

A Review of Environmental Impact of Azo Dyes

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ABSTRACT

Green plants are one of nature's factories, fixing inorganic chemicals into organic compounds through photosynthesis and other reactions. Microbes are one of the nature's most effective tools, converting organic materials—dead plants and animals—into inorganic forms through decomposition and mineralization. Together, green plants and microbes are responsible for maintaining a balance between the organic and inorganic worlds, but pollutants, such as synthetic dyes, can upset this balance. Compared with synthetic dyes, natural dyes are safer and more environmentally friendly, but they are also costlier and more difficult to apply, so they are not viable in most commercial applications. Azo dyes are low cost and have high intensity and color fastness, so they are the most frequently used class of synthetic dyes. The main threats related to the dumping of untreated azo dyes into the environment come from their primary byproducts—aromatic amines—which result from the cleavage of the central azo bonds, and which have been classified as significant carcinogenic compounds, representing a great risk to humans as well as the environment. To address this threat, the degradation of synthetic azo dyes is showing promise as an approach to treating azo-dye wastewater. In this review, the classification of azo dyes and their color mechanism is presented first, and then an overview of the research on their environmental impacts is provided, including their effects on biochemical and chemical oxygen demand, water ecosystems, plants, and crops. Finally, the legislation and guidelines on azo dyes and their byproducts are discussed.

Keywords: azo dyes; climate change; biochemical oxygen demand; chemical oxygen demand; aromatic amines

1. INTRODUCTION

Environmental pollution is the primary cause of climate change, and industries are the greatest polluters. In the textile industry, the dyeing process is responsible for the greatest amount of water pollution. This pollution can interfere with the penetration of sunlight through water, thus influencing photosynthetic organisms and the biochemical oxygen demand (BOD), with aesthetically and environmentally deleterious results [1].

Natural dyes were in use by 3,500 BC. The use of dyes in cave paintings reflects the early importance of color. Color has been associated with hierarchy, power, and leadership, is used symbolically in art, can represent a form of visual communication, and is a tool of expression [2–4]. Natural dyes have historically been obtained from mineral sources, plants—such as *Rubia tinctorum*, found in the Mediterranean, and *Paubrasilia echinata*, both of which contain anthraquinone pigments—and naphthoquinones from animals, such as those extracted from certain insect species, such as *Dactylopius coccus* [5,6].

Natural dyes have been made from mixtures of a variety of materials, including inorganic substances (e.g., water, clay, soil, minerals, metal salts, and even semi-precious stones, such as malachite) and organic materials, including animal fats and plants. Madder

root has a long tradition as a dyestuff because of its bright red color. The red uniforms of Napoleon's army and those of English soldiers in the 18th and 19th centuries were dyed with madder.

Natural dyes can be safer and more environmentally friendly than artificial dyes, but they are often more expensive to procure and more difficult to apply, so are not feasible for use in many commercial applications. In particular, typically only about 2% of the mass of natural dye sources is made up of the desired dyeing materials, so large quantities of raw material must be processed. This drives up costs and can make natural dyes unsuitable for mass production [5]. In 1856, William Henry Perkin accidentally synthesized the first synthetic dye—Mauveine [7]. Synthetic dyes replaced natural dyes over time due to their wider range of colors, cost-effectiveness, and resistance to fading by sunlight, water, or chemicals [8].

By the end of the 19th century, 10,000 synthetic dyes had been developed and manufactured. Currently, India, Eastern Europe, China, South Korea, and Taiwan together consume 600,000 tons of dye per annum [9]. About 15% of all synthetic dyes produced are discarded into the environment. This results in negative consequences, such as preventing sunlight from penetrating water bodies, which affects photosynthetic organisms and reduces the oxygen content of the water, causing metabolic stress, floral necrosis, death, and decreased faunal growth [10].

