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# Physicochemical characterization and Bioremediation perspective of textile effluent, dyes and metals by indigenous Bacteria

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ABSTRACT

Physicochemical and bacteriological status of a local textile mill effluent showed considerably high values of temperature (40 °C), pH (9.50), EC (3.57 μs/m), BOD (548 mg $l^{-1}$), COD (1632 mg $l^{-1}$), TSS (5496 mg $l^{-1}$), TDS (2512 mg $l^{-1}$), heavy metals ions (0.28–6.36 mg $l^{-1}$) and color above the prescribed fresh water limits. However, a considerable decline in almost all pollution indicators from source to sink indicated signs of natural remediation. Ten bacteria strains isolated from effluent showed comparatively higher resistance (MRL) (mg $l^{-1}$) (average) for 10 heavy metals than against four structurally different dyes tested on solid media of mineral salt. Overall bacterial resistance was quite high against $Fe^{3+}$ (2820), $Cr^{3+}$ (1203), $Zn^{2+}$ (1122), $Mn^{2+}$ (804) and $Pb^{2+}$ (435), whereas, it varied amid 300–500 in four dyes. Bacterial decolorization/degradation of dyes indicated on solid media was confirmed through experiments carried out in liquid broth.



## 1. Introduction

Textile industries consume large amount of water (60–400 l/kg of fabric) and chemicals for wet processing [1]. The chemical reagents used in textile sector are diverse in chemical composition ranging from inorganic to organic. The inputs of wide rage of chemicals, which, if not incorporated in the final products (fabric), become waste and turn out to be part of water ecology. Generally, textile effluent is colored, varying in hydraulic flow rate, having high; pH, temperature, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total suspended solids (TSS) [2–4].

Color is imparted to textile effluents because of various dyes and pigments used. Many dyes are visible in water at concentrations as low as 1 mg $l^{-1}$. Textile wastewaters, typically with dye content in the range of 10–200 mg $l^{-1}$ are therefore highly colored. In addition to dyes, various salts and chemicals are major sources of heavy metals in wastewater [5]. Sediments, suspended and dissolved solids are important repositories for toxic heavy metals and dyes [6,7] causing rapid depletion of dissolved oxygen leading to oxygen sag in the receiving water [8]. The metals and contaminants like dyes tend to persist indefinitely, circulating and eventually accumulating through out the food chain [9–11]. Various reports have mentioned the direct and indirect toxic effects of dyes and metals in the form of tumors, cancers and allergies besides growth inhibitions on different trophic levels like bacteria, protozoans, algae, plants and different animals including human being [6,12–15].

The key environmental issues associated with textile manufacture are: water use, treatment and disposal of aqueous effluent Textile effluents are mostly discharged after minimal or no pretreatment into the adjoining water channels, streams and estuaries [16,17]. The presence of dyes and metals causing severe damage to the aquatic biology. Consequently, the self-purification ability of the stream and conventional biological treatment systems is hindered [8]. Despite all aforementioned consequences, microbially mediated detoxification technologies are still being valued over physicochemical ones for the last few decades. This growing emphasis on biological remediation has been associated with their cost effective and long lasting nature. Many researchers have studied live microbial systems for remediaton of metals and dyes contaminated soils and waters [17–27]. Still, there is a need of valued research to unravel the potential of various microbes for the rehabilitation of our natural resources.

Relying on the diverse nature of textile effluents, the present study is focused on physicochemical and bacteriological evaluation of a local textile mill effluent. Self-purification ability of the wastewater is inspected besides analyzing the potential of

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**Table 1**
Methods used for analysis of different physicochemical parameters

| Parameter | Instrument | Method | Reference |
|---|---|---|---|
| pH | Orabeco portable pH meter model 64 | Direct at site | [28] |
| Electric conductivity (EC) | PW 9526 digital conductivity meter | Direct at site | |
| Total suspended solid (TSS) | Hot air oven, Memmert | Filtration and drying in oven at 100 °C | |
| Total dissolved solid (TDS) | Hot air oven, Memmert | Evaporation at 105 °C | |
| Chemical oxygen demand (COD) | COD reactor HACH) Perkin-Elmer, Shimadzu UV/VIS Spectrophotometer | Colorimetric method | |
| Biological oxygen demand (BOD) | | Wrinkler method | |

different heavy metals and dyes resistant bacterial isolates at laboratory scale.

