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Impact of '*Holi*' on the environment: A scientific study

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ABSTRACT

The festival of 'Holi' is proving to be an environmental risk due to the toxic colors used during the celebrations. Unlimited and uncontrolled use of such dyes can lead to grave consequences in terms of human health and ecological balance. These colors are highly structured polymers and are very difficult to decompose biologically. This study was taken up with an initiative to determine the extent of effects that the 'Holi' colors have on water and soil, respectively. The bacteria inhabiting these contaminated water and soil samples were isolated for biochemical analyses. Their ability to degrade these dyes was determined after optimization studies. Toxic trials were conducted on certain bacterial cultures and Triticum vulgare to check their impact on the other living flora and fauna in their surrounding environment. After carefully monitoring the dye degradation capacity of these bacteria, they were used for bioremediation purposes, giving promising results. The impact assessment and damage control led to safer methods of getting rid of the 'Holi' dyes, in order to save our environment.

Keywords: '*Holi*', physico-chemical parameters, dye degradation, toxicity trials, bioremediation.

INTRODUCTION

In India with the advent of spring season just after monsoons, it is the season of festivals and festivities for different communities, where a spectrum of culturally very different rituals and modes of festival celebrations are witnessed. With the festival season comes a disaster. Pollution of various types is generated in large amounts all across the country, thereby adding an even greater load of pollutants and contaminants to our already over polluted environment, overburdened rivers, lakes, and seas. Coupled with inadequate waste collection and waste disposal knowledge, the devastating impact is far reaching and is felt for a longer duration of time. This results in wide scale pollution and degradation of natural environment, thus threatening the sustainability of many species.

'Holi' is a religious spring festival celebrated by Hindus. *'Holi'* is also known as festival of colors. It is primarily observed in India and Nepal. The most celebrated *'Holi'* is in the Braj region, in locations connected to the Lord Krishna: Mathura, Vrindavan, Nandagaon, and Barsana. Bonfires are lit on the eve of the festival, also known as '*Holika Dahan*' (burning of Holika) or '*Chhoti Holi*' (little '*Holi*') after which prayers are offered. '*Holi*' is celebrated at the end of the winter season on the last full moon day of the lunar month Phalguna (February/March). In most areas, '*Holi*' lasts about two days. Every year, thousands of Hindus participate in the festival '*Holi*'.

Traditional '*Holi'* made use of natural colored powders having medicinal significance: the colors were traditionally made of *Neem, Kumkum, Haldi, Bilva*, etc. As the spring-blossoming trees that once supplied the colors used to celebrate '*Holi'* have become rarer, many safety issues have been found with all three forms of synthetic colors produced: pastes, dry colors and water colors. These are prepared from harmful substances like acids, mica, glass powder and alkalis, and are quite capable of causing serious skin complications and allergies. Often referred to as unholy colors of '*Holi*', artificial colors can lead to skin allergies, irritation, redness, rashes, itching and bumps [6]. A recent study conducted under the National Biodiversity Strategy and Action Plan revealed that chemical colors have all but wiped out India's wonderful vegetable dyes [8]. Colors give delightful pleasure to eyesight but at the same time they may act as serious pollutants when their origin is dyes and dyestuffs [9]. The colorant used in the dry colors, also called *gulals*, was found to be toxic, with heavy metals causing asthma, skin diseases and temporary blindness (*Toxics link* and *Vatavaran*, 2001). They reported that the wet colors might lead to skin discoloration and dermatitis and are also carcinogenic in nature. Lack of control over the quality and content of these colors is a problem, as they are frequently sold by vendors who do not know their origin. The large variety of dyes and chemicals used in an attempt to make more attractive and popular dyes for a competitive market render them very complex [13]. In India, these colors are prepared on a small scale and lack any quality checks. Use of such toxic colors should be discouraged, and all doctors should caution people against using synthetic dyes. There is an urgent need to put manufacturing of '*Holi'* colors under guidelines of the Food and Drug Cosmetic Act and the Bureau of Indian Standards [5]. The traditional '*Holika Dahan'* bonfire is believed to contribute to deforestation and air pollution. There is also concern about the large scale wastage of water and water-pollution due to synthetic colors during '*Holi'* celebration. Their presence in water, even at very low concentrations, is highly visible and undesirable. When these colored effluents enter rivers or any surface water system they upset biological activity. Ground–water systems are also affected by these pollutants because of leaching from the soil [1].