1.1 Dye Color Mechanism

Dyes possess one or more chromophores, which absorb light in the visible spectrum (400–700 nm) [11]. Chromophores contain atoms, such as nitrogen (N), oxygen (O), and sulfur (S), incorporated in azo ($-N=N-$), carbonyl ($=C=O$), sulfide ($C=S$), nitrous (NO or $N-OH$), and nitro ($-NO_2$ or $NO-OH$)

groups [12]. Chromophore groups are unsaturated and consist of heteroatoms or groups of heteroatoms in which there is a successive arrangement of single and double bonds that resonate, allowing the absorption of visible light [13].

A chromophore is colored because it absorbs light in the visible range of the spectrum at a particular wavelength. A small amount of dye in an aqueous solution can produce a vivid color, which corresponds to a high molar extinction coefficient. Color can be quantified by visible-spectrum spectrophotometry, chromatography (usually high-performance liquid chromatography), and high-performance capillary electrophoresis, (see Figure 1) [14]. Synthetic dyes, such as azo dyes, show considerable structural diversity and can therefore have substantially different chemical and physical properties [15]. Table 1 shows the main chromophores that determine the classification of synthetic dyes.

Most dyes have auxochrome groups, which are not responsible for color but do increase the intensity of the color—indeed, the meaning of the word “auxochrome” is “color enhancer.” These groups include hydroxyls ($-OH$), amines ($-NH_3$), carboxyls ($-COOH$), and sulfonates (HSO_3) [15,16]. Most chromophores are electron acceptors, whereas auxochrome groups are generally electron acceptors on the opposite side of the molecule. These groups also have the critical property of giving the compound a higher affinity to the fibers of the fabric. Another crucial part of the dye molecule is the chromogen—an aromatic structure, usually benzene, naphthalene, or anthracene. Synthetic dyes exhibit considerable structural diversity and thus possess a range of different chemical and physical properties. Azo dyes are the class of dyes most often employed on an industrial scale [25].

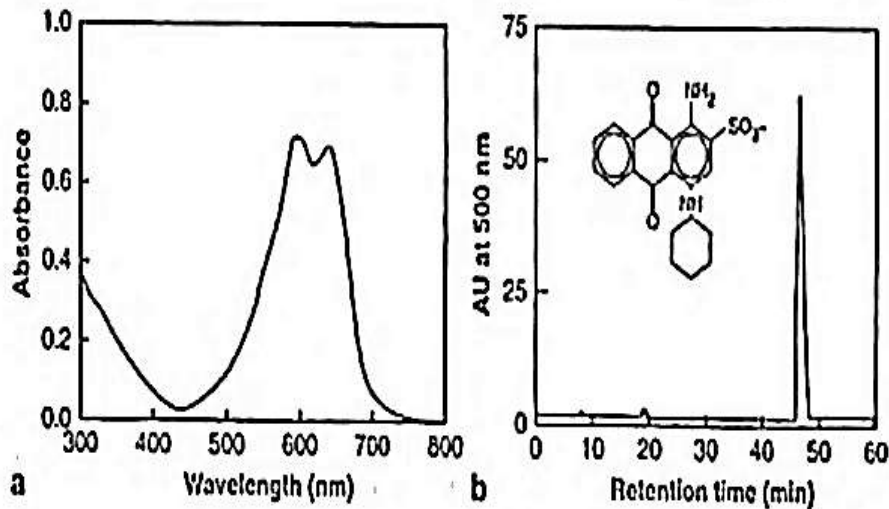


Figure 1: (a) Visible spectrum and (b) chromatogram (produced by high-performance liquid chromatography) of 1 mM Acid Blue 62.

The dyes most frequently used on an industrial scale are azo, anthraquinone, indigo, xanthene, and triarylmethane [15]. Azo dyes are the most common synthetic dyes used in the textile, tanning, pharmaceutical, cosmetics, paper, food, and

service industries [26]. Therefore, it is important to understand the severe problems associated with discharging azo dyes into the environment without treatment, and the viable alternatives for the biodegradation of azo dyes.

Table 1: Classification of synthetic dyes based on chromophore structure.

