

# Adverse effect of triphenylmethane dyes on environmental health and its detoxification for improved ecosystem

Gurwinder Kaur and Surojit Bera\*

Department of Microbiology, School of Biosciences and Bioengineering,  
Lovely Professional University, Phagwara, Punjab-144411, India.

**Abstract :** Synthetic dyes still remain a popular choice in industrial sector for long time due to its stability and low cost. However natural colors are gaining attention this day which may be from plant, microbe or dead biomass. On the other hand, synthetic dyes are continuously imposing serious threat to the environment due to their toxic and hazardous nature. These dyes mainly create serious health problems in different animals including humans. Additionally, these dye molecules accumulate in marine flora and fauna which undergoes bioaccumulation in fishes and ultimately reaches human plate. As a result, mankind is getting bi directional attack from synthetic dyes in critical terms. Triphenylmethane dyes like malachite green, brilliant green etc. are popular choice in textile industry which generates a huge effluent which contaminates the water bodies. Now a day, different bioremediation approaches being made along with chemical methods to treat these effluents for better environment. These treatments also helping the irrigation system to sustain and grow in contamination free environment. As a result, agricultural lands are becoming more fertile and usable. However, more cost effective and new approaches are required to detoxify these dye effluents to overcome this environmental threat. This current review shall be helpful to understand the problems associated with triphenylmethane dye usage and their possible detoxification procedures in detail.

**Key words – Triphenylmethane dye, industrial effluent, bioremediation, environmental pollution, flora and fauna.**

## I. INTRODUCTION

A large volume of water is used by the textile industry of which 90% is discarded as wastewater. This wastewater contains different type of dyes. Dye is considered as one of the most awkward pollutants which not only imparts obnoxious color to the waste effluent but is also recalcitrant in nature (Mondal, Baksi & Bose, 2017). Dye waste is non- biodegradable and can be seen with naked eyes (Mojsov et al., 2016). The disposal of dye containing waste effluent causes serious environmental pollution that adversely affects all types of living organisms (Subhatra et al., 2013). Dyes prevent the penetration of sunlight which reduce the photosynthetic activity (Hassan & Carr, 2018). It imposes a toxic effect on dissolved oxygen levels thus, affecting the entire aquatic ecosystem (Muhd Julkapli, Bagheri & Hamid, 2014). Dye adversely impacts on biological oxygen demand (BOD) and chemical oxygen demand (COD). Textile dyes and their metabolites act as mutagenic, toxic, and carcinogenic agents (Aquino et al., 2014, Khatri et al., 2018). They appear to cross the food chains, leading to biomagnification (Sandhya, 2010), so that species have a higher level of contamination at a higher trophic level relative to their prey (Newman, 2015).

The dye containing effluent emanating from the textile industries is considered as a major concern in the global economy as well as in daily lives. Currently, it is becoming one of the major causes of deterioration in the globe in terms of quality and quantity (Mondal, Baksi and Bose, 2017). The dye, and their stuffs contain composite mixture of organic and inorganic components (Fulekar, Wadgaonkar and Singh, 2013). Prior to 1800, dyes were prepared from natural sources like vegetables, roots, insects, flowers, woods, etc. However, due to increasing need and demand for industrial dyes, industries are dependent on the synthetic dyes manufactured from petrochemical sources. Because of their synthetic nature, dye removal from the effluent is very tough, as they have a high degree of structural complexity (Morales-Alvarez et al., 2018).

Dye removal has recently become an area of growing interest in science, as government regulation on the release of hazardous waste is becoming more stringent. Different methods for the removal of dyes from industrial effluent are quite time consuming and expensive with low performance, like specific coagulation, filtration, use of chemical flocculation and activated carbon have been employed (Verma, Dash and Bhunia, 2012). However these physiochemical methods are expensive and produces large amount of sludge which result in a subordinate level of land pollution (Shah, 2013). For this reason, there is a demand for eco-friendly and inexpensive removal technique of the polluting dyes. Bioremediation, usually by bacteria is becoming an important sector for treating the industrial effluent. This method is more eco-friendly, inexpensive, and has been reported as one of the best alternatives to the physiochemical methods (Banat et al., 1996; Shah, 2013). Probably, microbes such as fungi, bacteria, algae, yeast, have gained growing interests due to their cost-effectiveness, eco-friendly nature and their production of less sludge (Kalyani et al., 2009). Bacterial removal of dye can be either anaerobic or aerobic (Pandey et al., 2007). Generally bacteria from different taxonomic groups can attain various degrees of dye removal and process absolute mineralization of dyes under optimum conditions (Asad et al., 2007). Gentian violet (GV) is one of the important industrial dyes belonging to the triphenylmethane group and known for its mitotic and mutagenic poisoning nature. GV has been used in topical applications in domestic animals and humans and can be taken orally for the treatment of pinworms. It has the potential to manage fungal growth under various conditions. GV is mixed to the domesticated birds feed to control mould, thus exposing the human inhabitants directly or indirectly to GV due to its considerable commercial and medical applications. In Chinese hamster CHO cells, the cytogenic toxicity of GV in vitro has been studied. It was expressed that this composite is a clastogen in vitro as well as mitotic poison. In other five different mammalian cell types its clastogenic properties were

confirmed. Thus, the in vitro studies determined that, GV may be considered as a bio hazardous substances (Azmi, Sani and Banerjee, 1998).

## II. CHEMICAL STRUCTURE AND PROPERTIES

CV is a common cationic dye that has been used extensively in various other commercial textile operations as a biological stain, dermatological agent, temporary hair colourant, dyeing cottons, wools (Shengfang 2010; Senthilkumaar et al., 2006). It belongs to a class of organic compounds that are highly coloured and are collectively called triphenylmethane dyes. CV, which is a basic colourant with the molecular formula  $C_{25}H_{30}N_3Cl$ , is also known as hexamethyl pararosaniline chloride (Sharma et al., 2011).

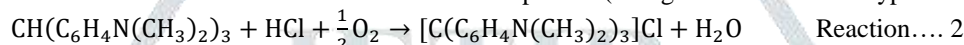
Tris (4-(dimethylamino) phenyl) methylum chloride, is the IUPAC name for crystal violet, which in colour, is blue-violet. The melting point and freezing point of the CV are respectively 205 and 40°C. It is highly soluble (13.78 per cent) in ethanol and less soluble (1.68 per cent) in water. With strong oxidising agents and strong acids, CV is found to be stable and incompatible. It is sensitive to light and is combustible. The structure and colour of crystal violet, however, depends largely on the medium's pH and temperature, which makes it an important acid-base indicator as well as an outstanding dye (Shah et al., 2013).

### 2.1 Uses and synthesis

The crystal violet dye can be prepared in laboratory conditions in a variety of possible ways. However, Caro and Kern (1883) developed the original method, which includes the chemical reaction of dimethylaniline with phosgene to intermediate 4, 41-bis (dimethylamino) benzophenone (Reinhardt and Travis 2000). In the presence of phosphorus oxychloride and hydrochloric acid, this intermediate form was then reacted with an additional dimethylaniline. Formaldehyde and dimethylene condensation are also synthesizable to produce a leuco-colorant which is a reduced Crystal violet, as shown in reaction 1 (Gessner and Mayer, 2002).



