

Earlier several microbes were reported to take effective part in bioremediation of industrial wastes [27]. The mechanism by which microorganisms act includes biosorption, intracellular accumulation and enzyme-catalyzed transformation [28]. On the basis of energetic requirements, biosorption seems to be the most common mechanism [29]. 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 [30].

Physico-chemical and Biological Characteristics of Sugar Mill Effluentl: India has been known as the original home of sugar and sugar cane. India is the second largest producer of sugarcane next to Brazil [31]. Generally, the effluent is generated from mill house, waste water from boiling house, waste water from boiler blow- down, condenser cooling water and soda and acid wastes [32]. Indian sugar mills generate 0.16-0.76m^3 of waste water for every tonnes of cane crushed by them. The pollution standards stipulate that BOD of waste water should be less than 30 mg/L for disposal into inland surface waters and less than 100 mg/L for disposal on land. Whereas, the combined sugar mill waste water had a BOD of 1,000 to 1,500 mg/L [33]. Nath and Sharma [34] reported that the untreated sugar mill effluent is toxic to plants when used for irrigation.

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The trends in developing countries to use sugar mill effluent as fertilizer has gained much importance as it is considered as a source of organic matter and plant nutrients and serves as good fertilizer. Sugar mill effluent contains considerable amount of potentially harmful substances including soluble salts and heavy metals like Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}. The long term use of this sugar mill effluent for irrigation which contaminates soil and crops to such an extent that it becomes toxic to plants and causes deterioration of soil [38].

The physico-chemical parameters of sugar mill effluent are, temperature (30°C), colour, turbidity (84.7 NTU), pH (8.1), EC (5530 dSm^{-1}), BOD (6856 mg/L), COD (7432 mg/L), total dissolved solids (2516 mg/L), chloride (1894 mg/L), total alkalinity (254 mg/L), total hardness (342 mg/L), sulphate (540 mg/L), phosphate (224 mg/L), total acidity (45 mg/L), calcium (364 mg/L) and magnesium (151 mg/L) and according to the permissible level suggested by Bureau of Indian standards (BIS) almost all the water quality parameters in the sugar effluents have been found to be very high and well above the permissible limits [39]. The physico-chemical characteristics of sugar industry effluent are high in TDS (422-608 mg/L), COD (1152-17680 mg/L) and BOD (380-650 mg/L) than ground water. There were some nutrients and heavy metals *viz*., P, N, K, Zn, Cu, Fe, Mn and Pb [40].

The physico-chemical properties such as silt, clay, electrical conductivity, water holding capacity, organic matter and total nitrogen contents, microbial population and cellulase activities were significantly higher in the samples collected from sugar industry wastes dump than in the non-dump sites [41]. Analysis of sugar mill effluents and soil samples had shown high metal content than the permissible limits except Pb. Further, analysis of plant samples have indicated the maximum accumulation of iron (Fe) followed by Mn and Zn in root, shoot, leaves and seeds of wheat and mustard [42]. The physico- chemical analysis showed that the sugar mill effluent was acidic in nature and yellowish in colour. It was rich in total suspended and dissolved solids with large amount of Biological oxygen demand and Chemical oxygen demand. Higher amount of chloride, calcium, magnesium, sodium, potassium and iron were also present in the effluent. These effluents severely affected the plants and soil properties when used for irrigation [43].

Parivash [44] reported that sugar mill effluent is typically associated with polluted problems related to high BOD, COD, toxicity, colour, suspended solids, etc. Sugar factory effluent when discharged into the environment, possess a serious health hazard to the rural and semi-urban populations where stream and river water was used for agriculture and domestic purposes. There are several reports of fish mortality and damage to the paddy crops in these areas due to wastewaters entering agricultural land [45]. Farmers have been using these effluents for irrigation, found that the growth, yield and soil health were reduced [46]. However, some potential fungal strains such as *Penicillium pinophilum, Alternaria gaisen, Aspergillus flavus, Fusarium monoliforme* and *Aspergillus niger* were isolated from sugarcane industrial effluent [47].

Arminder Kaur *et al*. [48] analyzed the effluents of sugar and textile industry and their deleterious effects on the soil microflora. Analysis of the sugar mill effluents showed that the pH (7.1-9.1); TSS (301- 494); TDS (2560-3978), BOD (2225-4526) and COD was (10896-16843). The microbial flora too is affected by it as compared to the control water sample due to the high BOD and COD values.

Ezhil Bama and Ramakrishnan [49] examined the occurrence and distribution of Arbuscular Mycorrhiza (AM) fungi on the sugar mill effluent polluted soil. Twenty three species of AM fungi belonging to five genera *viz., Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* were identified from the soil taken around ten plant species. Among ten plant species studied, only eight species were colonized and also the percentage root colonization was less in polluted soil. The number of AM spores were also less in polluted soil when compared to non-polluted soil. The increased levels of micronutrients and heavy metals were noticed in polluted soil, which caused reduction in the AM propagules.

