Impact of Untreated and Bioremediated Methyl Orange on Seed Germination, Growth and Yield of Flower Plants - Marigold (Yellow and Orange), Celosia Argentea

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Abstract

Worldwide the bioremediated textile effluent as a water source for agricultural irrigation has been introduced as an alternative for wastewater destination in the environment. Focusing on agricultural environmental aspects, the objectives of the present study was to analyse the physico-chemical parameters of textile effluent and methyl orange treated with the selected algae Oedogonium subplagiostomum AP1. To analyse the biometric parameters such as germination percentage, height (shoot and root length), vigour index, number of flowers, buds, branches, leaves, nodes, size of the flowers (cm), weight of the flower (g) and fresh weight of the test plants namely Marigold (yellow and orange) and Celosia argentea on 7th and 60th day using tap water (T_1), untreated methyl orange (T_2) and bioremediated methyl orange (T_3) solutions respectively. The physico-chemical characteristics of the soil before and after harvest were also analysed. The degraded metabolites in bioremediated methyl orange and untreated methyl orange solution were also checked for its toxicity against the selected bacterial and fungal isolates. The results revealed that the physicochemical parameters of the textile effluent, untreated and treated methyl orange falls within the limit prescribed by BIS. The biometric parameters analysed on 7th day shows that the germination percentage was maximum in control (T_1) followed by plants treated with bioremediated dye solution (T_3) and minimum in untreated dye solution (T_2). Similar trend was observed in all the biometric parameters tested for the selected plants on 7th and 60th day respectively. The soil parameters were found to be high in T_2 treatment when compared with control (T_1) and T_3. The microbial toxicity shows that there was no zone of inhibition in the control and treated dye solution whereas significant inhibition was recorded in untreated dye solution. Thus the present study validates the use of bioremediated methyl orange solution as an eco-friendly alternative water source for irrigation.

Keywords: Marigold (yellow and orange), Celosia argentea, methyl orange (MO), phytotoxicity, microbial toxicity

I. INTRODUCTION

India faces, serious water scarcity due to the population growth and industrial development. Groundwater is the major and important source of water for the agricultural and industrial sectors. The textile industries grow rapidly due to the availability of cheap water and labour. For instance, the textile valley in India, 'Tirupur', Tamil Nadu, is one of the largest and fastest growing urban areas also known as the textile hub of India. According to the Ministry of water resources, industrial water used in India stands at nearly 6% of total freshwater and the demand is expected to increase dramatically. In contrast, Indian agriculture sector claims 90% of total water resources and has caused groundwater depletion. Another major source of India's water supply is surface water, which is highly monsoon dependent. Climate change also has an effect on rainfall pattern and is make worse the depleting supply of water (Senthilkumar and Jaabir, 2013 and Prabha et al., 2013).

Universally, the ground water and surface water are polluted due to stirred economic growth and poor wastewater management practices and also the worsen issue of water scarcity. The wastewater generated by the textile industry is rated as polluting among all industrial sectors. Indiscriminate discharge of huge amount of wastewater by the industries into natural water bodies is the common practice in India, which pose serious problems to cultivated fields specially the area where water scarcity occurs, farmers are compelled to use of industrial wastewater for irrigation without knowing the direct and long term impacts of wastewater irrigation. Singh et al. (2006), Castro et al. (2015) and Carr et al. (2011) revealed that textile mill effluents particularly at higher concentration inhibit germination and growth of crop plant seedlings as well as affects the soil fertility and crop productivity.

Wastewater treatment is now receiving greater attention due to growing awareness on the impact of pollution on groundwater and surface water. During the last few years, new and strict regulations coupled with increased enforcement concerning wastewater discharges have been established in many countries. At the same time, physico-chemical treatment methods have been accepted for effluent treatment but they are expensive and generate the sludge. Accumulation of concentrated sludge poses a lot of practical difficulties in the disposal of wastewater. At the moment, sludge is being deposited into the lands by the textile industries converting them into waste lands (Anjaneyulu et al., 2005, Couto, 2009 and Robinson et al., 2001). Alternatively, biological treatment could

achieve greater efficiencies in the decolourisation and detoxification of textile wastewater by microorganisms. There are a lot of reports suggesting the use of algae that have the capacity to decolourise the dyes (Azza et al., 2013, Kumar et al., 2014, Khataee et al., 2013, Mausoud et al., 2012, Daneshvar et al., 2012, Ayca et al., 2012 and Mona et al., 2011).

