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ASSESSMENT OF DISTILLERY EFFLUENT IRRIGATION ON SOIL MICROBES AND ITS BIOREMEDIATION

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Abstract: The present study deals with the assessment of toxicity of distillery effluent on soil microorganisms and its quality improvement through bioremediation using *Pseudomonas spp.* Under lab scale experiment, different dilutions of distillery effluent *i.e.* 25%, 50%, 75% were used to examine effects on physico-chemical parameters of effluent and on soil microflora *e.g.* algae, bacteria, fungi and actinomycetes. The results revealed that dilution may reduce significantly the metal contents and other toxicants in the effluent as well as in the soil. Statistical analysis revealed that bioremediation of distillery effluent using *Pseudomonas spp.* caused significant reduction in BOD, COD, TDS, TN, TP and color. The study indicates that raw distillery effluent is harmful for soil microflora and bioremediation improves the quality of distillery effluent making it suitable as a soil amendment.
Keywords: Distillery effluent, CFU, Rhizosphere, Indigenous bacteria, Bioremediation.
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INTRODUCTION

Application of distillery effluent in agricultural soils is amongst the most economical resources for soil fertility amelioration through improvement in soil water-holding capacity, texture, structure, nutrient retention, root penetration, and soil pH reduction but it is also hazardous to human race by polluting the water bodies and soil. In certain areas, the scarcity of water has forced the farmers to use the effluent as a substitute for irrigation water over the years (Pal *et al*., 2012). Distillery effluents often have low pH, strong odor, dark brown color, and extremely high nutrients content. They often have chemical oxygen demand (COD) ranging from 80,000 to 100,000 mg/L biological oxygen demand (BOD) of 40,000–50,000 mg/L; and nutrients like nitrogen of 1,660–4,200 mg/L; phosphorus of 225–3,038 mg/L; and potassium of 9,600–17,475 mg/L. This disturbs the ecological balance and food chain is also ill affected with the toxic pollutants released from distillery effluents in environment (Pal *et al.,* 2012; Singh and Patel, 2012). Since the conventional methods of waste treatment are uneconomical, alternative methods like application of distillery effluents to agricultural land is receiving increasing attention. The increasing cost of fertilizers and most essential nutrients also demand the use of distillery effluent as a soil amendment. However, its application in soil also results in environmental problems (Cruz *et al.,* 1991) because apart from organic content and nutrients, it also includes toxic compounds, heavy metals, and phenolic derivatives (Chandra *et al.,* 2004).

Crop productivity basically depends on many factors in which irrigation water quality is most important. Irrigation water quality influences many soil microbiological properties, which further change the crop productivity. The factors influencing microbial community structure and function in soil include contaminant mixture type, soil type and time. Thus, soil microorganisms may be affected via variation in soil temperature, pH, nutrient status,

heavy metals, oxygen level and, which in turn, can affect the ecological processes linked with nutrients cycling by effluent application (Chandra *et al.,* 2008). Short-term evaluation of distillery effluent application in crop fields showed positive effect on crops growth (Bharagava *et al.,* 2008 and Ramana *et al.,* 2002) but in-depth understanding of the effect of distillery effluent is lacking on soil microorganism number and diversity. In recent years, people are facing many problems and water pollution is one among them. Environmentalist and government are looking for efficient, cheap and lasting solutions to distillery effluent treatment and recycling (Selvam *et al*., 2011). Physico-chemical methods of waste water treatment are inevitably cost intensive and cannot be employed in all industries especially in developing countries like India. Hence, the importance of biological treatment systems has attracted the attention of workers all over the world and has helped in developing relatively efficient, low cost waste treatment systems. Pseudomonads are one of the most important and well-studied bacteria because of its wide distribution in the environment and the relatively easy cultivation. Several studies indicate that the use of *Pseudomonas spp.* for the treatment of distillery effluent is significantly effective (Pal *et al.,* 2012 and Chaudhary *et al.,* 2013) but detailed study is required in subtropical countries. Therefore, the present study was carried out in Indian tropical agro ecosystems with the aim to evaluate the effect of varying amounts of distillery effluent on soil microbial population and use of *Pseudomonas sp.* for the treatment of distillery effluent.