Awasthi *et al*. [50] studied the fungal diversity in sugar mill effluent. In sugar industry the fungus *Aspergillus flavus*, *Aspergillus niger* found with maximum frequency, which were isolated from ETP water, stored molasses respectively. The fungus *Aspergillus* sp., *Cladosporium cladosporides*, *Acremonium butyri* were found with maximum density which were isolated from untreated effluent and Fresh molasses respectively. *Cladosporium cladosporides* was showed maximum abundance. The *Aspergillus niger* was found with maximum frequency, density, abundance in untreated effluent of sugar industry. The fungus *Aspergillus nidulans*, *Aspergillus candidus* was most frequent in sugar industry and untreated effluent respectively. *Trichoderma viride* and *Alternaria alternata* found with maximum density. *Aspergillus candidus* and *Trichoderma viride* were showed high abundance.

Bioremediation of Various Industrial Effluent and Toxic Substances by Microbial Isolates: The term “bioremediation” has been used to describe the process of using microorganisms to degrade or remove hazardous pollutants from the environment [51]. Bioremediation can also be defined as any process that uses microorganisms, green plants or their enzymes to return to the natural environment altered by contaminants to its original condition. Heavy metal bioremediation involves removal of heavy metal from waste water and soil through metabolically mediated or physico-chemical pathways. This natural and environmental friendly technology is cost effective, aesthetically pleasant, soil organism- friendly, diversity enhancer, energy derivation from sunlight [52] and more importantly, it is able to retain the fertility status of the soil even after the removal of heavy metals [53].

Bioremediation, the use of microorganisms to degrade environmental contaminants, is among these new technologies. Bioremediation has numerous applications, including clean-up of ground water, soils, lagoons, sludge and process-waste streams. Out of several methods that are used in the treatment of industrial effluents, biotreatment offers a cheaper and environmentally friendlier alternative for biological degradation of industrial effluents. Microbial treated industrial effluents contains an array of plant nutrients, trace elements, rich in silica which is essential for silicicolous plants like rice and sugarcane, for non lodging and also for improving resistance to pests and diseases. Industrial wastes for land application have been proven to be a cost effective [54].

Biological removal of contaminants from aquatic effluents offers great potential when the contaminants are present in trace amounts [55]. The term "bioremediation" has been used to describe the process of using microorganisms to degrade or remove hazardous pollutants from the environment [56]. Bioremediation can also be defined as any process that uses microorganisms, green plants or their enzymes to return to the natural environment altered by contaminants to its original condition. Heavy metal bioremediation involves removal of heavy metal from waste water and soil through metabolically mediated or physico-chemical pathways. This natural and environmental friendly technology is cost effective, aesthetically pleasant, harmless to soil organism, diversity enhancer, energy derivation from sunlight [57] and more importantly, it is able to retain the fertility status of the soil even after the removal of heavy metals [58].

Bioremediation of toxic industrial effluents by microorganisms serves as an effective method to substitute the conventional recovery and removal process. Fungal biomasses have huge capability of treating effluents discharged from various industries. White rot fungi are ubiquitous in nature and their adaptability to extreme conditions makes them good biodegraders. Their enzymatic degrading activity makes them effective decolorizer; they also remove toxic metals by biosorption ultimately rendering the effluents more ecofriendly [59].

Numerous research reports have been published from toxicological points of view, but these were concerned with the accumulation due to the active metabolism of living cells, the effects of metal on the metabolic activities of the microbial cells and the consequences of accumulation on the food chain [60]. However, further research has revealed that inactive/dead microbial biomass can passively bind metal ions via various physicochemical mechanisms. With these new findings, research on bioremediation became active, with numerous biosorbents of different origins being proposed for the removal of metals. Researchers have understood and explained that bioremediation depends not only on the type or chemical composition of the biomass, but also on the external physicochemical factors [61].

Hanife Buyukgungor [62] studied the bioaccumulation of lead from aqueous solutions by immobilized cells of *Citrobacter freundii*. *Corylus avellana* and *Juglans regia* shells which are agricultural wastes were used as support material and *Citrobacter freundii* cells were immobilized on the shell surface. Most of the higher organisms tend to accumulate heavy metals by ingesting metals bound to particles or sediments. Copper and lead were widely regarded as toxic metals for plants and native microorganisms.

Alexander *et al*. [63] isolated *Bacillus* sp. and *Pseudomonas* sp. from rhizosphere of *Trigonella* were capable of removing Cu (II) and *Rhizopus* sp. was found to be more resistant and efficient to accumulate larger amounts of Cd (II) than *Bacillus* sp. at all Cd (II) concentrations [64]. The primary absorption of cobalt at micromolar concentrations by *Azospirillium brasilense* cells was rapid and virtually complete, giving at least two major forms of cobalt species bound to the cells.

Semra Ihan *et al*. [65] studied on the selective biosorption of chromium, lead and copper ions by microorganisms from industrial waste waters. Liang-Ming Whang *et al*. [66] investigated the potential application of two biosurfactants, surfactin produced by *Bacillus* sp. and rhamnolipid produced by *Pseudomonas aeruginosa*, for enhanced biodegradation of contaminated water and soil and also reported that application of surfactin and rhamnolipid in stimulating indigenous microorganisms for enhanced bioremediation which was confirmed by their enhanced capability on both efficiency and rate of diesel biodegradation in soil systems.