The discharge of the toxic colors in the soil and water has a deleterious effect on the water resources, soil fertility, microorganisms living in these habitats and the ecosystem integrity on the whole. These colors are not readily degradable under natural conditions and are typically not removed from waste water by conventional waste water treatments. Thus, several bacteria have been found to decolorize, transform and completely mineralize colored soil and water in both aerobic and anaerobic conditions [10]. During the last decade, environmental issues associated with the dyestuff production and applications have grown significantly. Although widely recognized and discussed as an annually recurring problem involving many people, lack of any formal study on it has prompted us to undertake the present work. In this paper, we have studied the side effects of '*Holi'* on the environment and have utilized dye-degrading microorganisms to help in the removal of toxic colors from polluted soil and water samples. The metabolites produced after biodegradation are mostly non-toxic or comparatively less toxic in nature. We have also suggested healthier practices for a safe and environmental friendly '*Holi'*.

MATERIALS AND METHODS

Chemicals required: All the chemicals used during the research work were of analytical grade supplied by HI-media Laboratories Limited (India) and Sigma-Aldrich.

Sample Collection: Soil samples were collected a day after '*Holi'* festival, using sterilized polybags from three sites (site 1, site 2 and site 3) in Jaipur, India and kept in cold condition till they were brought in the laboratory. Water samples were also collected in sterilized bottles from the same three sites (site 1, site 2 and site 3). All the samples were collected during the day between 9:00 hrs and 13:00 hrs aseptically in sterile containers and placed in a cooler at room temperature and transported to the laboratory for analysis within 2 hours of collection. When immediate analyses were not possible, the samples were preserved at 4°C.

Isolation and Screening: 1.0g of each soil sample and 1.0ml of each water sample were suspended in sterile water blanks aseptically and various dilutions were made accordingly. About 1.0 ml of higher dilutions (10^{-5} to 10^{-7}) was spread on nutrient agar plates also. A loop full of sample from prepared nutrient broth was streaked on yeast extract medium agar plate for further screening. The colonies were observed for colonies showing clear zones which were then selected and serially diluted in 9 ml of sterilized distilled water, filtered and the filter paper was incubated on nutrient agar at 37^0C for 24 hrs. The single colonies on filter paper were picked up and cultured on yeast extract agar slants and stored at 4°C [4]. Purification and initial characterization of isolates was done by repeated streak plate and staining methods, respectively. The different isolates were inoculated in yeast extract medium and incubated at 37°C.

Discoloration Studies: The discoloration due to the inoculated isolates was monitored at every 24 hrs intervals. 250 ml Erlenmeyer flasks with 100 ml of yeast extract medium containing 24 hrs old culture of bacteria were taken for further analysis. All the studies were carried out at 30°C and at pH 7. Samples (2 ml) were withdrawn after various time intervals to monitor discoloration rate. Aliquots thus withdrawn were centrifuged at 8,000 rpm for 12 min, and the residual dye concentration in the supernatant was measured at 540 and 420 nm, respectively. Discoloration rate was expressed as percentage discoloration and calculated using the formula;

$$\% \text{ Discoloration} = A - B/A \times 100$$

Where A is initial absorbance and B is observed absorbance [12].

Optimization of culture conditions: Discoloration under different culture conditions was done by changing, one at a time, the factors with the basic conditions of temperature 37°C and pH 7.0. The effect of incubation temperature on the discoloration was studied by incubating the culture media under a range of temperatures (15°C, 25°C, 35°C, 45°C, 55°C and 65°C) at pH 7.0. For the study of effect pH on discoloration, colonies of an overnight grown culture was used to inoculate the culture media and the pH of the medium was adjusted to 3.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 11.0 at temperature 37°C. Above mentioned protocol was followed during the study of effect of different carbon sources like glucose, lactose, sucrose, maltose, and fructose, and increasing dye concentrations (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg/l) on discoloration. All assays were performed in triplicate with the uninoculated culture as the control.