## 2. Experimental

### 2.1. Chemicals

Drimarene Blue (Db) $K_2$RL (Reactive Anthraquinone) and Sulfur Black (Sb) (Anthraquinone) dyes were obtained from Kohinoor textile mill (KTM), Rawalpindi, Pakistan. Other chemical compounds including Acid Red (Ar 151) (di-azo) and Orange (Or) II (mono-azo) were purchased from BDH laboratory chemical division, Poole, Dorset, England, Sigma chemicals Co; St, Lois, E. Merck, Darmstadt, Germany.

### 2.2. Sampling and analysis of effluent

Sampling of KTM effluent was carried out during dyeing and washing at three sites [source ($S_1$), middle point ($S_2$) and sink ($S_3$)] spanning a distance of 500 m. Standard procedures (Spot or Grap) were followed during sampling. pH and temperature of the effluent were determined at the spots, whereas, rest of the physicochemical parameters were determined instantly after bringing the samples in the analytical chemistry laboratory, Environmental Protection Agency, Islamabad. Tables 1 and 2 precisely show the methods followed during physicochemical analysis of effluent. Effluent samples were also screened out for bacteria at Microbiology Research Laboratory, Quaid-i-Azam University, Islamabad.

### 2.3. Isolation and identification of bacterial strains from effluent

Effluent samples collected from textile mill were screened out for the isolation of potential bacterial strains. Samples after being serially diluted in sterile distilled water were plated onto nutrient agar plates and then incubated for 48 h at 30 °C. Discrete bacterial colonies that grew on agar plates were initially grouped on the basis of gram staining and different morphological characteristics, such as pigmentation, motility and colony forms. Bacterial isolates were then picked, sub-cultured and subjected to further biochemical tests for identification according to Bergey's Manual of Determinative Bacteriology (9th edition).

**Table 2**
Method used for analysis of different metal ions

| Metal | $\lambda_{max}$ (nm) | Determination limit (mg l$^{-1}$) | Sensitivity (mg l$^{-1}$) | Optimum concentration range (ng ml$^{-1}$) | Flame–gas | Instrument | Procedure | Reference |
|---|---|---|---|---|---|---|---|---|
| $Cd^{2+}$ | 228.80 | 0.002 | 0.025 | 0.02–50 | Air–acetylene | Atomic absorption Spectrophotometer (Solar Unicam) | Sample digestion with $HNO_3$ + filteration + analysis | [28] |
| $Cu^{2+}$ | 324.80 | 0.030 | 0.200 | 0.50–10 | | | | |
| $Pb^{2+}$ | 283.00 | 0.050 | 0.500 | 1.00–20 | | | | |
| $Zn^{2+}$ | 213.90 | 0.005 | 0.020 | 0.05–02 | | | | |
| $Fe^{3+}$ | 248.30 | 0.020 | 0.120 | 0.30–10 | | | | |
| $Cr^{3+}$ | 357.90 | 0.020 | 0.100 | 0.20–10 | | | | |
| $Ni^{2+}$ | 232.00 | 0.020 | 0.150 | 0.30–10 | | | | |
| $Mn^{2+}$ | 279.50 | 0.010 | 0.050 | 0.10–10 | | | | |

**Table 3**
Physicochemical and microbial characterization of the Textile effluent compared with National Environmental Quality Standards (N.E.Q.S)