Treated textile effluent can be used for irrigation, purpose and seems to be the most promising alternative method for irrigation. The reuse of treated effluent provides an opportunity for the local farmers to sustain their livelihood especially during the dry summer season (Asgher et al., 2009 and Lopez et al., 2006). The reuse of bioremediated methyl orange for agricultural irrigation is often viewed as a positive means of recycling water due to the potential large volumes of water that can be used.

Thus, the present study was aimed to assess the potential of bioremediated methyl orange on Marigold (yellow and orange) and Celosia argentea and also its effect on soil properties and microbial toxicity.

II. MATERIALS & METHODS

A. Physico-Chemical Characteristics of the experimental samples

The water samples namely untreated and treated effluent, untreated and treated methyl orange aqueous solution were analysed for the various physico-chemical parameters viz., colour, odour, pH, Electrical conductivity, Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Solids (TS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total hardness, total alkalinity, chloride, sulphate and nitrate as per the standard methods (Table 1).

Table – 1
Physico-chemical characterization of untreated and treated effluent and methyl orange dye

Physical parameters	*Method of analysis*	*Reference*
Colour	*Visual*	-
Odour	*Smell*	-
pH	*pH meter*	*APHA, 1998*
Electrical conductivity (µmhos/cm)	*Conductivity bridge*	*APHA, 1998*
Turbidity (NTU)	*Turbidity meter*	*APHA, 1998*
Alkalinity (mg/L)	*Titrimetry*	*APHA, 1998*
Total Dissolved Solids (mg/L)	*Filtration*	*APHA, 1998*
Total Suspended Solids (mg/L)	*Filtration*	*APHA, 1998*
Total Solids (mg/L)	*Calculation*	*APHA, 1998*
Total hardness (mg/L)	*Titrimetry*	*APHA, 1998*
Biological Oxygen Demand (mg/L)	*Titrimetry*	*APHA, 1989*
Chemical Oxygen Demand (mg/L)	*Titrimetry*	*APHA, 1998*
Chloride (mg/L)	*Titrimetry*	*Vogel, 1964*
Sulphate (mg/L)	*Turbidimetry*	*APHA, 1998*
Nitrate (mg/L)	*Colorimetry*	*APHA, 1998*

B. Phytotoxicity study

The experiment was conducted for the duration of 2 months and the crops were irrigated with tap water (T_1), untreated methyl orange dye solution (T_2) and bioremediated methyl orange dye solution (T_3). The meteorology of the study are indicated the temperature range from 20-30^{o}C with no rainfall and the moisture from 10-50%. The soil at the experimental site has the texture class of 60% clay, 20% silt, 14% fine sand and 6% coarse sand.

1) Selection of the experimental plant

Marigold (Family-Asteracea) and *Celosia argentea* (Family-Amaranthaceae) was selected for the phytotoxicity study. It is usually cultivated for ornamental purposes and it does not require special conditions for successful cultivation which makes it very popular among the gardeners worldwide. It requires adequate sunlight and well-drained soil for successful growth. Dyes extracted from the marigold flowers are used in textile and food industries.

The seeds of Marigold (yellow and orange) and *Celosia argentea* were collected from Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India. Healthy seeds were selected and surface sterilized with 0.1% $HgCl_2$ solution for 2 minutes and washed with tap water to remove the surface contaminants. For field level cultivation, nine square pits were made with 30X30X30cm (length, width, depth) and each treatment was replicated thrice. In each pit 20 healthy seeds were sown and irrigated with tap water (T_1), untreated (T_2) and treated methyl orange (T_3) solutions respectively.