Physico-chemical parameters: Various physico-chemical parameters (temperature, turbidity, BOD, COD, DO, TDS, total hardness, total alkalinity, chloride, nitrate contents and pH) of water samples were studied for the sample solutions [2, 14].

Microbial characteristics: Microbial studies were carried out by standard MPN methods [2, 11].
Toxic Trials:

- *Toxicity study of sample on bacteria.*

Mixed bacterial cultures were inoculated with glass spreader and 100µl of sample solution was added in the well of agar plate and the plate was incubated for 24 hrs and zones of inhibition were observed. Nature of dye was tested against *Escherichia coli*, *Proteus vulgaris*, *S. aureus*, *Bacillus subtilis*and *Klebsiellapneumoniae*.

- *Phytotoxicity.*

The phytotoxicity study was carried out at room temperature on *Triticum aestivum* by irrigating separately with 7ml of different concentration of sample and degraded product. Control set was carried out using plane water at the same time. Height of shoot and length of leaf and the % of germination was recorded after 15 days. The degraded product was extracted after 48 hrs of irrigation.

RESULTS AND DISCUSSION

Isolation and Screening: The soil and water samples were abundantly inhabited with many bacteria.All bacterial isolates used in this study were characterized by routine bacteriological procedures, including morphology and gram reaction, cultural characteristics, biochemical tests, and in some cases serological tests to determine their identities.

Discoloration Studies: The gram-negative organisms exhibited greater resistance to dyes than gram-positive organisms. Basic dyes are inhibitorier than acidic and neutral dyes at the same concentration. Several dyes not commonly used in diagnostic microbiology showed promising differentiation ability between gram-negative and gram-positive organisms. Effects of several synthetic dyes on a few selected groups of organisms were further tested in this study. The cultures showed dye degradation by varying concentration from 100mg-1000mg/l but at higher concentration, rate decrease with increase in toxicity. Three types of colonies showed discoloration on yeast extract media containing 200mg/l of dye resulting in different percentages of degradation after 48hrs. Higher degree of biodegradation and mineralization can be expected when co-metabolic activity within a microbial community complements each other. One organism may be able to cause a biotransformation of the dye which consequently renders it more accessible to another organism that otherwise is unable to attack the dye.

Optimization of culture conditions:

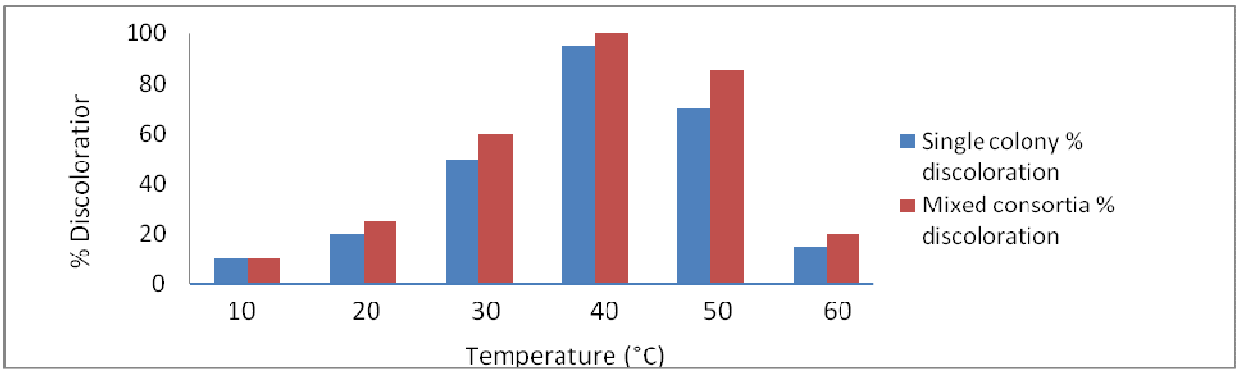
Effect of temperature

The dye decolorizing ability of the culture increased along with incubation temperature showing maximum activity at 40^0C (Table 1). Any further increase in incubation temperature up to 50^0C resulted in many fold reduction in discoloration activity of the culture. The dye discoloration activity of mixed consortia was found to increase with increase in incubation temperature showing maximum activity at 40°C (Figure 1).