| Parameters | Units | N.E.Q.S | Sample 1 (Source) | Sample 2 (Middle point) | Sample 3 (Semi-stagnant; Sink) | Average | S.E. (±) |
|---|---|---|---|---|---|---|---|
| Color | | | Brown/Blue | Dark blue/Dark grey | Black | | |
| Smell | | | Fishy | Fishy | pungent | | |
| Temperature | °C | 40 | 51.20 | 42.00 | 28.00 | 40.40 | 6.74 |
| pH | | 6 to 9 | 11.90 | 8.9 | 7.70 | 9.50 | 1.25 |
| EC | μs/m | | 5.81 | 3.84 | 1.07 | 3.57 | 1.37 |
| TSS | mg l$^{-1}$ | 200–400 | 415.63 | 626.00 | 15448.50 | 5496.71 | 4976.27 |
| TDS | mg l$^{-1}$ | 3500 | 1231.00 | 3849.67 | 2457.00 | 2512.56 | 756.46 |
| COD | mg l$^{-1}$ | 150–400 | 1728.00 | 2080.00 | 1088.00 | 1632.00 | 290.36 |
| BOD | mg l$^{-1}$ | 80–250 | 553.00 | 848.00 | 243.00 | 548.00 | 174.67 |
| $Cu^{2+}$ | mg l$^{-1}$ | 1 | 9.73 | 4.02 | 5.32 | 6.36 | 1.73 |
| $Cd^{2+}$ | mg l$^{-1}$ | 0.1 | 0.69 | 0.63 | 0.20 | 0.51 | 0.15 |
| $Zn^{2+}$ | mg l$^{-1}$ | 5 | 7.85 | 1.07 | 0.78 | 3.23 | 2.31 |
| $Fe^{2+}$ | mg l$^{-1}$ | 2 | 7.00 | 6.80 | 4.20 | 6.00 | 0.90 |
| $Cr^{3+}$ | mg l$^{-1}$ | 1 | 2.20 | 1.43 | 1.38 | 1.67 | 0.27 |
| $Mn^{2+}$ | mg l$^{-1}$ | 1.5 | 7.53 | 0.38 | 5.11 | 4.34 | 2.10 |
| $Ni^{2+}$ | mg l$^{-1}$ | 1 | 1.21 | 0.61 | 0.19 | 0.67 | 0.30 |
| $Pb^{2+}$ | mg l$^{-1}$ | 0.5 | 0.30 | 0.36 | 0.17 | 0.28 | 0.06 |
| Bacterial count | CFU/ml | | $0.21 \times 10^5$ | $0.87 \times 10^5$ | $11.5 \times 10^5$ | $4.19 \times 10^5$ | $3.7 \times 10^5$ |

### 2.4. Composition of mineral salt (MS) medium

Mineral salt medium was made by adding per liter of distilled water; acetic acid (99.9%) 0.15 ml, $(NH_2)_2CO$ 100.0 mg, $KH_2PO_4$ 67.0 mg, $NaHCO_3$ 840.0 mg, $MgSO_4{\cdot}7H_2O$ 38.0 mg, $CaCl_2$ 21.0 mg, $FeCl_3{\cdot}6H_2O$ 7.0 mg and Glucose 6.0 gm. Agar (1.5%) was added as solidifying agent.

### 2.5. Screening of bacterial isolates against dyes and metals

The bacterial isolates were sequentially adapted to higher concentrations (10–10,000 mg $l^{-1}$) of four different dyes and nine metals after repeated sub-culturing on solid culture media of mineral salt and agar in petri plates. Salts used for $Mn^{2+}$, $Zn^{2+}$, $Fe^{3+}$, $Cd^{2+}$, $Ni^{2+}$, $Cr^{3+}$, $Cr^{6+}$, $Cu^{2+}$ and $Pb^{2+}$ metal ions included $MnSO_4{\cdot}H_2O$, $ZnSO_4{\cdot}7H_2O$, $K_2Fe(CN)_6$, $(CH_3COO)_2Cd{\cdot}2H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Cr(NO_3)_3$, $K_2Cr_2O_7$, $CuCl_2{\cdot}2H_2O$ and $Pb(NO_3)_2$, respectively. The maximum resistance limits (MRL) beyond which the bacterial growth was completely inhibited at different concentration of dyes and metal ions were determined. In addition, apparent decolorization abilities of the bacterial strains for different dyes were also monitored at 37 °C for 8 days as described by Pasti-Grigsby et al. [29] and Lee et al. [27].