Second, the coloured cationic form is oxidised to this colourless compound: (Manganese dioxide is a typical oxidising agent).



The commercial preparations of triphenylmethane dye crystal violet (gentian violet or hexamethylpararosaniline) are relatively purified mixture of three rosaniline dyes: pentamethylpararosaniline, hexamethylpararosaniline and tetramethylpararosaniline. Varying mixtures are produced by different methods of synthesis. Through several processes, the manufacturing process of this dye was carried out by oxidising the mixture of p-toluene and aniline. Arsenic acid, an oxidising agent is most commonly used. Nitrobenzene has also been used. By the action of carbonyl chloride on N, N-dimethylaniline pure crystal violet has been made (Cook and Martin, 1951).

Triphenylmethane and closely related dyes are widely used as biological stains in food, silk, wood and cosmetic dyes (hair dyes). Brilliant blue (FCF) is a nontoxic dye of which surgical markers are made of; they typically contain 10% gentian violet as a solvent with 50% isopropanol. These dyes have been shown to decrease vascular activity, but they continue to be used to label conduits; The use of operative cutaneous markers to prevent implantation from twisting and kneading greatly affects the smooth muscle and endothelial function of (HSV) human saphenous vein (Eagle et al., 2011).

Several studies recommended administration of triple dye solution (prepared from a mixture of gentian violet, brilliant green, and proflavine hemisulfate) to newborn's umbilical cord in reducing colonisation by *Staphylococcus aureus* (Dandona et al., 2017). Gentian violet was used to prevent infection in patients with burns and to control intestinal parasites in humans before the discovery of antibiotics (Norman et al., 2017). Gentian violet has been used as a blood additive to prevent blood transfusion transmission of Chagas disease (Saye et al., 2020). Crystal violet has been used in poultry feed as a fungal growth controlling agent. Due to its ability to control diseases such as saprolegniasis, this compound was used as a fishery medicine in China's aquaculture industry (Song et al., 2020).

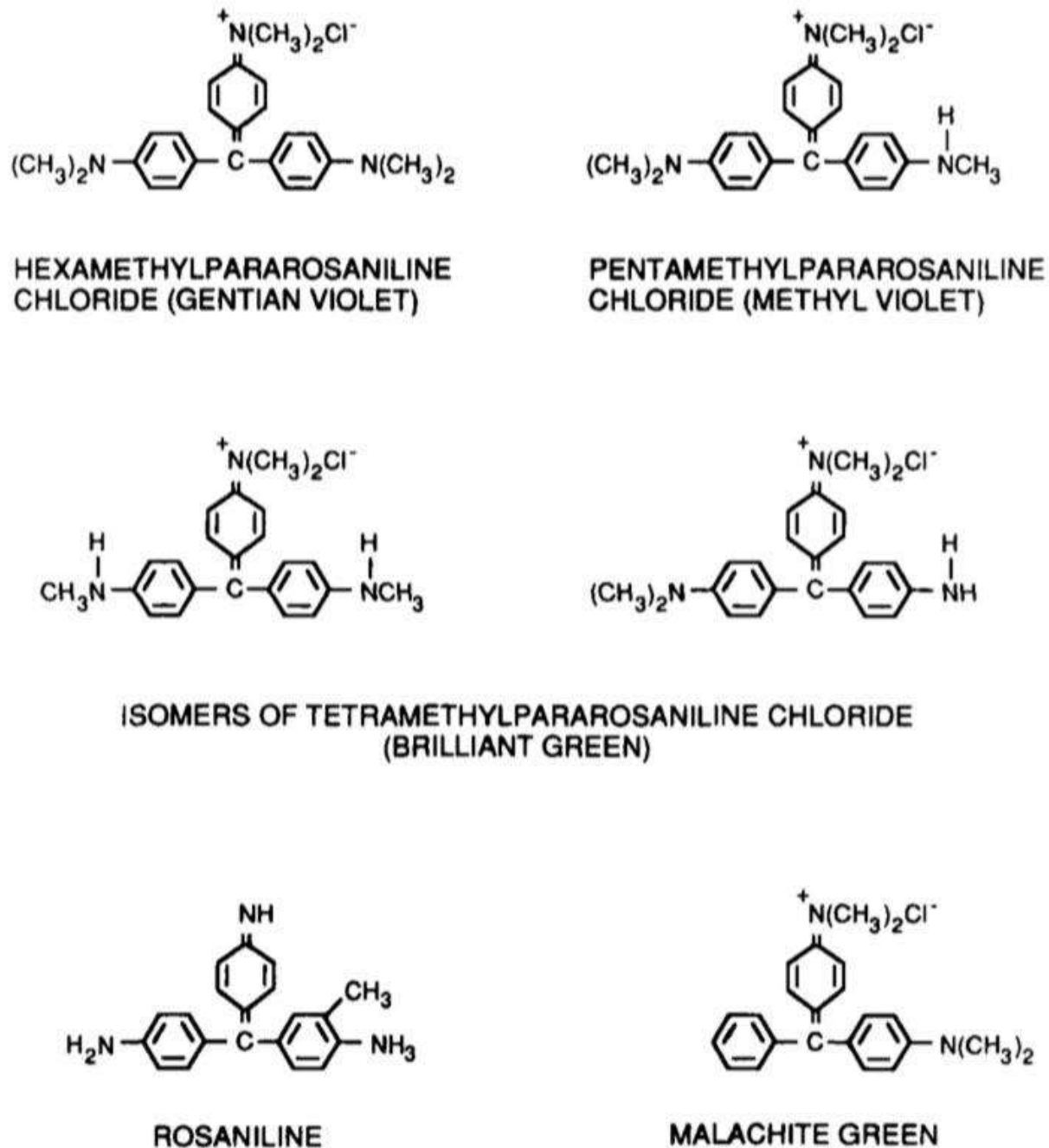


Fig.1 Gentian violet structural formulas and 5 associated compounds (Docampo and Moreno, 1990)

## 2.2 Impact on ecosystem

By triggering the Eutrophication phenomenon that interrupts the penetration of sunlight to the bottom of the marine environment, the presence of effluent in the aquatic environment, such as oceans, lakes, rivers, seas ... and insufficient biodegradability in typical ecological environments can demolish the essential conditions of individual environments. In aquatic environments, dyes high in concentration inhibit respiration activities, sunlight penetration, and also disturb photosynthetic and biological processes (Hassan & Carr, 2018). Dye effluent has a major impact on the population of plants, as plants are the necessary commercial products and are consumed by humans and animals, thus ultimately infecting the organs and creating life-threatening issues for humans and animals. In addition, plants may be used to determine the genetic toxicity of environmental pollutants as biosensors (Jadhav et al., 2010).

Moreover, fluctuating pH, a high degree of coloration and high levels of chemical oxygen demand (COD), biological oxygen demand (BOD), suspended solids (TSS), and total organic carbon (TOC) makes the presence of dyes in effluents very specific.