Table 1- Effect of temperature on % discoloration.

Temperature (˚C)	Single colony % discoloration	Mixed consortia % discoloration
10	10	10
20	20	25
30	50	60
40	95	100
50	70	85
60	15	20

Figure1 - Graph showing effect of temperature on % discoloration.



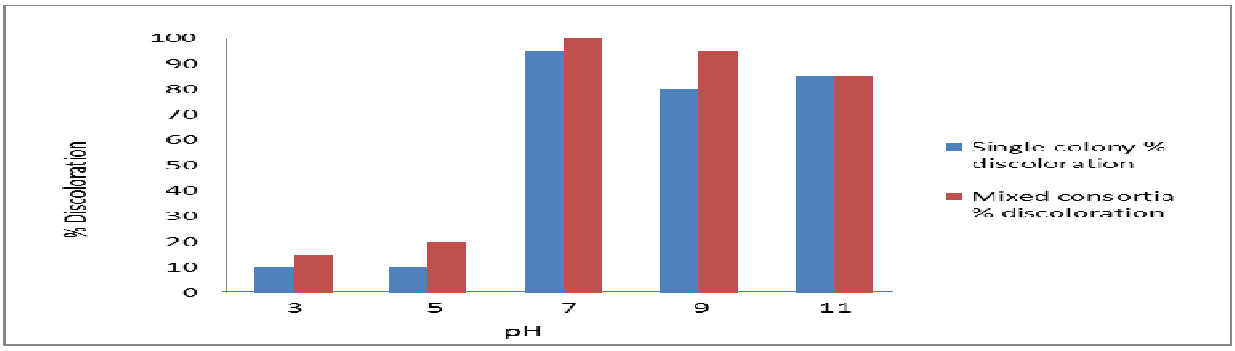
Effect of pH

It was found that change in pH significantly affected the discoloration rate. The culture showed growth at pH 7.0, 9.0 and 11.0, while at pH 3.0 and 5.0 the growth was found to be inhibited (Table 2). Bacterial cultures generally exhibit maximum discoloration at pH values near 7.0. Mixed consortia exhibited optimum discoloration activity in the narrow pH range from 7.0–8.0 with marked reduction in discoloration activity at pH 6.0 (Figure 2).

Table 2- Effect of pH on % discoloration.

pH	Single colony % discoloration	Mixed consortia % discoloration
3	10	15
5	10	20
7	95	100
9	80	95
11	85	85

Figure 2- Graph showing effect of pH on % discoloration.