EXPERIMENTAL

Experimental Design: Present study was performed at the wheat agricultural farm near Narang distillery, Nawabganj (26°52' N, 82°08' E and 98 m above the mean sea level) in northern India. Experiment was designed in randomized block design with four replicate plots of 5 × 5 m size for various doses of effluent amendment. Likewise an un-amended plot was also established as control. The field has been irrigated at a content of 25, 50, 75, 100 % distillery effluent. Wheat was sown in last week of November and harvested in last week of March 2012. Three irrigations and a pre-sowing (7 days before) irrigation each of 10 cm depth were applied to wheat crop on 20th, 40th and 90th day after sowing as the demand of wheat crop.

Soil and Effluent characterization: Soil and effluent samples were collected from the irrigation site. Soil sampling was performed fortnightly on 0, 20, 40, 60, 80, 100, 120, 140 and 160 days after sowing. On each sampling occasion three replicate from each treatments were taken. Composite soil samples were collected from A-horizon (0–20 cm soil depth) of plots from the experimental field at given time intervals. The electrical conductivity (EC), pH, Total solids (TS), TDS TSS, chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) of water were determined according to standard methods (APHA, 2005). In soil samples oxidisable organic carbon (C_{org}) and total Nitrogen (TN) were determined by the Walkley–Black method and soluble polyphenols by Beltran (1999) method. After HNO_3/$HClO_4$ digestion, the Phosphorus content was measured colorimetrically, Na and K by flame photometer and Ca, Mg, micronutrients and heavy metals by atomic absorption spectrophotometer. Some characteristics of the effluent of different dilutions and soil are listed in Table. 1 and 2 respectively.

Microbial population study: Microbial populations in both amended and unamended fresh soil samples of 1 g each (in triplicate) were enumerated following standard serial dilution plating technique on selective media using one-fourth strength Ringers solution and expressed as CFU /g dry soil. Culturally viable bacteria, fungi and actinomycetes were counted on nutrient glucose agar, Martin's Rose Bengal Agar (amended with 30 mg/L streptomycin sulphate), and Kenknight's Agar (amended with 0.05 g/L cyclohexamide), respectively. Plates were inoculated with 0.1 ml soil suspension cultured for 4, 7 and 10 days for heterotrophic bacteria, fungi and actinomycetes, respectively, at 28°C. Nitrogen-fixing bacteria enumeration in soil was based on the most probable number technique, using a semi-solid nitrogen-free combined carbon medium.

Bioremediation experiment: Screening of microorganisms having the ability to degrade melanoidin pigments and thereby decolorization in spent wash was done by enrichment. The tubes showing decolorization were subsequently subcultured and isolation of microbial culture was carried out on minimal salt glucose medium by spread plate technique. Pure cultures of different bacterial isolates were maintained on minimal salt glucose agar medium containing 15% spent wash. Further, pure culture of each isolate was transferred in minimal medium with 20% spent wash in 250 mL flask and incubated at room temperature. Dilution of spent wash was necessary to reduce the level of toxic ingredients in the spent wash, which otherwise inhibit the growth of the microorganisms. After 8 h interval, 5 mL aliquot was withdrawn for assaying decolorization. The decolorization was measured as decrease in optical density of supernatant of treated medium at 475 nm on UV-visible spectrophotometer (Toshniwal, TSUV 75). Uninoculated minimal salt medium and minimal medium with 10% spent wash were used as blank and control, respectively. Isolate showing better results as compared to others was selected for further investigation.