Vani *et al*. [67] isolated *Azotobacter* sp. from rhizosphere of *Allium cepa* L. polluted soils of sugar mill effluent. Nine different *Azotobacter* sp. were isolated and identified from the polluted study site. Sompong Meunchang *et al*. [68] reported that the inoculation of sugar mill by - products compost with N_2 fixing bacteria *viz*., *Azotobacter vinelandii, Beijerinckia derxii* and *Azospirillum* sp. may improve its quality by increasing total N and available P. Belimov *et al*. [69] isolated eleven cadmium-tolerant bacterial strains of *Bacillus* sp., *Azotobacter* sp., *Flavobacterium* sp. and *Rhodococcus* sp. which offer promise as inoculants to improve growth of the metal accumulating plant *Beijerinckia derxii* in the presence of toxic Cd and for the development of plant inoculant systems useful for phytoremediation of polluted soils.

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Sauidis Thomas *et al*. [72] have isolated *Aspergillus* sp. and *Rhizopus* sp. from the agricultural field and treated with sewage effluents could bioadsorb both metals *viz*., Cr and Cd, particularly *Rhizopus* sp. could bioadsorb higher concentration of both metals as compared to *Aspergillus* sp. The bacterial isolate *Aeromonas hydrophila* was shown to decolorize three triarylmethane dyes tested within 24 hrs with colour removal in the range of 72 to 96 % and highlighted the potential of *Aeromonas hydrophila* as a biotechnological tool for remediation and detoxification of textile and other industrial wastewaters containing triarylmethane dyes. Iqbal Ahmad *et al*. [73] isolated five different fungi *viz*., *Aspergillus* sp., *Rhizopus* sp., *Rhodotorula* sp., *Dreshlera* sp. and *Curvularia* sp. from soil which were used as a biosorbent for removal of Zn from wastewater.

Comte *et al*. [74] assessed the influence of pH on the metal biosorption of extracellular polymeric substances (EPS) extracted from two different activated sludge. The bacteria *Alcaligenes, Bacillus* and *Corynebacterium* isolated from sago industry effluent and effluent contaminated soil were found efficient in starch degradation and recorded 63% of degradation of starch in sago industry effluent. The effluent treated by aerobic microorganisms had no negative impact on the seed germination and shoot length the root length, fresh weight, dry weight and chlorophyll content showed an increase. Hence, the bioremediated effluent can be effectively used for irrigation [75].

Abou Shanab *et al*. [76] examined four bacterial isolates for their ability to increase the availability of water soluble Cu, Cr, Pb and Zn in soils and for their effect on metals uptake. Random Amplified Polymorphic DNA analysis was used to show that the bacterial cultures were genetically diverse. Bacterial isolates S3, S28, S22 and S29 had 16S rRNA gene sequences that were most similar to *Bacillus subtilis, Bacillus pumilus, Pseudomonas pseudoalcaligenes* and *Brevibacterium halotolerans* based on 100% similarity in their 16S rDNA gene sequence, respectively. Filtrate liquid media that had supported *Bacillus pumilus* and *Bacillus subtilis* growth significantly increased Cr and Cu extraction from soil polluted with tannery effluent and from Cu-rich soil, respectively. The highest concentrations of Pb, Zn and Cu were accumulated in shoots of *Zea mays* grown on Cu-rich soil inoculated with *Brevibacterium halotolerans*. The highest concentration of Cr was accumulated in *Sorghum bicolor* roots grown in tannery-effluent-polluted soil inoculated with a mixed inoculum of bacterial strains.

Xia- Fang Sheng *et al*. [77] have isolated two lead resistant bacteria *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G16 from grape roots grown in heavy metal contaminated soils. Strains G10 and G16 based exhibited different multiple heavy metal and antibiotic resistance characteristics and increased water- soluble Pb in solution and in Pb added soil. Increase in biomass production and total Pb uptake in the bacteria- inoculated plants were obtained compared to the control. The biosurfactant from *Azotobacter vinelandii* have the capability to recover oil upto 15% from oil sludge which suggested that both petrofilic consortia and biosurfactant addition stimulate the biodegradation and overcome the limitation of petroleum hydrocarbon degradation process.

Gaur and Dhankhar [78] studied the cyanobacterial biosorbent for uptake of zinc present in different types of industrial effluents. The *Azotobacter* sp. showed resistance to heavy metal like cadmium, copper, lead and zinc and showed bioaccumulation of heavy metal on the bacterial cell wall which resulted in overall reduction in the concentration of heavy metals in culture supernatant, thus, the isolate could be used to accelerate the *in situ* bioremediation of sites contaminated by loads of metals [79].