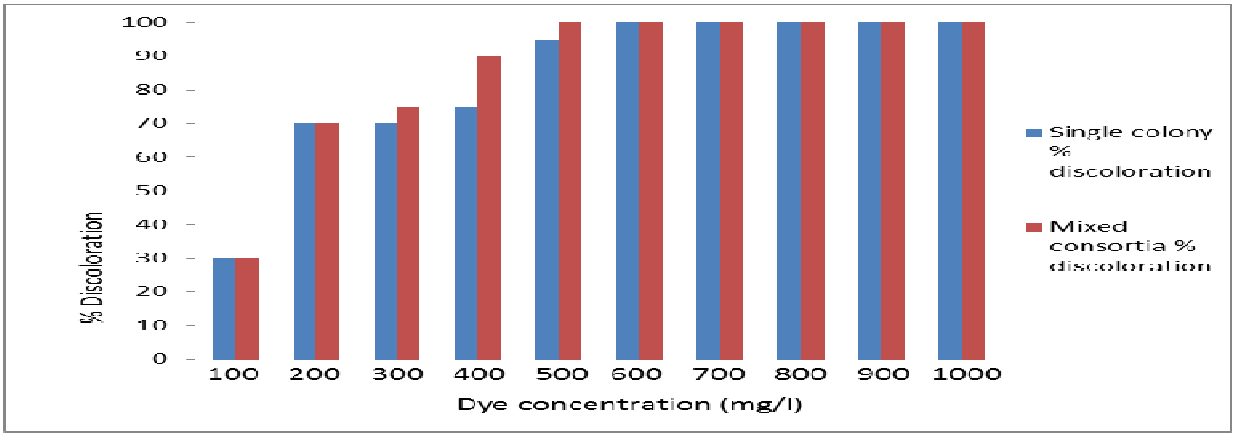
Effect of dye concentration

The isolated cultures showed discoloration activity at concentration 100-1000mg/l (Table 3). The discoloration activity of the culture was strongly inhibited at higher concentration of dye and it was concluded that dye concentration can influence the efficiency of microbial discoloration through combination of factors including the toxicity imposed by dye at higher concentration (Figure 3). Mixed consortia could discolor dyes much above the reported dye concentration.

Table 3- Effect of dye concentration on % discoloration.

Dye concentration (mg/l)	Single colony % discoloration	Mixed consortia % discoloration
100	30	30
200	70	70
300	70	75
400	75	90
500	95	100
600	100	100
700	100	100
800	100	100
900	100	100
1000	100	100

Figure 3- Graph showing effect of dye concentration on % discoloration.



Effect of carbon source medium

Different carbon sources were supplied with yeast extract like glucose, fructose, sucrose, lactose, maltose at 0.3% concentration (Table 4). The culture exhibited maximum discoloration activity in the presence of glucose and

lactose as compared to sucrose, fructose and maltose. The activity due to glucose, served as a source of a reducing equivalent and at lower glucose concentration the reducing equivalent generated discoloration (Figure 4). Alternately, glucose may enhance discoloration by allowing faster growth of actively respiring bacteria, resulting in rapid discoloration.

Table 4- Effect of carbon sources on % discoloration.

Carbon source (0.3%)	Single colony % discoloration	Mixed consortia % discoloration
Glucose	100	100
Fructose	30	70
Lactose	95	100
Sucrose	40	50
Maltose	20	35

Figure 4- Graph showing effect of carbon sources on % discoloration.