Table.1. Physico-chemical characteristics and metal contents in different dilutions of Distillery effluent

Parameters	No dilution	75% effluent	50% effluent	25% effluent
pH	8.5 ± 0.17	8.23 ±0.56	8.15±0.76	7.56±1.23
EC (mS/cm)	25960±1450	18660±1370	9020±933	6866±875
Total solids (mg/L)	37600±651	23193±452	17070±654	8532±562
TDS (mg/L)	25860 ±432	16413 ±453	12640± 233	6324±456
TSS (mg/L)	11740 ±239	6780 ±354	4430± 126	1633±345
COD (mg/L)	43522 ±985	17266± 934	14578± 876	6453±345
BOD (mg/L)	17633 ±343	8866 ±435	4800± 346	2154±254
Bicarbonate (mg/L)	12300 ±875	7300± 765	3200± 567	1433±123
TP (mg/L)	44.56 ±2.4	15.12± 2.23	8.65± 1.34	3.23±0.67
TK (mg/L)	8200±345	3500±345	2500±234	978±77.76
Calcium (mg/L)	1278 ±123	563.0± 23	390.0± 34	172±25.87
Magnesium (mg/L)	3452.3 ±345	1022.4± 564	710.22± 45.6	312±22.45
Sulphur (mg/L)	84.7±12.3	45.0±8.56	28.6±5.67	13.45±1.45
Sodium (mg/L)	476±55.67	298±55.45	232±76.5	112±12.34
Chlorides (mg/L)	5854± 456.5	4272± 234	3204± 122.5	1577±144.33
Iron (mg/L)	11.2 ±0.23	7.40 ±1.76	4.20± 1.04	2.12±0.34
Manganese (mg/L)	1234± 231	674 ±34.39	386± 55.65	181±11.45
Zinc (mg/L)	1.18±0.56	0.69±0.06	0.53±0.063	0.23±0.06
Copper (mg/L)	0.32± 0.05	0.17± 0.022	0.07± 0.045	0.032±0.004
Cadmium (mg/L)	0.04±0.004	0.03±0.004	0.010±0.005	0.0046±0.001
Lead (mg/L)	0.18 ±0.005	0.09 ±0.003	0.06± 0.004	0.034±0.002
Chromium (mg l^{-1})	0.067 ±0.003	0.037 ±0.002	0.015± 0.002	0.007±0.0002
Nickel (mg/L)	0.175 ±0.04	0.075±0.002	0.05± 0.0067	0.025±0.002

RESULTS AND DISCUSSION

Effect of distillery effluent on soil microbial population: Quantitative analysis of soil microbial populations showed variations in the groups of microflora populations in soils following amendment with distillery effluent (Figure.1). Aerobic heterotrophic, nitrifying and denitrifying bacteria were more sensitive to high dose of distillery effluent contamination than the other microbial groups under evaluation. A difference in the viable counts of fungal and actinomycetes were also significant; however, these two microbial groups seemed to be less sensitive to the effluent amendment. Results revealed that with increasing soil organic carbon and nutrients the counts of bacteria, fungi, and actinomycetes increased initially. The results shows that the effluent from distillery wastewater treatment plant may have potential as a beneficial soil amendment up to certain extent

for improving biological properties of the soil but at higher doses its contamination can create harm for the beneficial soil inhabitant microbial population. A few workers have also reported the similar results (Tripathi *et al.,* 2011).

Table.2. Physico-chemical characteristics and metal contents in experimental soil

Parameter	Soil
pH	6.8±0.27
Moisture (%)	70± 1.67
Organic Carbon (%)	1.09± 0.03
Phosphate (mg /kg)	265± 2.88
Phenol (mg /kg)	3.09± 0.21
EC (mS/cm)	1.55± 0.024
Total Nitrogen (mg /kg)	56.76± 2.34
Sodium (mg /kg)	13.67± 0.82
Chloride (mg /kg)	96.72± 1.51
Magnesium (mg /kg)	9.48± 0.32
Calcium (mg /kg)	11.55± 0.21
Aluminum (mg /kg)	3.94± 0.27
Potassium (mg /kg)	44.39± 0.017
Cadmium (mg /kg)	0.06± 0.0021
Chromium (mg /kg)	0.89± 0.014
Copper (mg /kg)	2.25± 0.043
Iron (mg /kg)	3.73± 0.069
Manganese (mg /kg)	1.86± 0.047
Nickel (mg /kg)	1.11± 0.021
Zinc (mg /kg)	12.18± 0.15
Lead (mg /kg)	4.31± 0.062