In addition, a long-term dye presence in watercourses induces the accumulation of dye in marine organisms and fish. Some of the dye effluents decompose, and the aquatic ecosystem may have a toxic influence on complementary hazardous compounds (Carmen and Daniela 2012). It was found from previous studies that the presence of dye effluents and related pollutants can be transferred to food in surface water (Tkaczyk and Kowalska 2016).

### III. BIOLOGICAL TREATMENT

An interesting alternative is to remove dye from the biological treatment of wastewater pollutants because it is based on activated sludge containing various aerobic and anaerobic microorganisms such as bacteria, fungi, and green algae. Bacterial isolated enzymes have been shown as an effective method for the treatment of dye effluents in recent studies (Hazirah et al., 2014). This method is ambiguous, divergent, and distinct for decolorization of industrial waste (Anjaneyulu, Chary and Raj, 2005). The activity and adaptability of microorganisms are associated with the most crucial factors affecting the effectiveness of biological dye treatment removal (Chen et al., 2003). Dyes themselves are not biologically degraded because colored constituents are not used as a source of nourishment by microorganisms. In Infield bioremediation and laboratory studies, microbial consortia have been used to clean up the effluent pollutant and usually believed that it is the most effective method. Therefore, bacteria express their complete degradation capacity in optimal conditions; temperature, pH, and other supplements have a high effect on their growth (Mao et al., 2012).

Biodegradation involves aerobic microorganisms that use molecular oxygen during the respiration process to reduce equivalent receptors. In anaerobic environment conditions (hypoxic and anoxic environment), biodegradation also occurs, and the survival of microorganisms is possible as electron acceptors by using nitrates, sulfates, and carbon dioxide (Harris et al., 1989). A low cost is the main advantage of biological treatment, the discharge of non-toxic mineralization substances in small amounts, the production of sludge, and in contrast to Physico-chemical treatment, approximately 70% of the organic matter expressed by COD<sub>Cr</sub> can be converted into biosolids (Anjaneyulu et al., 2005).

In large scale textile effluent treatment, activated sludge was the most used biological treatment, and biological aerated philtre (BAF) or trickling philtre is an alternative, removing 34-44 percent of dye color for various high dyeing loads of industrial effluents. Microorganisms are considered to be the most effective "weapon" against environmental pollution of triphenylmethane dye (Song et al., 2020). There are several strains isolated from various sources (such as lakes, textile effluents, soil) of bacteria and fungi that have the ability to degrade or decolorize triphenylmethane dyes. The main microorganisms that allow biodegradation of organic compounds are bacteria (e.g. *Streptomyces microflavus*, *Aeromonas Hydrophilia*, *Bacillus subtilis*, *Bacillus ceteus*, *Pseudomonas* species, *Klebsiella pneumoniae*, *Acetobacter liquefaciens*, *Pagmentiphaga kullae*, *Sphingomonas*, etc. (Zimmerman et al., 1982; Sani and Banerjee, 1999), fungi (e.g., white-rot fungi: *Hirschioporus larincinus*, *Inonotus hispidus*, *Phanerochaete chrysosporium*, *Coriolus versicolor*, *Phlebia tremellosa*, etc. (Gold and Alic, 1993; Swamy and Ramsay, 1999; Balan and Monteiro, 2001; Novotny et al., 2001), algae (e.g. *oscillatoria* and *chlorella* species, etc. (Dilek et al., 1999). Moreover, from a wide variety of habitat some bacteria, mixed microbial culture, white-rot fungus are found to degrade dye using enzymes such as lignin peroxidase (LiP), manganese dependent peroxidases (MnP), phenoloxidases, laccases, cellobiose dehydrogenase, and H<sub>2</sub>O<sub>2</sub> producing enzymes like glucose-1-oxidase and glucose-2-oxidase (Buntic et al., 2017).

Anaerobic biodegradation is primarily documented as a hydrogen oxidation-reduction reaction and the formation of hydrogen sulphide, methane, carbon dioxide, other gaseous compounds, and electrons are released, resulting in the decolorization of effluent by reacting with the colorant. The best option is thermophilic anaerobic treatment, as textile effluents are usually released at high temperatures (40-70 percent). The primary advantage of anaerobic biological treatment effluent decolorization is the production of biogas, which can be reused for power, and the generation of heat, thus reducing the cost of energy.

The textile wastewater is a major concern and is potentially carcinogenic and extremely toxic (Sharma, Dangi & Shukla 2018) such that it has become a significant danger to living organisms in an ecosystem as a result of different illnesses in human beings, animals and to environment deterioration (Khan & Malik 2018). Contaminants must also be broken down, degraded, or converted into non-toxic or less-toxic forms, thus eliminating, restoring, or removing contaminants from the atmosphere. In general, textile dyes cause diseases from central nervous system disorders to dermatitis (Khan & Malik, 2018) or can result in enzymatic activities being inactivated by enzymatic cofactors being substituted (Copaciu et al., 2013).

Table 1. Dye decoloration documented by various microorganisms in different literature.

Organism	Crystal violet concentration (ppm)	Growth time (h)	DT (h)	D (%)	Reference
<i>P. veronii</i>	20	168	96	95.1%	Song et al., 2020
<i>Rhodotorulae rubra</i> and <i>Rhodotorulae sp.</i>	10	24	96	99	Kwasniewska, 1985
<i>P.chrysosporium</i> BKM-F-1767	5	144	6	65	Bumpus and Brock, 1988
<i>Bacillus subtilis</i> IFO 13719	0.852	24	24	100	Yatome et al., 1991
<i>Nocardia globerula</i>	0.852	24	24	96	Yatome et al., 1991
<i>Nocardia corallina</i>	0.933	18	1.5	80	Yatome et al., 1993
<i>P. chrysosporium</i> ME446	5	144	72	62	Yesilada, 1995
<i>Coriolus versicolor</i>	5	144	72	92	Yesilada, 1995
<i>Funalia trogii</i>	5	144	72	82	Yesilada, 1995
<i>Laetiporus sulphureus</i>	5	144	72	86	Yesilada, 1995
<i>Cyathus bulleri</i>	29.38	192	96	96.3	Vasdev, 1995
<i>Cyathus stercoreus</i>	29.38	192	96	84.7	Vasdev, 1995
<i>Cyathus striatus</i>	29.38	192	96	75.5	Vasdev, 1995
<i>P. chrysosporium</i>	20	144	216	92	Knapp, 1995

NCIM 1197					
<i>P. chrysosporium</i> MTCC no. 787	5	120	70	90	Sani, 1998
<i>Pseudomonas aeruginosa</i>	20	48	24	80	Ogugbue and Morad, 2014
( <i>Agrobacterium radiobacter</i> , <i>Bacillus spp.</i> , <i>Sphingomonas paucimobilis</i> , and <i>Aeromonas hydrophila</i> )	50	24	2	91	Cheriaa et al., 2012
<i>Pseudomonas pseudomallei</i> 13NA	20.4	20	120	96	Yatome et al., 1981
<i>Enterobacter sp.</i> HSL69	50	24	72	81.25	Roy et al., 2018

Note :- D- decolorization percentage; DT- decolorization time; h- hours; ppm- parts per million.