Lina Velasquez and Jenny Dussan [80] tested Colombian *Bacillus sphaericus* native strains for the tolerance to As, Hg, Co, Fe and Cr, as well as the biosorption and bioaccumulation in living biomass and reported that the live and dead cells of *Bacillus sphaericus* OT4b31 and *Bacillus sphaericus* IV (4)10 showed biosorption of Cr and had the capacity to accumulate between 6 and 47% of Co, Hg, Fe and As.

For bioremediation of polluted soil, the lower pH value and higher enzyme activity in different bioremediation processes suggested the effectiveness of combination of effective microorganisms with ryegrass. The fraction analysis indicated a priority of low molecular hydrocarbon degradation in combination of microorganisms and ryegrass, polar fraction degradation in phytoremediation and aromatic fraction in microbial degradation [81]. All the immobilized isolates of heavy metal resistant bacteria isolated from electroplating industrial effluent samples *viz., Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. have potential application for the removal of Cu, Cd and Pb from industrial wastewater than dead bacterial cells [82].

Selvarathi *et al*. [83] proposed that the inoculation of *Serratia* sp. significantly increased the root biomass of *Zea mays* under Cd or Cu contaminated conditions. Similarly, the culture of *Bacillus* sp. showed the increased level of degradation rate of the dye from textile effluent [84]. The bioremediation treatment by various concentrations (1.5%, 2%, 2.5% and 3%) of biofertilizers *viz*. *Azotobacter* and *Phosphobacterium* with paper mill effluent individually on *L. esculentum* has brought out considerable increase on the growth and biochemical characteristics. Among the *Azotobacter* and *Phosphobacterium* analyzed, the *Azotobacter* exhibited better performance in reducing all the pollutants from the effluents than its counterparts.

Deepika Lakshmipath *et al*. [85] stated that the *Bacillus* sp. isolated with its biosurfactant production and heavy metal resistant activity could be used as potential strain and could be used as bioremediating agent. *Pseudomonas* sp. was found to be better microbial tool for bioremediation of heavy metal and can be explored to remove heavy metal load, present even in low concentration in waste water of pulp and paper mill effluent.

Ram Chandra Bajgai *et al*. [86] cultivated the filamentous yeast *Trichosporon cutaneum* on batch in Andreev medium containing biotin, thiamine and glucose. Metal chromium was added in concentration ranging from 0 - 1 0 mM ($K_2Cr_2O_7$) to the culture during the stationary phase of the culture growth. Viability, glucose consumption and removal efficiency of the strain was studied. 1.5 mM of $K_2Cr_2O_7$ was found to be lethal to the strain, consumes maximum glucose at 5 mM and removes maximum at 1 mM concentration. Removal efficiency decreases with increase in metal concentration.

Satpal Singh Bisht *et al*. [87] evaluated the heavy metal absorbing potential of bacteria isolated from industry effluent sites. From the effluent waste water, three bacteria were isolated and confirmed as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* after performing various biochemical tests. The heavy metal tolerant efficiency was determined by analyzing the growth of the bacteria in presence of heavy metal solutions and their optimum tolerance was determined by measuring the optical density at 600 nm after 24 hrs and 48 hrs of incubation.

Nagendra Kumar Chaurasia and Ram Krishna Tiwari [88][a] analyzed the heavy metals, namely Fe, Cu, Cd and Zn to assess the water quality of Saraya sugar factory, Saraya distillery, Biasy Nallah, Faren Nallah, Neckwar Ghat, Gorrah river and Rapti river. Their results pointed out that Saraya distillery showed significantly higher amount of heavy metals that run out in urban, sub-urban and rural areas through different water passages. The heavy metals infiltered into ground water which have been intoxicated both animal and plant life. However, the overall quality of water of this area was quit unsafe for domestic and agricultural purposes as the concentrations of Fe, Cu, Cd and Zn are not in the permissible level set by World Health Organization [89][b].

Sahar Alzubaidy [90] evaluated the resistance of *Serratia marcescens* obtained from soil and water to metals chlorides. Two isolates which was identified as *Serratia marcescens* and *Serratia marcescens* (S4) were selected for this study according to their resistance to five heavy metals. The ability of *Serratia marcescens* (S4) to grow in different concentrations of metals chloride (200-1200 µg/ml) was tested. The highest tolerance of heavy metals was exhibited by *Serratia marcescens* (S4) at a concentration of 1000 µg/ml for Zn^{+2}, Hg^{+2}, Fe^{+2}, Al^{+3} and Pb^{+2} and 300 µg/ml for Hg^{+2} in 24 hrs incubation at 37°C. The isolates showed the ability to grow in different pH values (4, 7 and 9) in presence of four metals in all pH values (1000 µg/ml) and unability to grow with 300 µg/ml Hg^{+2}. The highest Zn^{+2} removal ratio was 75% then Pb^{+2} 55% while Fe^{+2} has the lowest removal ratio (48%).