Decolorization ability of bacterial isolates was then analyzed for each dye (at 20 mg $l^{-1}$) in liquid culture of mineral salt (100 ml in 250 ml Erlenmeyer flask) under static condition for 8 days. Freshly prepared inocula (10%) of selected bacteria in nutrient broth were taken (at mid log phase) and used in the experiments. Samples of treated dye containing medium were drawn into plastic vials (size 2.5 ml). These samples were initially filtered through 0.2 μm syringe filter. The residual amounts of each dye in medium was monitored though Shimadzu UV/VIS spectrophotometer at its respective $\lambda_{max}$ (Ar 151 512, Or II 480, Db $K_2$RL 620, Sb 610). Absorbance units were converted to concentrations by using standard curves and %decolorizations were determined by the using the following formula:

$$\%\text{Decolorization} = \frac{\text{Initial concentration of dye} - \text{Final concentration of dye}}{\text{Initial concentration of dye}} \times 100$$

### 2.6. Statistical analysis

The results obtained in experiments were expressed in terms of means (average) and standard error (S.E.). Data was statistically defined by ANOVA (single factor) and LSD test by using Microsoft Excel and MSTAT softwares. Results in each experiment were interpreted rank wise in alphabetical order based upon probabilities. Probability ($p$-value) less than 0.05 and 0.01 was considered significant and highly significant, respectively.

## 3. Results

### 3.1. Physicochemical and microbiological status of textile effluent

Apparently, the effluent samples collected from KTM during dyeing and washing conditions were brown, blue ($S_1$) to black ($S_3$) in color, with pungent ($S_3$)—fishy ($S_1$ and $S_2$) smell and high in temperature [28–51 °C (average = 40.40 °C)]. Closed to source ($S_1$), the pH of the effluent was highly alkaline (11.9) but it reduced towards neutrality ($S_3$ = 7.7) at sink. Electric conductivity (EC) (μs/m) of the effluent was quite low (ave. = 3.57 μs $m^{-1}$) and it declined away from the source of emission (Table 3).

**Table 4**
Maximum resistance limits (MRL) of different bacterial isolates against heavy metals ions on solid culture media (MS + agar)

| Strain | MRL (mg $l^{-1}$) | | | | | | | | LSD | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $Zn^{2+}$ | $Ni^{2+}$ | $Cu^{2+}$ | $Mn^{2+}$ | $Fe^{3+}$ | $Cd^{2+}$ | $Cr^{6+}$ | $Cr^{3+}$ | $Pb^{2+}$ | Mean ± S.E. | Rank |
| *Aeromonas* spp. N1A | 130 | 200 | 30 | 2100 | 2450 | 50 | 0 | 1500 | 200 | 740.00 ± 329.87 | ABC |
| *Staphylococcus aureus* N2A | 130 | 150 | 15 | 550 | 2150 | 0 | 0 | 1500 | 500 | 555.00 ± 255.29 | ABC |
| *Lactobacillus* spp. N3A | 1600 | 150 | 0 | 65 | 600 | 50 | 800 | 1500 | 850 | 623.89 ± 206.11 | ABC |
| *Bacillus subtilis* N4A | 3000 | 150 | 0 | 65 | 4400 | 50 | 600 | 1500 | 0 | 1085.00 ± 532.35 | AB |
| *Micrococcus* spp. N5A | 1850 | 100 | 50 | 0 | 4400 | 0 | 100 | 1500 | 950 | 994.44 ± 486.73 | ABC |
| *Bacillus* spp. N6A | 1900 | 100 | 50 | 65 | 7600 | 50 | 900 | 1500 | 850 | 1446.11 ± 802.35 | A |
| *Bacillus megaterium* N7A | 130 | 150 | 10 | 2200 | 4400 | 50 | 0 | 1500 | 700 | 1015.56 ± 495.81 | AB |
| *Acinetobactor* spp. N8A | 2300 | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 272.22 ± 254.01 | BC |
| *Pseudomonas aeruginosa* N9A | 130 | 150 | 90 | 2700 | 2050 | 0 | 100 | 1500 | 200 | 768.89 ± 343.98 | ABC |
| *Escherichia coli* N10A | 50 | 100 | 200 | 300 | 150 | 50 | 10 | 30 | 100 | 110.00 ± 31.09 | C |
| | Mean ± S.E. | 1122 ± 355.3 | 140 ± 10.0 | 44.5 ± 19.6 | 804.5 ± 341.1 | 2820 ± 756.6 | 30 ± 8.2 | 251 ± 115.5 | 1203 ± 198.0 | 435 ± 119.5 | |
| LSD | Rank | B | C | C | BC | A | C | C | B | BC | |