Bioremediation of Distillery Effluent

Decolorization was followed spectrophotometrically by measuring the colour at 475 nm, which is A_{max} of melanoidin. Effects of various parameters on decolorization process were also studied. Decolorization was found to be maximum in the presence of glucose when compared with other sugars. 0.4% of glucose concentration as carbon source was optimized for decolorising activity and above 0.5 % glucose there was decrease in decolorization (Figure 2). This effect might be due to the acidic conditions produced in the medium after incubation; inhibiting to the microbial growth. Several researchers have investigated the role of microbial community in the degradation of melanoidins in the spent wash. *Bacillus cereus, B. megaterium* and *Xanthomonas fragarie* and in free and immobilised form are reported to degrade the colour causing material in the spent wash (Nakajima-Kambe *et al.,* 1999; Krzywonos, 2014). Few bacterial strains viz., *Pseudomonas, Enterobacter, Aeromonas, Acinetobacter* are reported to be capable of degrading some of the recalcitrant compounds in the anaerobically digested distillery spent wash (Dahiya *et al.,* 2001; Ghosh *et al.,* 2004; Mohana *et al.,* 2008). Treatment of anaerobically digested distillery spent wash in a two-stage bioreactor using *Pseudomonas putida* and *Aeromonas* spp. has achieved colour and COD reduction by 60 and 44.4%, respectively.

Maximum decolorization and COD reduction was found within the pH range of 6.8-7.2, the preferred range for the growth of the isolate. Temperature range of 30-35°C was found to be suitable for activity of the isolate. The medium composition was optimized as (in g/L), glucose, 4; KH_2PO_4, 0.2; $MgSO_4$, 0.009; pH range in between 6.8-7.2, temperature within the range of 30- 35°C was optimal. Under the optimum conditions the Glucose concentration, pH, temperature and spentwassh concentration; isolate *Pseudomonas* sp. was able to decolorize the spent wash by 61% and to reduce COD by 66% after 72 h of incubation (Table 3). Many workers have also reported the similar results (Pant *et al*., 2007; Sirianuntapiboon *et al*., 2004).