Munees Ahemad and Abdul Malik [91] isolated the most promising zinc resistant bacteria from heavy metal contaminated soils and assessed their metal accumulating ability. A total of 34 bacterial isolates from agricultural soils irrigated with metal polluted wastewater were characterized and identified as *Pseudomonas*, *Bacillus* and *Staphylococcus*. The zinc resistant bacteria (*Pseudomonas* isolate SN7, *Pseudomonas* isolate SN28 and *Pseudomonas* isolate SN30) were selected because of exhibiting co-resistance against Cu^{2+}, Hg^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Cr^{3+}, Cr^{6+} and in addition to Zn^{2+} and displayed high values of Minimum Inhibitory Concentrations (MIC) for each heavy metal. Further, the three isolates were assessed for their ability to remove zinc and copper from medium amended with these metals. The zinc resistant bacterial isolates SN7, SN28 and SN30 accumulated zinc maximum 29, 25 and 26 mg g^{-1} dry weight of cells, respectively at the zinc concentration of 1.6 mM. Similarly, bacterial isolates SN7, SN28 and SN30 accumulated copper maximum 20, 25 and 22 mg g^{-1} dry weight of cells, respectively at 2.92 mM of copper.

Recycling of Microbially Remediated Sugar Mill Effluent for Agricultural Use: Sugar industry is one of the most important agro based industries in India. All the industries consume huge quantity of water and throw back almost an equal quantity of effluent which contains highly toxic materials in dissolved or suspended form. If this water is properly used or it is purified to recycled, a part of water shortage will surely be solved [92]. The term “bioremediation” has been used to describe the process of using microorganisms to degrade or remove hazardous pollutants from the environment [93]. Bioremediation can also be defined as any process that uses microorganisms, green plants or their enzymes to return to the natural environment altered by contaminants to its original condition [94].

The beneficial use of treated effluents for agriculture is the major reuse application worldwide [95]. Effluents of particular interest for land application include municipal wastewater, farm effluents, wastewater from the agricultural and forest industries such as effluents from animal and plant processing plants, food processing plants and paper mills [96]. Currently, most countries still have no legal framework for wastewater reuse in agriculture. Many countries either refer to international guidelines [97] or do not mention the option of effluent irrigation in agriculture in their resource management act at all [98].

When considering effluent irrigation, the beneficial and harmful risks have to be assessed. Short term benefits associated with effluent irrigation, such as provision of nutrients for plant growth and development, improvement of soil structure and crop yield increase have been described in some detail [99]. The major risk of effluent irrigation is the potential for infectious diseases in animals grazing sewage-irrigated pastures and people directly or indirectly exposed to the effluents [100]. More recently, authorities are concerned about the potential long term tradeoffs associated with effluent irrigation, mainly the irreversible deterioration of groundwater quality. Elevated concentrations of anthropogenic contaminants such as heavy metals, effluent-derived hormones, pharmaceuticals and other organic chemicals [101] and soil - borne organic pollutants such as pesticides, have been identified in groundwater at effluent-irrigated sites.

Use of urban wastewater in agriculture is a centuries old practice, which has recently received renewed attention in many parts of the world, particularly in arid and semi-arid regions, due to the growing scarcity of water. Sewage or wastewater means untreated city effluent including industrial and domestic wastewater in this paper. High population growth rates and rapid industrialization imparts immense pressure on existing water and land resources; and has resulted in the discharge of large volumes of sewage. Wastewater use is increasing in urban and peri-urban agriculture and even in distant downstream rural areas where such use derives significant economic activity and supports the livelihood of resource poor farmers but alters environment quality. In countries where treatment and safe effluent disposal facilities are limited or non-existent, sewage is used to irrigate fodders, ornamental and food crops including vegetables [102].

Utility potential of industrial effluents for irrigation of crop-fields has been a controversial proposition due to the contradictory reports obtained on the effects of these effluents on crop plant responses [103]. Most of our water resources are gradually becoming polluted by addition of huge amounts of sewage and industrial effluents. These effluents contain toxic materials with varying properties from simple nutrients to highly toxic substances. The discharge of industrial effluents with varying amounts of pollutants has altered the water quality [104]. Sugar mill effluent at above 25 percent concentration significantly reduced the seed germination percentage of groundnut and paddy due to high concentration of solids in effluent [105]. Effluent of sugar mill showed toxic effects on rice and blackgram. Rice remained more sensitive and susceptible to the toxic effects of industrial effluents but blackgram proved to be more tolerant [106].

Metribuzin present in sugar mill effluent is weakly absorbed in soil and therefore leaches easily to lower soil profiles and results in loss of activity [107]. The lower concentration of sugar mill effluent showed promoting effect on seed germination, seedling growth, dry matter production and biochemical parameters whereas germination percentage and seedling growth were inhibited at 100% concentration [108]. The sugar factory effluent drastically reduced the growth and germination of wheat, jowar, raddish and bhindi [109].