**Table 5**
Maximum resistance limits (MRL) of different bacterial strains against four textile dyes on solid culture media (MS + Agar)

| Bacteria | | MRL (mg l$^{-1}$) | | | | LSD | |
|---|---|---|---|---|---|---|---|
| | | Ar 151 | Or II | Db $K_2$RL | Sb | Mean ± S.E. | Rank |
| *Aeromonas* spp. N1A | | 150 | 150 | 450 | 300 | 262.5 ± 71.80 | B |
| *Staphylococcus aureus* N2A | | 50 | 350 | 400 | 350 | 287.5 ± 80.04 | B |
| *Lactobacillus* spp. N3A | | 500 | 250 | 350 | 250 | 337.5 ± 59.07 | AB |
| *Bacillus subtilis* N4A | | 750 | 350 | 600 | 450 | 537.5 ± 87.50 | A |
| *Micrococcus* spp. N5A | | 600 | 600 | 250 | 300 | 437.5 ± 94.37 | AB |
| *Bacillus* spp. N6A | | 350 | 250 | 500 | 400 | 375.5 ± 52.04 | AB |
| *Bacillus megaterium* N7A | | 650 | 650 | 550 | 300 | 537.5 ± 82.60 | A |
| *Acinetobacter* spp. N8A | | 400 | 350 | 250 | 250 | 312.5 ± 37.50 | B |
| *Pseudomonas aeruginosa* N9A | | 250 | 300 | 350 | 350 | 312.5 ± 23.94 | B |
| *Escherichia coli* N10A | | 350 | 450 | 200 | 250 | 312.5 ± 55.43 | B |
| LSD | Means ± S.E. | 405 ± 70.49 | 370 ± 49.55 | 390 ± 42.69 | 320 ± 21.34 | | |
| | Rank | A | A | A | A | | |

TSS in the effluent was quite high in sample 3 (15448 mg l$^{-1}$) compared to the first two samples ($S_1$ = 415 mg l$^{-1}$; $S_2$ = 626 mg l$^{-1}$) but TDS decreased in sample 3 (2457 mg l$^{-1}$), however, increased from to $S_1$ (1231 mg l$^{-1}$) to $S_2$ (3850 mg l$^{-1}$). Overall, there was observed a significant high load of COD (average = 1632 mg l$^{-1}$) than BOD (average = 548 mg l$^{-1}$) though both followed same decreasing trend towards sink ($S_3$) after considerable increase at $S_2$ (Table 3).

Heavy metals analysis of the effluent released from textile industry showed their high amounts that decreased down the stream even then kept slightly higher than National Environmental Quality Standards (N.E.Q.S). The average amount (mg l$^{-1}$) of metals ions like $Cu^{2+}$ (6.35), $Fe^{3+}$ (6), $Mn^{2+}$ (4.34) and $Zn^{2+}$ (3.23), was considerably higher while it kept <2 mg l$^{-1}$ in case of $Cd^{2+}$, $Cr^{3+}$, $Ni^{2+}$ and $Pb^{2+}$ in the effluent. Different bacterial strains were isolated from effluent samples and they were morphological and biochemicals characterized for identification and are listed in Table 4. Six out of 10 bacterial isolates were gram positive in the effluent samples. The bacterial count (CFU) was significantly higher (11.5 × 105) at $S_3$ (sink) compared to $S_1$ (0.21 × 105) and $S_2$ (0.87 × 105) (Table 3).

### 3.2. Maximum resistance level (MRL) of bacterial isolates against heavy metals

The bacterial isolates showed a great deal of resistance (MRL) when acclimatized against higher concentration of different heavy metal ions after repeated sub-culturing on MS-agar plates (Table 4). MRL (mg l$^{-1}$) against total metal ions was comparatively higher in *Bacillus subtilis* N4A (1085.00 ± 532.35), *Micrococcus* spp. N5A (994.44 ± 486.73), *Bacillus* spp. N6A (1446.11 ± 802.35) and *Bacillus megaterium* N7A (1015.56 ± 495.81). It was highest against; $Zn^{2+}$ (3000) in *Bacillus subtilis* N4A, $Ni^{2+}$ (200) in *Aeromonas* spp. N1A, $Cu^{2+}$ (200) in *Escherichia coli* N10A, $Mn^{2+}$ (2700) in *Pseudomonas aeruginosa* N9A and $Fe^{2+}$ (7600) in *Bacillus* spp. N6A. It was 900 against $Cr^{6+}$ in *Bacillus* spp. N6A, while 1500 against $Cr^{3+}$ in all the bacterial strains except *Acinetobactor* spp. N8A and *Escherichia coli* N10A. Against $Cd^{2+}$, the bacterial resistance kept ≤50 mg l$^{-1}$, while in case of $Pb^{2+}$, *Lactobacillus* spp. N3A, *Micrococcus* spp. N5A, *Bacillus* spp. N6A and *Bacillus megaterium* N7A showed MRL ranging from 700 to 950 mg l$^{-1}$.