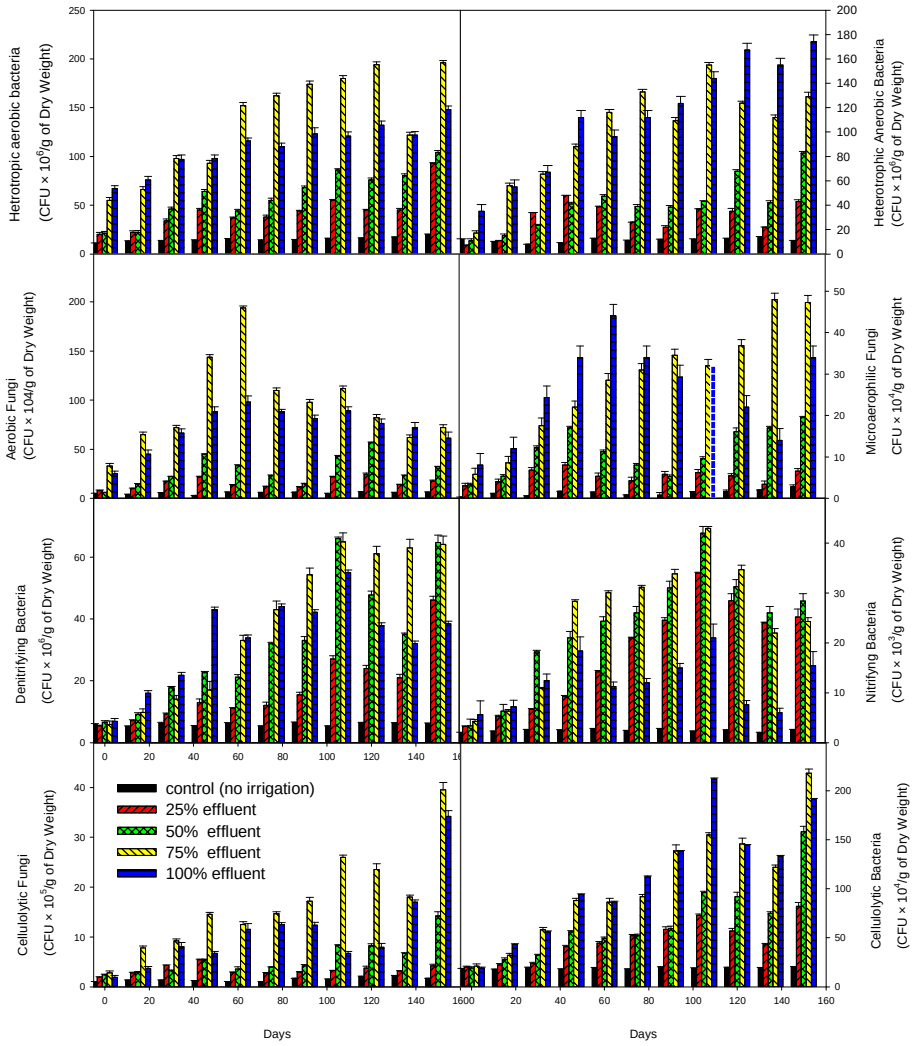

Figure 1: Effect of different dilutions of distillery effluent on soil microorganisms

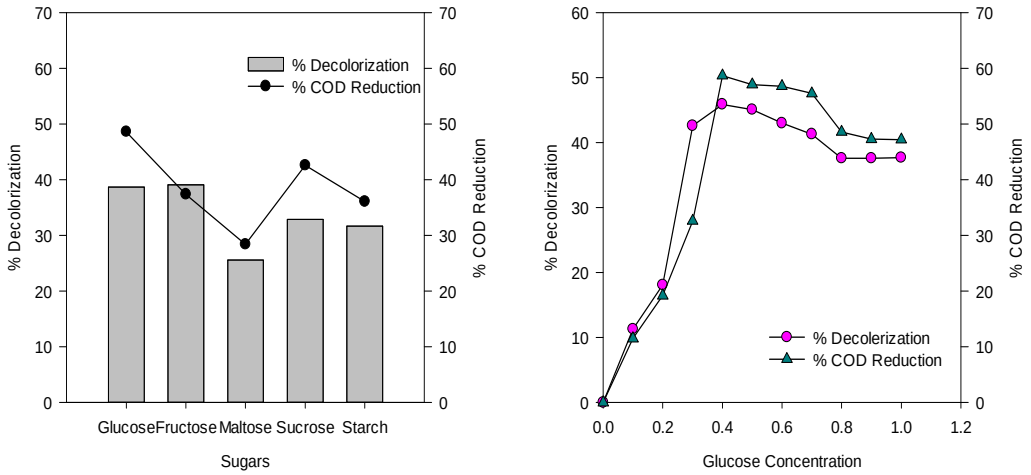

Figure 2: Effect of different carbon sources and Glucose concentration on decolorization and COD removal

Table 3: Bioremediation of Distillery Effluent

Parameters	Before treatment	After treatment	% Reduction
BOD (mg/L)	2154± 145	603±22.5	72
COD (mg/L)	6453± 254	2195±125.5	66
TDS (mg/L)	6324± 456	2353±133.6	63
TN (mg/L)	3.23±0.2	10.87±0.15	42
TP (mg/L)	978±55.7	525±64.6	46
Colour (HU)	8200	3200	61

CONCLUSION

Results from present study revealed that application of distillery effluent without dilution increased organic matter, electrical conductivity, heavy metals and toxic organic contaminants up to toxic level in soil. Statistical analysis shows that soil microbial population increases initially and further decreased. Effluent from distilleries may have potential as a beneficial soil amendment up to certain extent for improving biological properties of the soil but at higher doses its contamination can create harm for the beneficial soil inhabitant microbial population and their activities. Results indicate that soil consortium having *Pseudomonas* population is able to used for bioremediation of distillery effluent. Under the optimum conditions, glucose concentration, pH, temperature and spent wash concentration; isolate *Pseudomonas* sp. was able to decolorize the spent wash by 61% and to reduce COD by 66% after 72 h of incubation.

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