The seed germination and seedling growth of wheat, garden pea, black gram and mustard were significantly reduced with increase in concentration of mixed industrial (distillery and sugar) effluent [110]. The sugar factory effluent was found to affect plant growth but bore water favoured seedling growth of greengram (*Vigna radiata*) and maize (*Zea mays*). All growth parameters of greengram (*Vigna radiata*), biochemical contents, enzyme activities and yield parameters were found to increase at 10% effluent concentration and decreased from 25% effluent concentration onwards. Increase in sugar mill effluent concentration resulted in corresponding decrease in germination rate of Soybeans (*Glycine max*), whereas 10% dilution of effluent enhanced seed germination and 100 % effluent completely inhibit for both seed germination and seedling growth [111]. The per cent root colonization and the number of AM spores was less in sugar mill effluent polluted soil when compared to non polluted soil which resulted in decreased germination rate of sunflower (*Helianthus annus* L.) [112].

Ragen *et al*. [113] experimented on the sugar factory effluent using a pilot upflow anaerobic sludge blanket reactor. After bioremediation of sugar mill effluent using *Trichoderma* sp., the physico - chemical parameters of the effluent were found to be within the permissible limit and the seed germination of green gram and maize was effective even at 100% bioremediated effluent were as in untreated effluent the seed germination stopped at 50% [114]. Similarly, the amount of amino acid, protein and chlorophyll content gradually increased with increasing concentration of the bioremediated effluent in tomato [115] and in barely, the plumule length and number of lateral roots significantly increased from 50%.

Panoras *et al*. [116] studied the effects of effluent from reclaimed either by activated sludge with microorganism or by stabilization ponds on field-grown corn and reported that there is a potential risk of facing problems related to soil salinity and alkalinity if no consideration for soil reclamation is taken into account. Chandra *et al*. [117] studied the effect of sugar mill effluent on *Phaseolus aureus* and found that sugar mill effluent decreased the chlorophyll content. Similarly untreated sugar mill effluent decreases the seedling growth, chlorophyll and amylase contents in green gram [118].

Hulugalle *et al*. [119] reported that the treated sewage effluent using *Aspergillus niger* contained considerable amounts of nitrogen, phosphorus and high concentrations of sodium salts. Effluent water was moderately saline and compared with river water, had higher concentrations of Na, nitrate-N and K and lower concentrations of Ca and Mg. Irrigation with treated sewage effluent caused large increase in nitrate-N, small increase in exchangeable Mg, Na and K and small decrease in heavy metals.

Sompong Meunchang *et al*. [120] stated that sugar mill by-products inoculated with N_2 fixing bacteria *viz*., *Azotobacter* sp., *Beijerinckia* sp. and *Azospirillum* sp. may be good soil amendment to promote tomato growth. Similarly, the higher concentration of the treated effluent using fungi (*Aspergillus niger*) had no effect on seed germination of *Triticum aestivum* and also the lower concentration of sugar factory effluent increased the seedling growth, chlorophyll and amylase contents in green gram seedlings.

Kamlesh Nath *et al*. [121] studied on *Phosphobacterium* treated distillery and sugar factory mixed effluent to investigate its effect on seed germination and seedling growth in wheat and black gram. Among the effluent analysed, the treated sugar mill effluent exhibited better performance in growth rate of wheat and black gram.

Laine and Jurgensen [122] found that mixing of *Bacillus megaterium*, along with nutrient addition degraded the heavy metals in the sugar mill contaminated soil and increased the germination percentage of Onion. Similarly, distillery contaminated soils were degraded using *Termitomyces clypeatus* and used in cultivation of olives, the length and number of new shoots, diameter of the main trunk, leaf area and dry weight of the plants were enhanced under the influence of treated soil.

Ashutosh Kumar Tripathi *et al*. [123] stated that water pollution through industrial discharges, which is mainly in the form of effluent or waste water, is one of the biggest problems. These effluents have strong concentrations of chemical oxygen demand, phenol and its derivatives, contains metals, inorganic compounds, proteins, cyanides, chlorinated lignin and dyes.

Abou Shanab *et al*. [124] examined four bacterial isolates for their ability to increase the availability of water soluble Cu, Cr, Pb and Zn in soils and for their effect on metals uptake. Random Amplified Polymorphic DNA analysis was used to show that the bacterial cultures were genetically diverse. Bacterial isolates S3, S28, S22 and S29 had 16S rRNA gene sequences that were most similar to *Bacillus subtilis, Bacillus pumilus, Pseudomonas pseudoalcaligenes* and *Brevibacterium halotolerans* based on 100% similarity in their 16S rDNA gene sequence, respectively. Filtrate liquid media that had supported *Bacillus pumilus* and *Bacillus subtilis* growth significantly increased Cr and Cu extraction from soil polluted with tannery effluent and from Cu-rich soil, respectively. The highest concentrations of Pb, Zn and Cu were accumulated in shoots of *Zea mays* grown on Cu-rich soil inoculated with *Brevibacterium halotolerans*. The highest concentration of Cr was accumulated in *Sorghum bicolor* roots grown in tannery effluent-polluted soil inoculated with a mixed inoculum of bacterial strains.