### 3.3. Screening of bacterial isolates against four textile dyes

The bacterial isolates adapted to four different dyes (Ar 151, Or II, Sb and Db $K_2$RL) after repeated sub-culturing on MS-agar plates showed MRLs ranging from 50 to 750 mg l$^{-1}$ (Table 5). The average MRL values of 10 bacterial strains against four different dyes though not differed significantly but was maximum against Ar 151 i.e., 405 ± 70.49 mg l$^{-1}$ and it was 390 ± 42.69, 370 ± 49.55 and 320 ± 21.34 mg l$^{-1}$ against Db $K_2$RL, Or II and Sb, respectively. The highest MRL (average) was observed in case of *Bacillus subtilis* N4A (537.5 ± 87.50) and *Bacillus megaterium* N7A (537.5 ± 82.60) against four dyes. Specifically the most resistant bacterial strains were including *Bacillus subtilis* N4A against Ar 151 (750), Db $K_2$RL (600) and Sb (450) and *Bacillus megaterium* N7A against Or II (650). Decolorization of dyes specifically azo (Ar 151 and Or II) was apparently associated with cellular wall uptake (adsorption) mechanism. In addition, there was observed a slow manifestation of varying decolorization zones around bacterial colonies (only after 8 days) on different dyes containing plates. Bacterial decolorization abilities of multiple natures were then confirmed through experiments conducted in liquid broth under static condition (Table 6). The decreasing decolorization trend of four dyes was like; Ar 151 > Db $K_2$RL > Sb > Or II. Specifically, the most decolorizing bacterial strains was *Bacillus subtilis* N4A (66.72) in Ar 151, *E. coli* (48%) in Or II, *Acinetobactor* spp. N8A (47%) in case of Sb, while *Aeromonas* spp. N1A and *Micrococcus* spp. N5A (54%) in Db $K_2$RL.

## 4. Discussion

Physicochemical status of colored effluent samples of KTM revealed a reasonably high load of pollution indicators compared to the prescribed N.E.Q.S (Table 3).

Color is imparted to a water body by dissolved constituents (dyes and pigments) that absorb white light and emit light at specific wavelengths. There was a gradual change in the color from brown/blue to grey/black of the effluent from source to the sink indicating sign of decolorization. The decreasing color intensity of the effluent has been related to adsorption/chemical transformation of dyes (including metal complex) by biotic and abiotic component of the effluent [30–32]. The increasing bacterial count at sink might have been responsible for such color change in the present study.

Initially the temperature of the effluent generated from KTM was considerably high (51 °C), however, declined to mesophilic status (28 °C) at sink ($S_3$), which ultimately could have favored biologically mediated remediation of effluent. High temperature reduces solubility of gases in water that ultimately express as high BOD/COD. BOD and COD levels recorded in effluent samples declined down the stream, specifically BOD which almost touched the permissible limits of N.E.Q.S. Nevertheless, high values of BOD/COD as observed in present case demands significant amount of dissolved oxygen for enhanced intrinsic remediation of wastewater.