#### IV. TOXICITIES

With permanent damage to the cornea and conjunctiva, the CV dye is recorded to cause mild eye irritation and painful sensitization to light. It is highly toxic to mammalian cells and, if ingested via the skin in harmful amounts, can cause skin irritation and irritation of the digestive tract. In extreme conditions, it may also lead to respiratory and kidney failure (Mittal et al., 2010). The oncogenic and protection potential as it interacts with DNA cells is controversial (Maley and Arbiser, 2013). It functions as a mitotic poison as well as a clastogen (Au et al., 1978).

Increased hepatocellular carcinoma in mice is indicated by excess use of crystal violet (Culp et al., 2006), while in FDA studies there has been an rise in the number of thyroid cancers in rats fed crystal violet (Littlefield et al., 1989). It prevents the hypothyroidism thyroid peroxidase and induces thyroid-stimulating hormone to duplicate thyroid cells from the pituitary gland (Arbiser, 2009).

The use of crystal violet without contraindication is extremely secure and reliable. No cases of crystal violet linked cancer have been found for decades. The toxicity of gentian violet is restricted to humans, as found in studies and case reports concerning the use of gentian violet (Berrios & Arbiser, 2011), and the FDA approved the selling of gentian violet over the counter.

It induces gastrointestinal discomfort if taken orally (John 1968), and causes depression of white blood cells through intravenous injection (Young and Hill 1924).

##### 4.1. Antibacterial Response

Triphenylmethane dye has anti-fungal, anti-angiogenic, anti-bacterial, anti-trypanosomal, anti-helminthic, and anti-tumor properties. It has been a long tradition of use as a cure and as monotherapy for a variety of diseases (Maley and Arbiser 2015).

Gentian violet's antibacterial action is stronger at high pH (Adams 1967). Organisms (like anaerobic bacteria) have a strong reduction mechanism as Gentian violet tolerant, whether gramme-positive or gram-negative (Ingraham 1933). Although the exact mode of action is unclear, an anti-microbial effect has been suggested for Crystal violet: free radical formation (Zhang et al., 2011), inhibition of glutamine synthesis and protein synthesis (Hoffmann et al., 1995), inhibition of decreased oxidases of nicotinamide adenine dinucleotides phosphate (NADPH) (Perry et al., 2006), inhibition of bacterial cell wall formation (Wu and Wood, 2018) or disruption of oxidative phosphorylation (Moreno et al., 1988). A photodynamic effect of crystal violet has been identified in bacteria and in *T. cruzi* mediated by free-radical mechanism (Docampo & Moreno 1990).

As an anti-microbial, Bakker et al in 1992 retraced the use of dye for dermatological diseases. In addition to *Candida albicans*, the investigators applied triphenylmethane dye to bacterial species (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus A & B*, *Proteus*). The result showed Gentian violet to be very effective with low concentration against species *Streptococcus*, *Staphylococcus*, and *Candida* and forms adducts with it because of its ability to penetrate and bind to proteins in the bacterial cell wall. Owing to its inability to penetrate the bacterial cell wall, Crystal violet is much less effective against *Mycobacterium* and gram-negative bacteria. This has been in clinical use for more than a century, as this is the basis of Gram staining.

It has been used for a long time to avoid bacterial umbilical stump infection following childbirth (Zupan et al., 2004).

Gentian violet also has low inhibitory MRSA efficacy in the setting of ulcers with no significant side effects (Okano et al., 2000). It has emerged as a possible treatment for infectious MRSA, successfully used for graft infection with prosthetic vascular bypass (Igari et al., 2011), mediastinitis (Kato et al., 2006), MRSA nasal carriage (Okano et al., 2000) and notitis media (Kayama et al., 2006).

In 2003, the FDA cleared the foam dressing of gentian violet polyvinyl alcohol (PVA) as a bacteriostatic dressing (refers to capable of inhibiting the reproduction or growth of bacteria) for use on diabetic ulcers, pressure ulcers, venous stasis ulcers, superficial burns, arterial ulcers, donor sites, trauma wounds, post-operative incisions, lacerations, and abrasions (Edwards 2016).

##### 4.2. Genotoxic action

Gentian violet has been used as a chromatin or nuclear stain for several years. It binds to DNA and interacts with two adjacent base pairs of A-T, causing extreme binding or/and kinking, accompanied by DNA double helix coupled unwinding (Wu and Wood, 2018). This causes damage to DNA and thereby contributes to the creation of malignant tumors. Moreover, (Kim and Norden, 1993) evidence shows that gentian violet is linked to the major DNA groove. In addition, the vast majority of small molecules and natural products that bind nucleic acid tend to inhabit the narrower minor groove, where hydrophobic and van der Waals interactions with the groove's walls and floor are maximized (Nunez et al., 2019).

For any of the five strains of *Salmonella typhimurium*, or *Saccharomyces cerevisiae* strain XV185-14C (Shahin and Von Borstel, 1978), mutagenicity analysis of various dyes interpreted that gentian violet was not mutagenic. With free radical formation, Gentian violet was shown to have oxidation-reduction ability. A thiazole antibiotic called thioestrepton (TS) further increased the cytotoxic activity of Gentian violet, which suppresses the expression of oncogenic transcriptional factor FOXM1, which is primarily needed for cell cycle progression and also shows resistivity to oncogenic oxidative stress (Garufi et al., 2014).

#### 4.3. Antimycotic Action

Gentian violet has an antifungal activity against many candida species. Several studies have demonstrated the effectiveness of crystal violet against candida in catheter infection settings. Catheters coated with gentian violet were found to be superior in reducing the burden of E.coli in many species of microorganisms in the urine, bladder and colony (Hachem et al., 2009). It is used for treating parasitic nematodes of *Enterbium* (pinworm) (Bumbalo & Gustina, 1955) and *Strongyloides* (Browne et al., 1957).

GV is also used as an oral thrush remedy; for 90 years, it has been an effective and safe remedy to treat oral candidiasis by painting an infant's mouth with oral thrush (Maley and Arbiser 2013). Oral candidiasis is the most common infection in HIV- infected patients (Blignaut, 2007). In addition, Candida's ability to form biofilms depends on both the host immune status and antibiotic resistance (Stewart and Costerton 2001). The biofilms produced by candida isolated from HIV infected patients are inhibited by Gentian violet (Mukherjee et al., 2017). For the treatment of Candida biofilms, antifungals are widely used (Kuhn et al., 2002; Mukherjee et al., 2003). It is important to grow antibiofilm to treat oral candidiasis. In HIV infected patients, triphenylmethane dye is used as a fungicidal agent against planktonic candida cells to treat oral candidiasis at a concentration of 0.5 percent -1 percent (Traboulski et al., 2008).

The toxicity of gentian violet has been reported in some of the studies for the treatment of oral candidiasis including necrosis (John 1968) and oral mucosal irritation (Vucicevic et al., 2005), obstructive laryngotracheitis (Baca, Drexler and Cullen 2001), and difficulty with breastfeeding (Utter 1990).