Nagajyoti *et al*. [125] tested the *Arachis hypogaea* grown in pots for a period of 30 days where the soils were treated with different effluent concentrations (25, 50, 75 and 100%). The distribution of heavy metals in the soils and corresponding accumulations in the experimental crop was investigated on different experimental days 10, 15, 20, 25 and 30th day. The metals Cr, Cu, Mn, Fe, Co, Ni, Pb, Cd and Zn in plants and soil samples were analyzed by AAS technique. In *Arachis hypogaea*, Fe was high in 100% effluent at 10, 15, 20 and 25th days. It has been observed that at 25% effluent concentration, there is growth in the root length, an increase in shoot length, germination percentage, total chlorophyll content in *Arachis hypogaea* L. chlorophyll content have increased upto the 20th day and then decreased from 25th day.

Nagajyoti *et al*. [126] investigated the effects of biomass power plant effluent on the carbohydrates, amino acids, nitrite and nitrite enzyme activities and proline of *Arachis hypogaea* L. var TCGS 320 under controlled pot culture methods. Plants were cultivated with 25, 50, 75 and 100% of the effluent and a control without the effluent. The treatment of the crop with 25% of the effluent has shown stimulatory effect on all the biochemical parameters studied. Carbohydrates, starch, amino acids, protein, nitrate and nitrite reductase enzymatic activities have increased in 10, 15, 20 DAS (days after sowing). In 25 and 30 DAS, all biochemical parameters have decreased due to environmental factors.

Parmila Rani and Sanjeev Kumar [127] assessed the effect of periodic watering with different concentration of sugarcane industrial effluent on different parameters such as length (root, shoot and spike), no. of leaves, no. of grain/ spike, leaf area and chlorophyll level of *Triticum aestivum*. The effluent reflected promotory effect of different concentration of sugarcane industrial effluent on chlorophyll level, growth and yield of plant. The experiment suggested that effluent can be used as fertilizer after dilution.

Lakshmi and Sundaramoorthy [128] has explained that the microbes mixed polluted soil showed good results in morphological, biochemical and yield parameters of blackgram than the untreated soil. Albino Wins and Murugan [129] has stated that the sugar mill effluent can be safely used for irrigation purposes with proper treatment and dilution at 25% for growing blackgram (*Vigna mungo*). Similarly, germination of *Phaseolus aureus* and *Abelmoschus eseulentus* seeds where affected adversely when raw textile effluent was used, whereas as the bioremediated effluent increased the seed germination, total sugars, starch, reducing sugars and chlorophyll content of the plant than control. The bacteria *viz*., *Bacillus cereus* and *Bacillus thuringiensis* played an important role in degrading Cd, Ni and Zn in soil and increasing soil fertility by removal of heavy metals for Millet and Green gram plants [130].

Kailas Doke *et al*. [131] assessed physico - chemical parameters of treated waste water effluents from a sugar industry and determined the effect of various concentrations (0%, 20%, 40%, 60%, 80 % and 100%) of effluent on seed germination, germination speed, peak value and the germination value of Mung (*Vigna angularis*), Chavali (*Vigna cylind*rical) and Jowar (*Sorghum cernum*) seeds. The low effluent pH (4.35), total dissolved solids, (TDS, 720 mg/L) and chemical oxygen demand, (COD, 1330 mg/L) indicated the high inorganic and organic content with an acidic load. Germination percentages and germination values decreased with increasing concentration of effluent in all the seeds tested.

Vijayaragavan *et al*. [132] studied the effect of sugar mill effluent on plant growth and biochemical constituents of *Raphanus sativus* in a pot culture experiment. The radish plants were grown upto 60 days, in the soil irrigated with different concentrations of sugar mill effluent. All pots were irrigated (500 ml) with respective concentration of test solutions daily. Plants were thinned to a maximum of three per pots, after a week of germination. The higher sugar mill effluent concentrations were found to affect plant growth and decreased total chlorophyll, caroteinoids, total sugar, amino acids and protein contents, but diluted effluent favoured the plant growth and biochemical contents.

Sajani Samuel and Muthukkarupan [133] studied the physico-chemical parameters of sugar mill effluent and contaminated soil and the effect of various concentrations of the effluent on seed germination, germination speed of Paddy (*Oryza sativa* L.). The low effluent pH (4.20), total dissolved solids, (TDS, 1480 mg/L) and chemical oxygen demand, (COD, 3140 mg/L) indicate the high inorganic and organic content with an acidic load. Germination percentages and germination values were decreased with increasing the concentration of effluent in the seeds tested.

Anuradha Mehta and Nagendra Bharadwaj [134] analyzed germination percentage and seedling growth of Vigno and Cicer plants and showed considerable reduction in case of untreated effluents of the sugar mill industries. The treated effluent also showed inhibitory effect to some extent. Root and shoot length of *Vigna* seedling reduced upto 58.66% and 69.06% respectively while in *Cicer* the reduction was upto 53.62% and 67.91% in untreated effluent of RI as compared to control. Minimum reduction in root and shoot length was observed in treated effluent of SSI in both *Vigna* and *Cicer.* Maximum phytotoxicity was observed in untreated effluent of RI for *Vigna* and in untreated effluent of SSI for *Cicer*. Treated effluent of NEI and SSI showed minimum phytotoxicity. *Cicer* was more sensitive towards effluent application as compared to *Vigna*.