Generally alkaline pH of textile effluents is associated with the process of bleaching [1–3] and it is extremely undesirable in water ecology [19]. Both chemically and biologically mediated adsorp-

**Table 6**
Decolorization (%) of dyes in liquid culture medium by different bacterial isolates

| Bacteria | Decolorization | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ar 151 | | | | Or II | | | | Db $K_2$RL | | | | Sulfur black | | | | Total Dyes | | | |
| | Ave. | S.E. (±) | Rank | *N* | Ave. | S.E. (±) | Rank | *N* | Ave. | S.E. (±) | Rank | *N* | Ave. | S.E. (±) | Rank | *N* | Ave. | S.E. (±) | Rank |
| *Aeromonas* spp. N1A | 23.20 | 0.16 | J | 3 | 10.21 | 1.18 | F | 3 | 53.79 | 2.26 | A | 3 | 7.48 | 3.15 | F | 3 | 23.67 | 10.61 | A |
| *Staphylococcus aureus* N2A | 43.02 | 0.76 | G | 3 | 6.69 | 2.09 | F | 3 | 43.49 | 1.96 | B | 3 | 14.64 | 1.52 | E | 3 | 26.96 | 9.55 | A |
| *Lactobacillus* spp. N3A | 39.13 | 0.23 | H | 3 | 31.38 | 3.92 | C | 3 | 35.13 | 2.20 | C | 3 | 19.85 | 1.98 | D | 3 | 31.37 | 4.15 | A |
| *Bacillus subtilis* N4A | 66.72 | 1.41 | A | 3 | 18.07 | 2.11 | E | 3 | 12.47 | 2.86 | F | 3 | 32.44 | 0.88 | C | 3 | 32.43 | 12.18 | A |
| *Micrococcus* spp. N5A | 45.34 | 0.11 | F | 3 | 45.27 | 2.82 | AB | 3 | 53.66 | 2.24 | A | 3 | 30.07 | 4.81 | C | 3 | 43.59 | 4.92 | A |
| *Bacillus* spp. N6A | 56.71 | 0.57 | C | 3 | 43.01 | 2.91 | B | 3 | 27.74 | 1.42 | D | 3 | 42.31 | 1.11 | B | 3 | 42.44 | 5.92 | A |
| *Bacillus megaterium* N7A | 50.73 | 0.81 | D | 3 | 16.20 | 2.53 | E | 3 | 21.19 | 2.15 | E | 3 | 31.34 | 2.69 | C | 3 | 29.86 | 7.64 | A |
| *Acinetobacter* spp. NA8 | 59.90 | 0.64 | B | 3 | 25.27 | 2.18 | D | 3 | 23.88 | 2.65 | DE | 3 | 47.38 | 1.57 | A | 3 | 39.11 | 8.78 | A |
| *Pseudomonas aeroginosa* NA9 | 47.64 | 0.31 | E | 3 | 8.64 | 2.10 | F | 3 | 50.63 | 2.79 | A | 3 | 43.10 | 2.60 | AB | 3 | 37.50 | 9.74 | A |
| *Escherichia coli* N10A | 29.47 | 1.32 | I | 3 | 48.13 | 2.11 | A | 3 | 52.58 | 5.05 | A | 3 | 19.22 | 4.21 | DE | 3 | 37.35 | 7.85 | A |
| | Ave. | 46.19 | | | 25.29 | | | | 37.46 | | | | 28.78 | | | | | | |
| Bacteria | S.E. (±) | 0.19 | | | 0.17 | | | | 0.25 | | | | 0.30 | | | | | | |
| | Rank | A | | | D | | | | B | | | | C | | | | | | |

tion/reduction of dyes are initiated with decreasing pH level under redox-mediating compounds [33–35]. Decrease in pH i.e., 11.9 to 7.7 of KTM effluent down the stream significantly improved bacterial count and thereby associated remediation.

Conductivity or specific conductance is measured to establish a pollution zone around an effluent discharge. It is sensitive to variation in dissolved ions and mineral salts [36]. Electric conductivity noted in KTM effluent was considerably low and it gradually declined and paralleled with decreasing metal ions concentrations ($S_1$ to $S_3$) thereby suggesting their biotic [37–39] and abiotic removal [40]. Divalent cations are considered to be important bridging agents between negatively charged expolymers and bacterial surfaces [41]. In addition, the decreasing metal ions concentrations in effluent could also be linked to their leaching into the soil bordering effluent channel [42].

TSS and TDS in effluents correspond to filterable and non-filterable residues, respectively. There was observed an increase in TSS in effluent of KTM from source to sink compared to desired limits of N.E.Q.S. Though, decrease in TDS at $S_3$ with an increase in bacterial count and declining pH and EC suggested process of flocculation (as TSS). Microbial community (both aerobic and anaerobic) establishes itself in granulated floc as activated sludge plays a vital role in biodecolorization/bioremediation of wastewater [43,44].