#### 4.4. Cytogenic toxicity

In cell culture, the dye also induces cytogenic toxicity. In the culture of CHO cells, HeLa & L cells, human lymphocytes, and fibroblastic cell lines, it smashes chromosomes. Gentian violet in CHO cells also induces chromatid exchange. In vivo assay, since it failed to induce sister chromatid exchange, the dye was not clastogenic. In addition, it was shown to be highly toxic to the developing embryos of chicks at high dosages (Docampo and Moreno 1990).

CV has also been found to cause a decrease in the synthesis of RNA and proteins and decreased consumption of oxygen in the tissue of rabbit granulation. The deposition of crystal violet and malachite green in sediments and water was recorded in the Buffalo River, New York, USA, (Nelson and Hites, 1980). These chemicals have been suggested to be responsible for promoting tumour growth in several fish species that feed on the bottom (Cho et al., 2003). Therefore, both the aquatic environment and the human population pose a serious threat.

### V. CONCLUSION AND FUTURE PROSPECTUS

Bioremediation has been established in recent years as a possible solution for a significant number of wastewater treatment processes. However, in practical applications, engineering scale degradation work is very minimal, generally concentrated in the experimental procedure.

In human and veterinary medicine, Gentian violet (GV) /Crystal Violet (CV), a triphenylmethane dye, has been commonly used as a biological stain, as a textile dye in the textile processing industry and also to provide paints and printing ink with a deep violet colour. Genetic violet has been used for nearly 100 years in medicine: for external use as an antiseptic, oral administration as an anti-Helminthic agent; and more recently as a blood additive, to prevent the transmission of Chagas' disease. To avoid fungal growth in poultry feed, GV is also used as a mutagenic and bacteriostatic agent in medical solutions and as an antimicrobial agent. FDA proved gentian violet as a bacteriostatic dressing on ulcers. To date, there have been no recorded serious side effects when used externally. GV has been reported as a recalcitrant dye molecule, despite its many uses, that persists in the environment for a long time and poses toxic environmental effects. In some species of fish, it functions as a mitotic toxin, a potent carcinogen and a potent clastogen that promotes tumour development. GV is therefore recognised as a biohazard material. It is still considered a challenging task to effectively eliminate dye micro-pollutants from industrial waste water supplies. While several physico-chemical methods are recorded for the removal of GV, such as coagulation, adsorption and ion-pair extraction, these methods are inadequate for the complete removal of GV from industrial waste water and also generate large quantities of secondary pollutant-containing sludge. However, for industrial wastewater treatment, biological methods are considered to be cost-effective and eco-friendly, but these methods also have some limitations. There is an urgent need, to establish certain eco-friendly and cost-effective biological treatment methods for the protection of the environment, as well as for human and animal welfare, it may effectively extract dye from industrial waste water.

It is important for the recycling and reuse of their wastewater in industries that use large water volumes such as textiles, leather, paints, acrylic, cosmetics, plastics and pharmaceutical, etc. The previous GV account shows that because of its adverse effects on the environment and extreme health threats posed to living organisms, this dye has now become one of the most discussed and controversial compounds. It is therefore concluded that GV is a pollutant that is recalcitrant and has harmful effects on both marine and terrestrial habitats. It also serves as a mitotic poisoning agent, a carcinogen and a potent clastogen that promotes tumour growth in some fish species. Therefore, for the degradation and detoxification of waste water containing GV, a regular and efficient treatment system should be employed. In this respect, methods of physical and chemical treatment are very expensive and contain a large number of different forms of secondary contaminants as well. Therefore, an environmentally sustainable and cost-effective biological method for the effective degradation and detoxification of GVs for environmental protection needs to be developed urgently.

**VI. REFERENCES**

1. Adams, E. 1967. The antibacterial action of crystal violet. *Journal of Pharmacy and Pharmacology*, 19(12): 821-826.
2. Anjaneyulu, Y., Chary, N.S. and Raj, D.S.S. 2005. Decolourization of industrial effluents—available methods and emerging technologies—a review. *Reviews in Environmental Science and Bio/Technology*, 4(4): 245-273.
3. Aquino, J.M., Rocha-Filho, R.C., Ruotolo, L.A., Bocchi, N. and Biaggio, S.R. 2014. Electrochemical degradation of a real textile wastewater using  $\beta$ -PbO<sub>2</sub> and DSA® anodes. *Chemical Engineering Journal*, 251: 138-145.
4. Arbiser, J.L. 2009. Gentian violet is safe. *Journal of the American Academy of Dermatology*, 61(2): 359.
5. Asad, S., Amoozegar, M.A., Pourbabae, A., Sarbolouki, M.N. and Dastgheib, S.M.M. 2007. Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. *Bioresource technology*, 98(11): 2082-2088.
6. Au, W., Pathak, S., Collie, C. J. and Hsu, T. C. 1978. Cytogenetic toxicity of gentian violet and crystal violet on mammalian cells in vitro. *Mutation Research/Genetic Toxicology*, 58(2-3): 269-276.
7. Azmi, W., Sani, R. and Banerjee, U. 1998. Biodegradation of triphenylmethane dyes. *Enzyme and Microbial Technology*, 22(3): 185-191.
8. Baca, D., Drexler, C. and Cullen, E. 2001. Obstructive laryngotracheitis secondary to gentian violet exposure. *Clinical pediatrics*, 40(4): 233-235.
9. Bakker, P., Van Doorne, H. A. N. S., Gooskens, V. and Wieringa, N. F. 1992. Activity of gentian violet and brilliant green against some microorganisms associated with skin infections. *International journal of dermatology*, 31(3): 210-213.
10. Balan, D. S. and Monteiro, R. T. 2001. Decolorization of textile indigo dye by ligninolytic fungi. *Journal of Biotechnology*, 89(2-3): 141-145.
11. Banat, I. M., Nigam, P., Singh, D. and Marchant, R. 1996. Microbial decolorization of textile-dyecontaining effluents: a review. *Bioresource technology*, 58(3): 217-227.
12. Berrios, R. L. and Arbiser, J. L. 2011. Effectiveness of gentian violet and similar products commonly used to treat pyodermas. *Dermatologic clinics*, 29(1): 69-73.
13. Blignaut, E. 2007. Oral candidiasis and oral yeast carriage among institutionalised South African paediatric HIV/AIDS patients. *Mycopathologia*, 163(2): 67-73.
14. Browne, D. C., Contacos, P. G., Welch, G. E. and McHardy, G. 1957. Treatment of *Strongyloides stercoralis* infection with intravenous gentian violet. *The American Journal of Tropical Medicine and Hygiene*, 6(6): 1066-1067.
15. Bumbalo, T. S. and Gustina, F. J. 1955. The treatment of pinworm infection (enterobiasis) with gentian violet suspension. *The Journal of pediatrics*, 47(3): 311-314.
16. Bumpus, J.A. and Brock, B.J. 1988. Biodegradation of crystal violet by the white rot fungus *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology*, 54(5): 1143-1150.
17. Buntić, A. V., Pavlović, M. D., Antonović, D. G., Šiler-Marinković, S. S. and Dimitrijević-Branković, S. I. 2017. A treatment of wastewater containing basic dyes by the use of new strain *Streptomyces microflavus* CKS6. *Journal of cleaner production*, 148: 347-354.
18. Carmen, Z. and Daniela, S. 2012. Textile organic dyes—characteristics, polluting effects and separation/elimination procedures from industrial effluents—a critical overview. In *Organic pollutants ten years after the Stockholm convention—environmental and analytical update* (Vol. 10, p. 32373). London, UK: IntechOpen.
19. Caro, H. and Kern, A. 1883. Manufacture of dye-stuff. US290856: 25.
20. Chen, K.C., Wu, J.Y., Liou, D.J. and Hwang, S.C.J. 2003. Decolorization of the textile dyes by newly isolated bacterial strains. *Journal of Biotechnology*, 101(1): 57-68.
21. Cheriaa, J., Khairiddine, M., Rouabhia, M. and Bakhrouf, A. 2012. Removal of triphenylmethane dyes by bacterial consortium. *The Scientific World Journal*.
22. Cho, B. P., Yang, T., Blankenship, L. R., Moody, J. D., Churchwell, M., Beland, F. A. and Culp, S. J. 2003. Synthesis and characterization of N-demethylated metabolites of malachite green and leucomalachite green. *Chemical Research in Toxicology*, 16(3): 285-294.
23. Cook, E. F., Martin, E. W. and Remington, J. P. 1951. *Remington's practice of pharmacy*. Mack.
24. Copaciu, F., Opris, O., Coman, V., Ristoiu, D., Niinemets, Ü. and Copolovici, L. 2013. Diffuse water pollution by anthraquinone and azo dyes in environment importantly alters foliage volatiles, carotenoids and physiology in wheat (*Triticum aestivum*). *Water, Air, and Soil Pollution*, 224(3): 1478.
25. Culp, S. J., Mellick, P. W., Trotter, R. W., Greenlees, K. J., Kodell, R. L. and Beland, F. A. 2006. Carcinogenicity of malachite green chloride and leucomalachite green in B6C3F1 mice and F344 rats. *Food and Chemical Toxicology*, 44(8): 1204-1212.
26. Dandona, R., Kochar, P. S., Kumar, G. A. and Dandona, L. 2017. Use of antiseptic for cord care and its association with neonatal mortality in a population-based assessment in Bihar State, India. *BMJ open*, 7(1).
27. Dilek, F. B., Taplamacioglu, H. M. and Tarlan, E. 1999. Colour and AOX removal from pulping effluents by algae. *Applied Microbiology and Biotechnology*, 52(4): 585-591.
28. Docampo, R. and Moreno, S. N. 1990. The metabolism and mode of action of gentian violet. *Drug metabolism reviews*, 22(2-3): 161-178.
29. Eagle, S., Brophy, C. M., Komalavilas, P., Hocking, K., Putumbaka, G., Osgood, M. and Cheung-Flynn, J. 2011. Surgical skin markers impair human saphenous vein graft smooth muscle and endothelial function. *The American surgeon*, 77(7): 922-928.
30. Edwards, K. 2016. New twist on an old favorite: gentian violet and methylene blue antibacterial foams. *Advances in wound care*, 5(1): 11-18.