2) Biometric observations

The plants were uprooted on 7th and 60th day after sowing and washed in running water to remove the soil particles adhered on the roots and dried with filter paper to remove the water contents. On the 7th day, germination percentage, root length, shoot length and vigour index were calculated as follows

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed sown}} \text{ X } 100$$

Vigour index = Germination percentage X (root length + shoot length)

(Abdul-Baki and Anderson, 1973).

Similarly, on 60th day height of the plant, number of flowers, buds, branches, leaves, nodes, size of the flowers (cm) and flower weight (g) of the test plants were recorded.

C. *Soil Characteristics of Irrigated Land*

The soil samples were collected (15cm depth) after harvest of the crops with a wooden spade (treatment wise soil samples were collected). The collected samples were air dried and ground to pass through 105mm sieve and stored in plastic bottles before analysis. The soil samples were analysed for different physical and chemical properties as per the standard procedure.

Parameters	*Method of analysis*	*References*
pH	*pH meter*	*Jackson, (1973)*
EC (µmhos/cm)	*Conductivity bridge*	
Macronutrients		
Total nitrogen (kg/ha)	*Titration*	*Subbaih and Asija, (1956)*
Total phosphorus (kg/ha)	*Flame photometry*	*Olsen et al. 1954*
Total potassium (kg/ha)		
Sodium (kg/ha)		
Calcium (kg/ha)	*Titration*	*Subbaih and Asija, (1956)*
Micronutrients		
Iron (mg/kg)	*Atomic absorption spectrophotometry*	*Lindsay and Norvell, (1978)*
Copper (mg/kg)		
Zinc (mg/kg)		
Nickel (mg/kg)	*Spectrophotometry*	*APHA, (1998)*
Manganese (mg/kg)	*Atomic absorption spectrophotometry*	*Lindsay and Norvell, (1978)*

D. *Microbial Toxicity Study*

The microbial toxicity was performed to examine the toxic effects of the degradation products of methyl orange in relation to microbial flora. The bacterial isolates selected for the present study includes Klebsiella pneumoniae, Streptococcus epidermis, Vibrio cholerae, Pseudomonas aeruginosa, Shigella species, Bacillus cereus, Proteus vulgaris, Staphylococcus aureus, Escherichia coli, Salmonella enteritis, Enterococcus faecalis, Yersinia enterocolitica and Acinetobacter species. The fungal isolates are Aspergillus flavus, Aspergillus niger, Acremonium species, Rhizopus species and Trichoderma viride.

Agar well diffusion was carried out to assess the microbial toxicity against the selected bacterial and fungal isolates (Bauer et al., 1966). Three wells were bored on sterile Mueller-Hinton agar (bacteria) and Rose Bengal Chloramphenicol agar (fungi) plates using a sterile cork bore separately. Each plate was swabbed with 100µl of the selected bacterial and fungal isolates separately. To each well in the respective medium distilled water (T_1), untreated methyl orange (T_2) and treated methyl orange (T_3) solutions were added separately and the plates were incubated at 37°C for 24hrs (bacteria) and room temperature for 5days (fungi) to measure the zone on inhibition. The size of inhibition zone (mm) surrounding the well represented the index of toxicity against the pollutants.

III. RESULTS AND DISCUSSION

A. *Physico-chemical characteristics of experimental water and soil samples*

The physico-chemical characteristics of untreated and treated effluent, untreated and bioremediated methyl orange solution samples are illustrated in Table 2. The colour of the bioremediated methyl orange solution was observed to be light green. All the tested parameters (colour, odour, turbidity, pH, turbidity, EC, TDS, TS, TSS, BOD, COD, total hardness, total alkalinity, chloride, sulphate and nitrate) in the bioremediated dye solution (T_3) were within the BIS limit whereas the limits exceed in untreated dye solution (T_2).