Ten bacterial isolates from KTM showed varying resistance (MRL) of multiple natures for different metal ions ($110.00 \pm 31.09$ to $1446.11 \pm 802.35$ mg $l^{-1}$). Similar high incidence of metals' resistance has been reported in different bacterial strains (*Bacillus* spp. Enterobacter, Pseudomonas, Alcaligenes, Micrococcus and Caulobacter). Such bacterial strains resistance was associated with their continuous exposure to different metal ions that eventually developed bioaccumulation capability in them [27,45–51] related to specific cellular metal binding peptides or excreted polymers [52–54]. Moreover, the macromolecular composition of biosorbent could also be manipulated by cultivation conditions (hard and soft acid and base principles) to produce stronger ligands of transition metals than those naturally present on the microbial surfaces [55,56].

Most of the bacterial isolates from KTM were previously reported to be used in the degradation of dyes and related products [57–63]. Bacterial isolates sequentially adapted against higher concentration of four different dyes (Ar 151, Or II, Sb and Db $K_2$RL) on solid medium exhibited varying MRLs ranging from 50 to 750 mg $l^{-1}$. Besides, diffused decolorization halos around bacterial colonies were evident in at least 8 days (Table 5). Similarly, Pasti-Grigsby et al. [29] examined slow biodecolorization of dyes on solid culture medium compared to liquid culture. MRL against dyes was considerably higher in bacterial isolates which already exhibited higher resistance against heavy metal ions in present study. Sequential adaptation of bacterial cultures with increasing concentration of Or II and Or I reported to develop higher resistance and degradation abilities [64]. Similarly, a co-culture consisted of *H. palleronii* $S_1$ and *Agrobacterium radiobacter* $S_2$ developed to grow with 4-carboxy-4′-sulfoazobenzene as a sole source of carbon and energy [65]. However, subsequent high concentrations of dyes proved to be limiting bacterial growth in current research and it was complementary to the findings of different other reports [66–68]. There was observed signs of dyes' adsorption to the cell walls of bacterial isolates grown on solid culture medium. Such dye (Acid Orange 7)-binding ability was also linked with cell membrane and was considered relatively independent from decolorization dynamics of *A. faecalis* [68]. However, adsorption could be variable, depending on the dye, with subsequent reduction [69]. Otherwise, the dye may remain in the cell wall [70] though not inhibiting the reduction rate of microbes [71]. Nevertheless, lucid decolorization

zones around bacterial colonies in present study also suggested indirected confirmation of extracellur enzymatic mineralization of dyes [68].

Overall, biodecolorization abilities of the bacterial isolates confirmed through experiments in liquid broth under anoxic (static) conditions remained below 50% (Table 6). It clearly indicated need of improvements in culture conditions (aeration and agitation) to further augment the decolorization processes. Generally, azo dyes undergo restricted degradation under aerobic/anoxic condition and this was somewhat depicted in case of Or II (monoazo dye) which decolorized minimum out of four dyes tested. On the contrary, Ar 15, besides being diazo, decolorized maximum demonstrating either varying azo reductase type enzyme production against it in bacterial strains or dye might have adsorption onto bacterial surfaces due to its reactive nature [68]. Similar reactive nature seemed to be favoring decolorization of Db $K_2$RL (removal through dye adsorption) over Sb although both also have shared same anthraquonone structural basis [68]. Nonetheless the removal of Db $K_2$RL type dyes (like Reactive Blue 2) has also been well documented through enzymatic mechanism [72] and indicated in present results. Therefore, consideration of multiple aspects in degradation studies of such chemicals cannot be ignored.

## 5. Conclusions

Kohinoor textile mill effluent clearly imparting a high load of chemicals indicated in the form of different pollution indicators. But, somehow, the phenomenon of natural remediation seemed to be occurring on-site and it was furthermore confirmed through laboratory studies where the bacterial isolates indicated high resistant and transformation abilities for heavy metal ions and dyes. A detailed physiological understanding of such microbes is much needed for standardization of bioremediation technologies in future.

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