31. Fulekar, M. H., Wadgaonkar, S. L. and Singh, A. 2013. Decolourization of dye compounds by selected bacterial strains isolated from dyestuff industrial area. *Int J Adv Res Technol*, 2(7): 182-192.
32. Garufi, A., D'Orazi, V., Arbiser, J. L. and D'Orazi, G. 2014. Gentian violet induces wtp53 transactivation in cancer cells. *International journal of oncology*, 44(4): 1084-1090.
33. Gessner, T. and Mayer, U. 2000. Triarylmethane and diarylmethane dyes. *Ullmann's Encyclopedia of Industrial Chemistry*.
34. Gold, M. H., and Alic, M. 1993. Molecular biology of the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Microbiology and Molecular biology reviews*, 57(3): 605-622.
35. Hachem, R., Reitzel, R., Borne, A., Jiang, Y., Tinkey, P., Uthamantil, R. and Raad, I. 2009. Novel antiseptic urinary catheters for prevention of urinary tract infections: correlation of in vivo and in vitro test results. *Antimicrobial agents and chemotherapy*, 53(12): 5145-5149.
36. Hafshejani, M. K., Ougubue, C. J. and Morad, N. 2014. Application of response surface methodology for optimization of decolorization and mineralization of triazo dye Direct Blue 71 by *Pseudomonas aeruginosa*. *3 Biotech*, 4(6): 605-619.
37. Harris, J. A., Birch, P. and Short, K. C. 1989. Changes in the microbial community and physico-chemical characteristics of topsoils stockpiled during opencast mining. *Soil Use and Management*, 5(4): 161-168.
38. Hassan, M. M. and Carr, C. M. 2018. A critical review on recent advancements of the removal of reactive dyes from dyehouse effluent by ion-exchange adsorbents. *Chemosphere*, 209: 201-219.
39. Hoffmann, M. E., Jang, J., Moreno, S. N. and Docampo, R. 1995. Inhibition of protein synthesis and amino acid transport by crystal violet in *Trypanosoma cruzi*. *Journal of Eukaryotic Microbiology*, 42(3): 293-297.
40. Igari, K., Jibiki, M., Kudo, T., Sugano, N. and Inoue, Y. 2011. Drainage surgery followed by postoperative irrigation with gentian violet for prosthetic graft infection caused by methicillin-resistant *Staphylococcus aureus*. *European Journal of Vascular and Endovascular Surgery*, 41(2): 278-280.
41. Ingraham, M. A. 1933. The bacteriostatic action of gentian violet and dependence on the oxidation-reduction potential. *Journal of bacteriology*, 26(6): 573.
42. Jadhav, J. P., Phugare, S. S., Dhanve, R. S. and Jadhav, S. B. 2010. Rapid biodegradation and decolorization of Direct Orange 39 (Orange TGLL) by an isolated bacterium *Pseudomonas aeruginosa* strain BCH. *Biodegradation*, 21(3): 453-463.
43. John, R. W. 1968. Necrosis of oral mucosa after local application of crystal violet. *British medical journal*, 1(5585): 157.
44. Kalyani, D. C., Telke, A. A., Dhanve, R. S. and Jadhav, J. P. 2009. Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas* sp. SUK1. *Journal of Hazardous Materials*, 163(2-3): 735-742.
45. Kato, T., Takagi, H., Matsuno, Y., Imaizumi, M. and Umemoto, T. 2006. High-pressure irrigation and gentian-violet application for mediastinitis following replacement of ascending aorta and aortic valve. *Heart and Vessels*, 21(6): 392-394.
46. Kayama, C., Goto, Y., Shimoya, S., Hasegawa, S., Murao, S. I., Nakajo, Y. and Nibu, K. I. 2006. Effects of gentian violet on refractory discharging ears infected with methicillin-resistant *Staphylococcus aureus*. *Journal of otolaryngology*, 35(6).
47. Khan, S. and Malik, A. 2018. Toxicity evaluation of textile effluents and role of native soil bacterium in biodegradation of a textile dye. *Environmental Science and Pollution Research*, 25(5): 4446-4458.
48. Khatri, J., Nidheesh, P. V., Singh, T. A. and Kumar, M. S. 2018. Advanced oxidation processes based on zero-valent aluminium for treating textile wastewater. *Chemical Engineering Journal*, 348: 67-73.
49. Kim, S. K. and Nordén, B. 1993. Methyl green: A DNA major-groove binding drug. *FEBS letters*, 315(1): 61-64.
50. Knapp, J. S., Newby, P. S. and Reece, L. P. 1995. Decolorization of dyes by wood-rotting basidiomycete fungi. *Enzyme and Microbial Technology*, 17(7): 664-668.
51. Kuhn, D. M., George, T., Chandra, J., Mukherjee, P. K. and Ghannoum, M. A. 2002. Antifungal susceptibility of *Candida* biofilms: unique efficacy of amphotericin B lipid formulations and echinocandins. *Antimicrobial agents and chemotherapy*, 46(6): 1773-1780.
52. Kwasniewska, K. 1985. Biodegradation of crystal violet (hexamethyl-p-rosaniline chloride) by oxidative red yeasts. *Bulletin of environmental contamination and toxicology*, 34(1): 323-330.
53. Li, S. 2010. Removal of crystal violet from aqueous solution by sorption into semi-interpenetrated networks hydrogels constituted of poly (acrylic acid-acrylamide-methacrylate) and amylose. *Bioresource technology*, 101(7): 2197-2202.
54. Littlefield, N. A., Gaylor, D. W., Blackwell, B. N. and Allen, R. R. 1989. Chronic toxicity/carcinogenicity studies of gentian violet in Fischer 344 rats: two-generation exposure. *Food and chemical toxicology*, 27(4): 239-247.
55. Maley, A. M. and Arbiser, J. L. 2013. Gentian Violet: a 19th century drug re-emerges in the 21st century. *Experimental dermatology*, 22(12): 775-780.
56. Mao, J., Luo, Y., Teng, Y. and Li, Z. 2012. Bioremediation of polycyclic aromatic hydrocarbon-contaminated soil by a bacterial consortium and associated microbial community changes. *International Biodeterioration & Biodegradation*, 70: 141-147.
57. Mittal, A., Mittal, J., Malviya, A., Kaur, D. and Gupta, V. K. 2010. Adsorption of hazardous dye crystal violet from wastewater by waste materials. *Journal of colloid and interface science*, 343(2): 463-473.
58. Mojsov, K. D., Andronikov, D., Janevski, A., Kuzelov, A. and Gaber, S. 2016. The application of enzymes for the removal of dyes from textile effluents. *Advanced technologies*, 5(1): 81-86.
59. Mona, S., Kaushik, A. and Kaushik, C. P. 2011. Waste biomass of *Nostoc linckia* as adsorbent of crystal violet dye: Optimization based on statistical model. *International Biodeterioration & Biodegradation*, 65(3): 513-521.
60. Mondal, P., Baksi, S. and Bose, D. 2017. Study of environmental issues in textile industries and recent wastewater treatment technology. *World Scientific News*, 61(2): 98-109.
61. Morales-Alvarez, E. D., Rivera-Hoyos, C. M., Poveda-Cuevas, S. A., Reyes-Guzmán, E. A., Pedroza-Rodríguez, A. M., Reyes-Montañón, E. A. and Poutou-Piñales, R. A. 2018. Malachite green and crystal violet decolorization by ganoderma