Table – 2
Physico chemical analysis of textile effluent and methyl orange dye solution

Parameters analysed	*Untreated textile effluent*	*Treated textile effluent*	*Untreated methyl orange dye solution*	*Treated methyl orange dye solution*	*Tolerance limits*
Physical parameters					
Colour	*Dark orange*	*Light green*	*Orange*	*Light green*	*-*
Odour	*Bad odour*	*Obnoxious*	*Disagreeable*	*Agreeable*	*-*
pH	*10.76*	*7.74*	*8.16*	*7.27*	*5.5-9.0 (BIS, 1981)*
Electrical conductivity (µmhos/cm)	*6.12*	*2.1*	*5.73*	*1.7*	*<2.25 (BIS, 1981)*
Turbidity (NTU)	*Turbid*	*Clear*	*Turbid*	*Clear*	*-*
Chemical parameters (mg/L)					
Alkalinity	*470*	*64.97*	*650*	*54.2*	*270 (BIS,1993)*

Total Dissolved Solids	*7650*	*1840*	*5190*	*1580*	*2100 (BIS, 1981)*
Total Suspended Solids	*312*	*95*	*1043*	*93*	*100 (BIS, 1981)*
Total Solids	*7962*	*1935*	*6233*	*1487*	*-*
Total hardness	*895*	*140*	*768*	*140*	*250 mg/I (BIS,1974)*
Biological Oxygen Demand	*95*	*24*	*80*	*15*	*30 (BIS, 1981)*
Chemical Oxygen Demand	*878*	*223*	*742*	*190*	*250(BIS, 1981)*
Chloride	*3569*	*950*	*2309*	*649*	*1000 (BIS, 1981)*
Sulphate	*1896*	*507*	*1558*	*420*	*1000 (BIS, 1981)*
Nitrate	*159*	*25.58*	*126*	*18.6*	*50 (BIS, 1981)*

Values are mean of three replicates, BIS - Bureau of Indian standards

B. Phytotoxicity study

Use of untreated and treated dye solution for agricultural purpose has direct impact on the fertility of soil. Therefore, it is of concern to assess the phytotoxicity of dye solution before and after degradation by any mode of treatment. Seed germination and plant growth bioassays are the most common techniques used to evaluate the phytotoxicity (Jadhav *et al*., 2010, Saratale *et al*., 2010 and Forss and Welander, 2009).

The commercially available flowering namely plants Marigold (yellow, orange) and *Celosia argentea* have been tested for phytotoxicity using tap water (T_1), untreated methyl orange solution (T_2) and biologically treated methyl orange (T_3). There was a remarkable performance of germination percentage (93%, 96% and 98%) in Marigold (yellow, orange) and *Celosia argentea* seeds irrigated with bioremediated methyl orange on 7th day when compared with (T_2). Control (T_1) plants exhibited maximum germination percentage grown in all treatments. Similar trend was noticed in shoot length, root length and vigour index of all the test plants treated with T_1, T_2 and T_3 (Table 3, 4 and 5).

Table – 3

Biometric parameters of 7th day old seedlings of Marigold (yellow) grown with different treatments

Treatments	*Germination Percentage*	*Shoot length (cm)*	*Root Length (cm)*	*Vigour index*
T1	*97*	*2.88±0.66*	*1.80±0.40*	*454*
T2	*63*	*1.45±0.01*	*0.63±0.07*	*131*
T3	*93*	*2.60±0.83*	*1.71±0.33*	*401*
SED	-	*0.19006*	*0.14627*	-
CD (5%)	-	*0.38281*	*0.29461*	-

(Values are mean ± SD, T_1 –Control (tap water), T_2 - Untreated methyl orange dye solution, T3 - Bioremediated methyl orange dye solution)

Table – 4

Biometric parameters of 7th day old seedlings of Marigold (orange) grown with different treatments

Treatments	*Germination Percentage*	*Shoot length (cm)*	*Root Length (cm)*	*Vigour index*
T1	*98*	*3.95±0.65*	*1.91±0.86*	*574*
T2	*54*	*1.52±0.09*	*0.58±0.59*	*113*
T3	*96*	*2.37±0.41*	*1.68±0.38*	*389*
SED	-	*0.19006*	*0.14627*	-
CD (5%)	-	*0.38281*	*0.29461*	-