Siva Santhi and Suja Pandian [135] assessed the waste water quality parameters of treated sugar effluent and their effect at various concentrations like 0%, 25%, 50%, 75% and 100% on germination, speed of germination, peak value and germination value of two selected seeds i.e. peanut (*Arachis hypogaea*) and greengram (*Vigna radiata*). Germination percentage decreases with increasing concentration of effluent in all the tested seeds, whereas the germination speed, peak value and germination value increased from control to 25% and 50% concentration and decreases from 50% to 75% and 100% effluent.

Charu Saxena and Sangeeta Madan [136] compared the efficacy of natural adsorbents, *viz*., activated charcoal, wood ash and bagasse pith for the removal of pollutants from sugar mill effluent. Experimental pots were filled with the adsorbents mixed soil separately and the surface sterilized seeds of *Solanum melongena* were equidistantly sown. Triplicates for a given adsorbent were maintained. Effluent irrigation to pots was done with its four concentrations *i.e.*, 25%, 50%, 75% and 100% and the leachate collected from each treatment was analyzed. Pollutant removal efficiency of the adsorbents was found to be in the order of activated charcoal> bagasse pith> wood ash.

Satpal Singh Bisht *et al*. [137] evaluated the heavy metal absorbing potential of bacteria isolated from industry effluent sites. From the effluent waste water, three bacteria were isolated and confirmed as *Staphylococcus aureus* and *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* after performing various biochemical tests. The heavy metal tolerant efficiency was determined by analyzing the growth of the bacteria in presence of heavy metal (Cu, Hg, Co and Zn) solution and their optimum tolerance was determined by measuring the optical density at 600nm after 24hrs and 48hrs of incubation. *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus saprophyticus* were found to grow in heavy metal solutions.

Sahar Alzubaidy [138] evaluated the resistance of *Serratia marcescens* obtained from soil and water to metals chlorides (Zn^{+2}, Hg^{+2}, Fe^{+2}, Al^{+3} and Pb^{+2}). Four isolates, identified as *Serratia marcescens* and *Serratia marcescens* (S4) were selected for this study according to their resistance to five heavy metals. The ability of *Serratia marcescens* (S4) to grow in different concentrations of metals chloride (200-1200 µg/ml) was tested. The highest concentration that *Serratia marcescens* (S4) tolerate was 1000 µg/ml for Zn^{+2}, Hg^{+2}, Fe^{+2}, Al^{+3} and Pb^{+2} and 300 µg/ml for Hg^{+2} through 24 hrs incubation at 37°C. The isolates showed the ability to grow in different pH values (4, 7 and 9) in presence of four metals in all pH values (1000 µg/ml) and unability to grow with 300 µg/ml of Hg^{+2}. The highest Zn^{+2} removal ratio was 75% than Pb^{+2} 55% while Fe^{+2} has the lowest removal ratio (48%).

Munees Ahemad and Abdul Malik [139] isolated the most promising zinc resistant bacteria from heavy metal contaminated soils to assess their metal accumulating ability. A total of 34 bacterial isolates from agricultural soils irrigated with metal polluted wastewater were characterized and identified as *Pseudomonas*, *Bacillus* and *Staphylococcus*. The zinc resistant bacteria (*Pseudomonas* isolate SN7, *Pseudomonas* isolate SN28 and *Pseudomonas* isolate SN30) were selected because of exhibiting co-resistance against Cu^{2+}, Hg^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Cr^{3+} and Cr^{6+} in addition to Zn^{2+} and displaying high values of Minimum Inhibitory Concentrations (MIC) for each heavy metal. Further, the three isolates were assessed for their ability to remove zinc and copper from medium amended with these metals. The zinc resistant bacterial isolates SN7, SN28 and SN30 accumulated zinc maximum 29, 25 and 26 mg g^{-1} dry weight of cells, respectively at the zinc concentration of 1.6 mM. Similarly, bacterial isolates SN7, SN28 and SN30 accumulated copper maximum 20, 25 and 22 mg g^{-1} dry weight of cells, respectively at 2.92 mM of copper.

CONCLUSION

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. Sugar factory effluent, when discharged into the environment, poses a serious health hazard to the rural and semi-urban populations that uses stream and river water for agriculture and domestic purposes, with reports of fish mortality and damage to the agricultural crops due to wastewaters entering agricultural land. Among the effluent discharging industries, sugar mills play a major role in polluting the water bodies by discharging large amount of effluents.

In developing countries including India, farmers are irrigating their crop plants with industrial effluents having high level of several toxic metals due to the non- availability of alternative sources of irrigation water. Untreated effluent used for irrigation is highly toxic to the plant, fish and other aquatic life. The need for economical, effective and safe methods for disposal of pollutant in effluent has resulted in the search for unconventional materials that may be helpful in reducing the pollutant in the effluent. Reuse of treated effluent that is normally discharged to the environment from sugar mill waste water treatment plants is receiving an increasing attention as a reliable water resource.

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