- lucidum and pleurotus ostreatus supernatant and by rGILCC1 and rPOXA 1B concentrates: molecular docking analysis. *Applied biochemistry and biotechnology*, 184(3): 794-805.
62. Moreno, S. N., Gadelha, F. R. and Docampo, R. 1988. Crystal violet as an uncoupler of oxidative phosphorylation in rat liver mitochondria. *Journal of Biological Chemistry*, 263(25): 12493-12499.
  63. Muhd Julkapli, N., Bagheri, S. and Bee Abd Hamid, S. 2014. Recent advances in heterogeneous photocatalytic decolorization of synthetic dyes. *The Scientific World Journal*.
  64. Mukherjee, P. K., Chandra, J., Kuhn, D. M. and Ghannoum, M. A. 2003. Mechanism of fluconazole resistance in *Candida albicans* biofilms: phase-specific role of efflux pumps and membrane sterols. *Infection and immunity*, 71(8): 4333-4340.
  65. Mukherjee, P. K., Chen, H., Patton, L. L., Evans, S., Lee, A., Kumwenda, J. and Freedberg, K. A. 2017. Topical gentian violet compared to nystatin oral suspension for the treatment of oropharyngeal candidiasis in HIV-1 Infected participants. *AIDS (London, England)*, 31(1): 81.
  66. Nelson, C. R. and Hites, R. A. 1980. Aromatic amines in and near the Buffalo River. *Environmental Science & Technology*, 14(9): 1147-1149.
  67. Newman, M. C. 2009. *Fundamentals of ecotoxicology*. CRC press.
  68. Norman, G., Christie, J., Liu, Z., Westby, M. J., Jefferies, J. M., Hudson, T. and Dumville, J. C. 2017. Antiseptics for burns. *Cochrane Database of Systematic Reviews*, (7).
  69. Novotný, Č., Rawal, B., Bhatt, M., Patel, M., Šašek, V. and Molitoris, H. P. 2001. Capacity of *Irpex lacteus* and *Pleurotus ostreatus* for decolorization of chemically different dyes. *Journal of Biotechnology*, 89(2-3): 113-122.
  70. Nuñez, O., Chavez, B., Shaktah, R., Garcia, P. P. and Minehan, T. 2019. Synthesis and DNA binding profile of monomeric, dimeric, and trimeric derivatives of crystal violet. *Bioorganic chemistry*, 83: 297-302.
  71. Nur Hazirah, R., Nurhaslina, C. R. and Ku Halim, K. H. 2014. Enhancement of biological approach and potential of *Lactobacillus delbrueckii* in decolorization of textile wastewater-A review. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 8(11).
  72. Okano, M., Noguchi, S., Tabata, K. and Matsumoto, Y. 2000. Topical gentian violet for cutaneous infection and nasal carriage with MRSA. *International journal of dermatology*, 39(12): 942-944.
  73. Pandey, A., Singh, P. and Iyengar, L. 2007. Bacterial decolorization and degradation of azo dyes. *International Biodeterioration & Biodegradation*, 59(2): 73-84.
  74. Perry, B. N., Govindarajan, B., Bhandarkar, S. S., Knaus, U. G., Valo, M., Sturk, C. and Losken, A. 2006. Pharmacologic blockade of angiopoietin-2 is efficacious against model hemangiomas in mice. *Journal of investigative dermatology*, 126(10): 2316-2322.
  75. Perry, B. N., Govindarajan, B., Bhandarkar, S. S., Knaus, U. G., Valo, M., Sturk, C. and Losken, A. 2006. Pharmacologic blockade of angiopoietin-2 is efficacious against model hemangiomas in mice. *Journal of investigative dermatology*, 126(10): 2316-2322.
  76. Reinhardt, C. and Travis, A. S. 2000. The Chemist as Inventor. In *Heinrich Caro and the Creation of Modern Chemical Industry*. Springer, Dordrecht. 25-176.
  77. Roy, D. C., Biswas, S. K., Saha, A. K., Sikdar, B., Rahman, M., Roy, A. K. and Tang, S. S. 2018. Biodegradation of Crystal Violet dye by bacteria isolated from textile industry effluents. *PeerJ*, 6, e5015.
  78. Rybczyńska-Tkaczyk, K. and Kornilowicz-Kowalska, T. 2016. Biosorption optimization and equilibrium isotherm of industrial dye compounds in novel strains of microscopic fungi. *International Journal of Environmental Science and Technology*, 13(12): 2837-2846.
  79. Sandhya, S. 2010. Biodegradation of azo dyes under anaerobic condition: role of azoreductase. In *Biodegradation of azo dyes*. Springer, Berlin, Heidelberg. 39-57.
  80. Sani, R. K. and Banerjee, U. C. 1999. Decolorization of triphenylmethane dyes and textile and dye-stuff effluent by *Kurthia* sp. *Enzyme and Microbial Technology*, 24(7): 433-437.
  81. Sani, R. K., Azmi, W. and Banerjee, U. C. 1998. Comparison of static and shake culture in the decolorization of textile dyes and dye effluents by *Phanerochaete chrysosporium*. *Folia microbiologica*, 43(1): 85-88.
  82. Sayé, M., Gauna, L., Valera-Vera, E., Reigada, C., Miranda, M. R. and Pereira, C. A. 2020. Crystal violet structural analogues identified by in silico drug repositioning present anti-*Trypanosoma cruzi* activity through inhibition of proline transporter TcAAP069. *PLoS neglected tropical diseases*, 14(1): e0007481.
  83. Senthilkumaar, S., Kalaamani, P. and Subburaam, C. V. 2006. Liquid phase adsorption of crystal violet onto activated carbons derived from male flowers of coconut tree. *Journal of hazardous materials*, 136(3): 800-808.
  84. Shah, M. P. 2013. Microbial degradation of textile dye (Remazol Black B) by *Bacillus* spp. ETL-2012. *Journal of Applied & Environmental Microbiology*, 1(1): 6-11.
  85. Shahin, M. M. and Von Borstel, R. C. 1978. Comparisons of mutation induction in reversion systems of *Saccharomyces cerevisiae* and *Salmonella typhimurium*. *Mutation Research/Environmental Mutagenesis and Related Subjects*, 53(1): 1-10.
  86. Sharma, B., Dangi, A. K. and Shukla, P. 2018. Contemporary enzyme based technologies for bioremediation: a review. *Journal of environmental management*, 210: 10-22.
  87. Song, J., Han, G., Wang, Y., Jiang, X., Zhao, D., Li, M. and Mu, Y. 2020. pathway and kinetics of malachite green biodegradation by *Pseudomonas veronii*. *Scientific reports*, 10(1): 1-11.
  88. Stewart, P. S. and Costerton, J. W. 2001. Antibiotic resistance of bacteria in biofilms. *The lancet*, 358(9276): 135-138.
  89. Subhathra, M., Prabakaran, V., Kuberan, T. and Balamurugan, I. 2013. Biodegradation of Azo dye from textile effluent by *Lysinibacillus sphaericus*. *Sky Journal of Soil Science and Environmental Management*, 2(1): 1-11.
  90. Swamy, J. and Ramsay, J. A. 1999. The evaluation of white rot fungi in the decoloration of textile dyes. *Enzyme and Microbial Technology*, 24(3-4): 130-137.