(Values are mean ± SD, T_1 –Control (tap water), T_2 - Untreated methyl orange dye solution, T3 - Bioremediated methyl orange dye solution)

Table - 5

Biometric parameters of 7th day old seedlings of *Celosia argentea* grown with different treatments

Treatments	*Germination Percentage*	*Shoot length (cm)*	*Root Length (cm)*	*Vigour index*
T1	*99*	*3.52±0.12*	*1.94±0.76*	*541*
T2	*61*	*1.56±0.69*	*0.66±0.85*	*136*
T3	*98*	*2.32±0.59*	*1.27±0.34*	*352*
SED	-	*0.19006*	*0.14627*	-
CD (5%)	-	*0.38281*	*0.29461*	-

(Values are mean ± SD, T_1 –Control (tap water), T_2 - Untreated methyl orange dye solution, T3 - Bioremediated methyl orange dye solution)

Thus the bioremediated methyl orange solution favoured the growth of the test plants in bioremediated methyl orange solution. From the results, it was obvious that the untreated methyl orange dye solution reduced the seed germination in selected plants

(Table 3, 4 and 5). The biometric parameters namely the height (shoot and root length), number of flowers, buds, branches, leaves, nodes, size of the flowers (cm) and weight of the flower (g) plant irrigated with tap water (T_1), untreated methyl orange solution (T_2) and biologically treated methyl orange (T_3) were presented in Table 6, 7 and 8.

Among all the tested plants, the plants grown with bioremediated methyl orange showed better growth when compared to that of untreated methyl orange solution (T_2). T_3 plants exhibited significant yield when compared with control plants (T_1). These findings are in accordance with the finding of Rehman et al. (2009) who found that treated effluent showed 100 percent germination of Raphanus sativus L, Brassica raapa L and Brassica compastris L and also in agreement with the findings of Kaushik et al. (2005).

Table – 6

Biometric parameters of Marigold (yellow) grown with different treatments on 60th day

Treatments	*Height (shoot and root length) (cm)*	*No. of Flowers*	*No. of Buds*	*No. of Branches*	*No. of Leaves*	*No. of nodes*	*Size of the Flowers (cm)*	*Weight of the flower (g)*
T1	*57.97±0.34*	*14.27±1.11*	*4.83±0.13*	*6.21±0.55*	*28.44±1.04*	*4.87±0.12*	*5.48±0.41*	*5.57±0.06*
T2	*17.34±0.89*	*2.69±0.52*	*2.67±0.38*	*2.67±0.45*	*14.67±0.38*	*2.59±0.07*	*3.88±0.75*	*2.28±0.29*
T3	*50.68±1.80*	*9.60±0.33*	*3.33±0.15*	*4.05±0.30*	*26.41±0.02*	*3.57±0.17*	*4.92±0.19*	*5.64±0.01*
SED	*1.1751*	*0.7321*	*0.2472*	*0.4445*	*0.8194*	*0.6402*	*0.1284*	*0.5041*
CD (5%)	*2.8755*	*1.7915*	*0.6049*	*1.0878*	*1.7924*	*1.5665*	*0.3143*	*1.2335*

(Values are mean ± SD, T1 –Control (tap water), T2 - Untreated methyl orange dye solution, T3 - Bioremediated methyl orange dye solution)6

Table – 7

Biometric parameters of Marigold (orange) grown with different treatments on 60th day

Treatments	*Height (shoot and root length) (cm)*	*No. of Flowers*	*No. of Buds*	*No. of Branches*	*No. of Leaves*	*No. of nodes*	*Size of the Flowers (cm)*	*Weight of the flower (g)*
T1	*61.13±0.27*	*18.88±0.97*	*6.52±0.17*	*7.35±0.64*	*35.52±1.33*	*7.92±0.17*	*6.85±0.25*	*6.71±0.11*
T2	*11.21±0.17*	*1.89±0.32*	*2.78±0.80*	*2.90±0.84*	*12.54±0.82*	*2.65±0.13*	*2.38±0.63*	*2.76±0.39*
T3	*59.81±1.64*	*17.56±1.19*	*5.78±0.38*	*6.49±0.71*	*32.21±0.19*	*5.68±0.29*	*6.52±0.76*	*6.23±0.33*
SED	*1.1985*	*0.8271*	*0.2945*	*0.5791*	*0.5671*	*0.7168*	*0.1427*	*0.6247*
CD (5%)	*2.9295*	*1.7749*	*0.8078*	*1.0971*	*1.1276*	*1.6172*	*0.6349*	*1.3184*