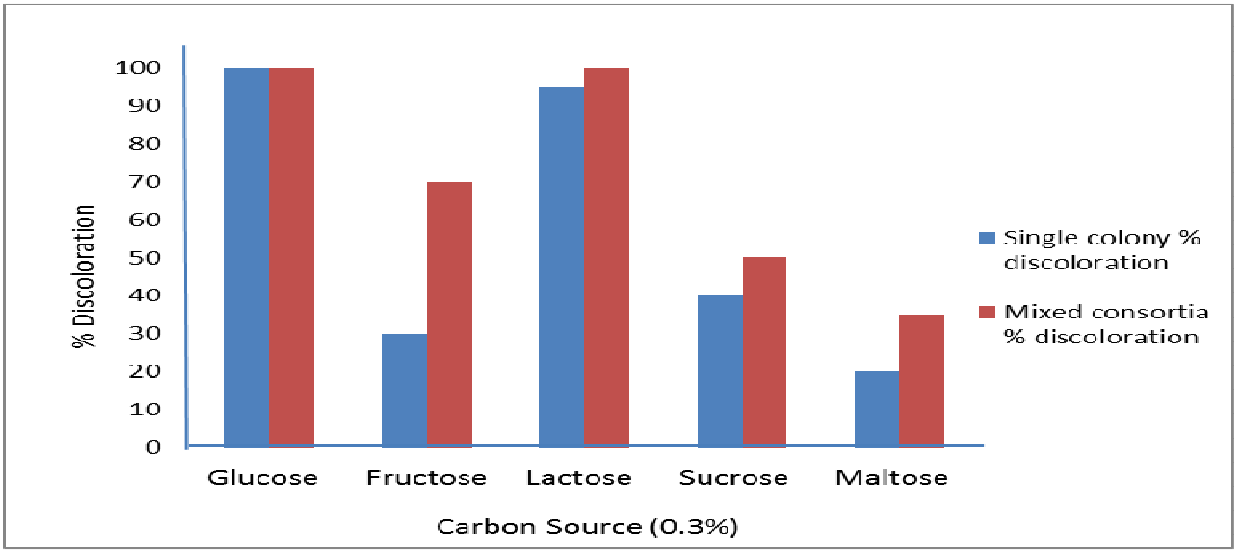


Table 5- Estimation of physico-chemical parameters

S. No.	Parameter	Method	Site 1	Site 2	Site 3	Average
1	Temperature	Digital Thermometer	18.3˚C	21.5˚C	25.3˚C	21.7˚C
2	pH	Digital pH meter	7.1	8.3	8.9	8.1
3	Turbidity	Nephlometry	170 NTU	130 NTU	154 NTU	151.3 NTU
4	BOD	Titration	2.20 mg/l	1.8 mg/l	2.0 mg/l	2 mg/l
5	COD	Titration	309 mg/l	228 mg/l	250 mg/l	262.3 mg/l
6	DO	Iodometry	13.4 mg/l	11.0 mg/l	9.8 mg/l	11.4 mg/l
7	TDS	Filtration	360 mg/l	371 mg/l	342 mg/l	357.6 mg/l
8	Total Hardness	Titration	1.17 mg/l	2.3 mg/l	2.19 mg/l	1.88 mg/l
9	Total Alkalinity	Titration	35 mg/l	39 mg/l	28 mg/l	34 mg/l
10	Chlorides	Silver nitrate method	7.1 mg/l	5.2 mg/l	1.9 mg/l	4.73 mg/l
11	Nitrates	Brucine method	2.8 mg/l	1.2 mg/l	0.7 mg/l	1.56 mg/l

Physico-chemical parameters: The various physico-chemical parameters were studied yielding the following results (Table 5).

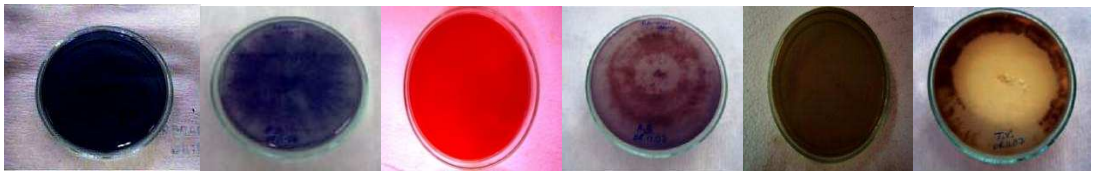
Microbial characteristics:
It was difficult to understand the biological phenomena fully because the chemistry of water reveals much about the metabolism of the ecosystem that explains the general hydro-biological relationship between the two. High levels of coliform bacteria were present in water samples taken from all the sources. Total coliform counts in most cases were >250 CFU/100ml, this was far above the accepted WHO standard (0 CFU/100ml). In general, the characteristics of the organisms corresponded to the descriptions found in Bergey's Manual.

Toxic Trials:

- *Toxicity study of sample on bacteria.*

Bacterial cultures of *Escherichia coli, Proteus vulgaris, S. aureus, Bacillus subtilis*and *Klebsiellapneumoniae* were tested against dyes in volume 100µl supplemented in solid as well as liquid culture media, zones of inhibition and discoloration of the dye ascertaining significant activity. Results were compared with a control without inoculation (Figure 5 & 6).

Figure 5- Bacteria from Site1, Site 2 and Site 3 showing dye degradation in solid culture media.



Control Test Control Test Control Test

Figure 6- Bacteria from Site1, Site 2 and Site 3 showing dye degradation in liquid culture media.

Control Test Control Test Control Test Control Test

- *Phytotoxicity.*

The phytotoxicity study was carried out at room temperature. *Triticum aestivum* revealed considerable decrease in growth in the presence of higher concentration of dye (Table 6). Similarly results were observed in % germination of *Triticum aestivum* where it was found to be less. Results were compared with *Triticum aestivum* grown without dye as a control (Figure 7).

Figure 7- *Triticum aestivum* grown in soil containing dye of varying concentrations.