91. Traboulsi, R. S., Mukherjee, P. K., Chandra, J., Salata, R. A., Jurevic, R. and Ghannoum, M. A. 2011. Gentian violet exhibits activity against biofilms formed by oral *Candida* isolates obtained from HIV-infected patients. *Antimicrobial agents and chemotherapy*, 55(6): 3043-3045.
92. Utter, A. R. 1990. Gentian violet treatment for thrush: can its use cause breastfeeding problems?. *Journal of Human Lactation*, 6(4): 178-180.
93. Vasdev, K., Kuhad, R. C. and Saxena, R. K. 1995. Decolorization of triphenylmethane dyes by the bird's nest fungus *Cyathus bulleri*. *Current Microbiology*, 30(5): 269-272.
94. Verma, A. K., Dash, R. R. and Bhunia, P. 2012. A review on chemical coagulation/flocculation technologies for removal of colour from textile wastewaters. *Journal of environmental management*, 93(1): 154-168.
95. Vucicevic Boras, V., Brailo, V., Andabak Rogulj, A., Vidovic Juras, D., Gabric, D. and Vrdoljak, D. V. 2015. Oral adverse reactions caused by over-the-counter oral agents. *Case reports in dentistry*.
96. Wu, J. and Wood, G. S. 2018. Analysis of the effect of gentian violet on apoptosis and proliferation in cutaneous T-cell lymphoma in an in vitro study. *JAMA dermatology*, 154(10): 1191-1198.
97. Yatome, C., Ogawa, T. and Matsui, M. 1991. Degradation of crystal violet by *Bacillus subtilis*. *Journal of Environmental Science & Health Part A*, 26(1): 75-87.
98. Yatome, C., Ogawa, T., Koga, D. and Idaka, E. 1981. Biodegradability of azo and triphenylmethane dyes by *Pseudomonas pseudomallei* 13NA. *Journal of the Society of Dyers and Colourists*, 97(4): 166-169.
99. Yatome, C., Yamada, S., Ogawa, T. and Matsui, M. 1993. Degradation of crystal violet by *Nocardia corallina*. *Applied Microbiology and Biotechnology*, 38(4): 565-569.
100. Yesilada, O. 1995. Decolorization of crystal violet by fungi. *World J. Microbiol. Biotech*, 11: 601-602
101. Young, H. H. and Hill, J. H. 1924. The treatment of septicemia and local infections: by intravenous injections of mercurochrome-220 soluble and of gentian violet. *Journal of the American Medical Association*, 82(9): 669-675.
102. Zhang, X., Zheng, Y., Fried, L. E., Du, Y., Montano, S. J., Sohn, A. and Lu, J. 2011. Disruption of the mitochondrial thioredoxin system as a cell death mechanism of cationic triphenylmethanes. *Free Radical Biology and Medicine*, 50(7): 811-820.
103. Zimmermann, T., Kulla, H. G. and Leisinger, T. 1982. Properties of purified Orange II azoreductase, the enzyme initiating azo dye degradation by *Pseudomonas* KF46. *European Journal of Biochemistry*, 129(1): 197-203.
104. Zupan, J., Garner, P. and Omari, A. A. 2004. Topical umbilical cord care at birth. *Cochrane Database of Systematic Reviews*, (3).