(Values are mean ± SD, T1 –Control (tap water), T2 - Untreated methyl orange dye solution, T3 - Bioremediated methyl orange dye solution)

Table – 8

Biometric parameters of Celosia argentea grown with different treatments on 60th day

Treatments	*Height (shoot and root length) (cm)*	*No. of Flowers*	*No. of Buds*	*No. of Branches*	*No. of Leaves*	*No. of nodes*	*Size of the Flowers (cm)*	*Weight of the flower (g)*
T1	*74.62±3.27*	*8.63±0.68*	*13.37±1.59*	*11.39±0.62*	*89.59±1.17*	*7.93±0.45*	*5.87±0.44*	*3.23±0.25*
T2	*32.57±1.19*	*3.27±0.52*	*3.53±0.05*	*3.29±0.15*	*14.18±1.46*	*3.15±0.31*	*2.83±0.35*	*1.09±0.18*
T3	*71.52±3.89*	*8.01±0.55*	*12.11±0.81*	*10.22±0.68*	*85.77±1.97*	*6.76±0.54*	*5.67±0.67*	*2.94±0.05*
SED	*2.7197*	*0.5437*	*0.8837*	*0.4907*	*21.6090*	*0.4141*	*0.4495*	*0.1564*
CD (5%)	*6.6552*	*1.3306*	*2.1625*	*1.2008*	*52.8778*	*1.0133*	*1.0999*	*0.3828*

(Values are mean ± SD, T1 –Control (tap water), T2 - Untreated methyl orange dye solution, T3 - Bioremediated methyl orange dye solution)

C. *Physical and Chemical characteristics of soil samples*

The soil parameters in the field irrigated with untreated methyl orange (T_2) was found to be increased after harvesting. It shows significant difference when compared with other soil samples T_1 and T_3 (Table 9). This indicates that the untreated methyl orange has a tendency to inhibit the growth of plants as well as decreased the soil fertility.

Table – 9

Physical and Chemical characteristics of soil samples

Parameters	*Control (T_1)*	*Untreated dye soil (T_2)*	*Treated dye soil (T_3)*	*Tolerance limits*
**pH*	*7.64*	*12.04*	*8.01*	*6.5- 8.4*
**EC (µmhos/cm)*	*0.60*	*3.0*	*0.92*	*<1 (normal), 1-4 (critical), >4 (injurious to crop)*
***Macronutrients*				
Total Nitrogen (kg/ha)	*221*	*134*	*282*	*0-280 (low), 280-450 (medium), >450 (high)*
Total Phosphorus (kg/ha)	*30*	*7*	*21*	*0-11 (low), 11-22 (medium), >22 (high)*
Total Potassium (kg/ha)	*211*	*86*	*233*	*0-118 (low), 118-280 (medium), >280 (high)*
Sodium (kg/ha)	*275*	*93*	*240*	*0-118 (low), 118-280 (medium), >280(high)*

Calcium (kg/ha)	*17.8*	*0.4*	*8.2*	*0.5 (low), 5-10 (medium),10-20(normal), >20 (high-excess)*
****Micronutrients*				
Iron (mg/kg)	*4.3*	*1.9*	*3.8*	*<2.5 (low), 2.5-4.5 (marginal) >4.5 (adequate)*
Copper (mg/kg)	*0.66*	*0.2*	*0.51*	*0.72 (FAO, 1980)*
Zinc (mg/kg)	*1.37*	*4.92*	*1.34*	*<0.2 (very low), 0.2-0.7 (low) 0.8-2.4 (medium), 2.5-8.0 (high), >8.0 (too high)*
Nickel (mg/kg)	*1.9*	*0.3*	*1.1*	*2-5 (WHO,1989)*
Manganese (mg/kg)	*21.6*	*14*	*27.41*	*<4 (very low), 4-14 (low) 15-50 (medium) , 51-170 (high), >170 (too high)*