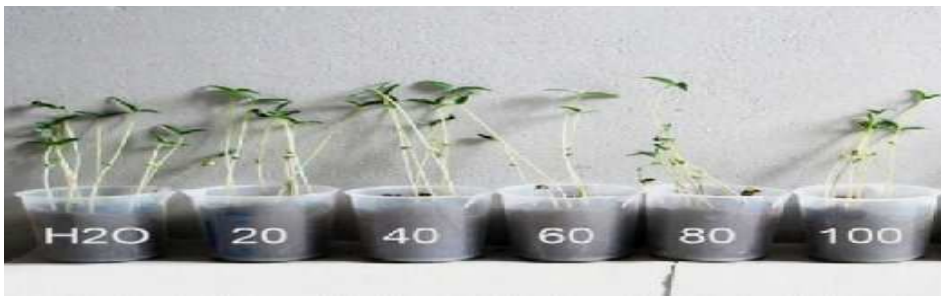


Table 6- Effect of dye on % Seed germination, Root and Shoot length.

Triticum aestivum	Control	20 µl	40 µl	60 µl	80 µl	100 µl
% Seed germination	10 %	9 %	7 %	6 %	6 %	5 %
Root length	20 mm	18 mm	17 mm	14 mm	8.7 mm	4.5 mm
Shoot length	15 mm	14.5 mm	14 mm	13.5 mm	12 mm	12 mm

CONCLUSION

'*Holi*' has become more of an environmental hazard now-a-days. This study showed the microbiological impact of toxic colors used during '*Holi*'. Soil and water samples containing dye-degrading bacteria were isolated and screened for bioremediation purposes. The physic-chemical properties were studied extensively where the average temperature and pH of the water samples taken from all 3 sites were 21.7°C and 8.1 respectively. The average turbidity was recorded to be 151.3 NTU. Titration methods to determine BOD, COD and DO resulted in 2 mg/l, 262.3 mg/l and 11.4 mg/l respectively. This showed that oxygen content in all the 3 sites was utilized by the bacteria in the water for their growth and proliferation. The contaminated water samples from 3 sites recorded an average amount of 357.6 mg/l TDS, 1.88 mg/l total hardness and 34 mg/l alkalinity. Chlorides and nitrates were recorded at 4.73 mg/l and 1.56 mg/l respectively. This was due to the '*Holi*' color in the water samples that completely changed the physic-chemical parameters of the water collected for this research purpose. Because of the widespread use and potential carcinogenicity of certain dyes, there has been a growing interest in assessing the hazards associated with dyes available in the local market. Such dyes are openly sold without any information regarding their chemical nature, purity, toxicity or possible mutagenicity. The aim of this research work was to biologically degrade the toxic '*Holi*' colors and lessen their ill effects. Such bacteria have well adapted themselves in this polluted environment. Toxic trials of these dyes on bacteria and *Triticum vulgare* showed decreased growth and reproduction. Utilization of these novel bacteria in biodegradation of '*Holi*' colors will eventually help in the long run. Taking into consideration the findings of this research, we must spearhead bioremediation practices in order to detoxify the after effects of '*Holi*' in our country. Such promising results have not been documented till date using soil and water samples contaminated with '*Holi*' colors. In recent years, several non-governmental organizations have started campaigning for safe practices related to the use of colors. Some are producing and marketing ranges of safer colors derived from natural sources such as vegetables and flowers. Alternatives like vegetable dyes prepared from various plant extracts have been developed in the Indian Toxicology Research Centre (ITRC) and National Botanical Research Institute (NBRI). They lack the mechano-abrasive and phototoxic properties of synthetic dyes. Use of such non-toxic colors should be encouraged, and doctors should caution people against using synthetic dyes such as '*Holi'* colors. The NGO, Society for Child Welfare, is being assisted by the Delhi government's environment department in its work. These products, along with those manufactured by some other NGOs are tested by Centre for Scientific and Industrial Research and other research laboratories [7]. We believe that large-scale efforts to increase public awareness regarding the health hazards of harmful colors, widespread availability of safer alternatives at affordable prices, and governmental regulatory control on the production and selling of hazardous chemicals will go a long way in a safer and environment-conscious celebration of this vibrant festival.

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