* denotes the limits prescribed by Arnold (1984)
**denotes the limits prescribed by Gupta (2007)
*** denotes the limits prescribed by GUBRETAS (2010)

D. Microbial Toxicity Study

The zone of inhibition exhibited by control (T_1), untreated methyl orange solution (T_2) and treated methyl orange solution (T_3) against the selected bacteria and fungal isolates were presented in Table 10. Untreated methyl orange dye solution (T_2) exhibited maximum zone of inhibition whereas in contrast, the bioremediated methyl orange (T_3) did not show any growth inhibition in all tested microorganisms. However, the control (T_1) also showed no zone in all the microorganisms. Similar trend was observed in fungal isolates (Table 11).

Table – 10
Microbial toxicity (bacteria) studies on methyl orange and its metabolites formed after biodegradation

S. No	*Name of the bacteria*	*Zone of inhibition (mm)*		
		Control (T_1)	*Untreated methyl orange (T_2)*	*Treated methyl orange (T_3)*
1.	*Klebsiella pneumoniae*	*NI*	*11.7±0.30*	*NI*
2.	*Streptococcus epidermis*	*NI*	*11.8±0.52*	*NI*
3.	*Vibrio cholerae*	*NI*	*10.2±0.11*	*NI*
4.	*Pseudomonas aeruginosa*	*NI*	*11.5±0.16*	*NI*
5.	*Shigella species*	*NI*	*11.2±0.50*	*NI*
6.	*Bacillus cereus*	*NI*	*13.7±0.18*	*NI*
7.	*Proteus vulgaris*	*NI*	*11.3±0.75*	*NI*
8.	*Staphylococcus aureus*	*NI*	*11.2±0.20*	*NI*
9.	*Escherichia coli*	*NI*	*9.5±0.55*	*NI*
10.	*Salmonella enteritis*	*NI*	*8.5±0.30*	*NI*
11.	*Enterococcus faecalis*	*NI*	*12.2±0.20*	*NI*
12.	*Yersinia enterocolitica*	*NI*	*11.3±0.15*	*NI*
13.	*Acinetobacter species*	*NI*	*10.2±0.11*	*NI*

The values are mean±SD, NI-No zone of inhibition

Table – 11
Microbial toxicity (fungus) studies on methyl orange and its metabolites formed after biodegradation

S. No	*Name of the fungus*	*Zone of inhibition (mm)*		
		Control (T_1)	*Untreated methyl orange (T_2)*	*Treated methyl orange (T_3)*
1.	*Aspergillus flavus*	*NI*	*12.1±0.25*	*NI*
2.	*Aspergillus niger*	*NI*	*10.5±0.31*	*NI*
3.	*Acremonium species*	*NI*	*12.5±0.72*	*NI*
4.	*Rhizopus species*	*NI*	*13.7±1.10*	*NI*
5.	*Trichoderma viride*	*NI*	*14.6±1.54*	*NI*

The values are mean±SD, NI-No zone of inhibition

Thus, phytotoxicity study shows good germination as well as significant growth in Marigold (yellow, orange) and Celosia argentea against the selected treatments. The growth of microorganisms on dye degraded metabolites indicated that the metabolites generated after biodegradation of methyl orange is less toxic when compared to the original dye.

IV. CONCLUSION

The overall results of wastewater irrigated with three different flower plants namely Marigold (yellow, orange) and Celosia argentea shows good yield and the biometric parameters indicate the positive impact of wastewater on the irrigated water. The physico-chemical characteristics of the bioremediated samples falls within the BIS limits. Hence bioremediated waste water may have a promising future as an alternative source for safe irrigation.

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