Dye Chemical Classes	Chromophore Structure	Examples of Dyes	Characteristics	Reference
Azo		Methyl Orange Congo Red Orange G Amaranth	Azo dyes are frequently used (60%). These dyes have a functional group (-N=N-) linking two alkyl or aryl radicals, symmetrical and or asymmetrical, identical or non-azofe.	[17]
Anthraquinone		Remazol Brilliant Blue R Reactive Bright Blue X-BR Reactive Blue 4 Alizarin Red S	Anthraquinone dyes are the second most widely used dyes due to their low price, accessibility and performance in the dyeing process. They have anthraquinone chromophore groups comprising two carbonyl groups on either side of a benzene ring.	[18]
Triphenylmethane		Malachite Green Crystal Violet Bromophenol Blue Light Green SP	These molecules have a central sp ³ hybridised carbon atom, bonded to three aryl groups and belong to the most commonly used synthetic dyes in the textile industry.	[19]
Nitro and Nitroso		Naphthol Yellow S Disperse Yellow 26 Disperse Yellow 14	In nitro dyes, a nitro group conjugates to an electron donor group via an aromatic system. Nitro dyes always contain a hydroxyl group as a donor.	[20]
Indigoid		Indigo Carmine Ciba Blue 2B	Synthetic indigo is the most widely used dye in the textile industry worldwide. It is highly resistant to light and high temperatures.	[21]
Xanthene		Rhodamine 6G Rhodamine 123 Fluorescein	Xanthenes are dyes used in the food, cosmetics, paper and ink manufacturing industries because of their superior dyeing and colouring properties, but are poorly biodegradable, and some of them are very toxic.	[22]
Acridine		Acridine orange Basic Yellow 9	Acridine dyes are heat-resistant, although they have low lightfastness. They are currently not very important commercially.	[23]
Phthalein		o-cresolphthalein Thymolphthalein Disylenolphthalein Phenolphthalein	Phthalein dyes are employed to titrate weak acids. Phthalein dyes are insoluble in water but soluble in alcohol. There are frequently in the construction, coatings, electronics and electrical industries.	[24]

1.2 Azo Dyes

Azo dyes have the lowest cost, highest intensity, and greatest color fastness of all synthetic dyes [27], and are the most frequently used class (making up 60% of the market), due to their dyeing performance, easy accessibility, and low cost [28]. Appropriate dye selection depends on the affinity and bond stability of the dye with the fiber, as well as the dye's diffusion, reactivity, cost, and fixing characteristics [27]. In the paper industry, a significant amount of wastewater is associated with handcrafted paper dyeing [29].

The pharmaceutical industry also uses food-approved colors, based on the Codex Alimentarius Commission standards [30,31]. Therefore, it is common to find synthetic dyes in pharmaceutical products, including Amaranth (E123), tartrazine (E102), azorubine/carmoisine (E122), Sunset Yellow (E110), Ponceau 4R (E124), Bright Blue (E133), and Allura Red (E129). The structures of some azo dyes are shown in Table 2 [31].

Synthetic azo dyes are organic chemical compounds that can be applied in different

fields. Characterized by their stability and chemical versatility, high fixation, and resistance to light and moisture, all of which directly impact their ability to be degraded, they represent a serious environmental issue [32,33].

The compounds of the synthetic azo group are chemically represented as R-N=N-R', where -N=N- is the chromophore group referred to as azo. According to the classification scheme of the International Union of Pure and Applied Chemistry, azo dye compounds are derivatives of diazine (HN=NH) with replacement of the hydrogen (H) atoms by azobenzene, hydrocarbyl, or diphenyldiazene groups. They may contain one to three azo bonds linking phenyl and naphthyl rings, which may be substituted by chloro, amino, nitro, and/or hydroxyl groups. Azo dyes are characterized by their intense coloration, and it is estimated that they account for two-thirds of the synthetic dyes produced today. They are the class of commercial organic dyes with the most structural diversity and the broadest range of use [34,35].

Table 2: Classification of azo dyes based on number of azo linkages.

Chromophore	Azo dye	Chemical structure
Monoazo	Methyl Orange	
Diazo	Red Ponceau S	
Triazo	Direct Blue 71	
Poliazo	Direct Red 80	
Naphthol	Naphthol Yellow S	
Azo lakes	Lithol Rubine BK	
Benzimidazolone	Benzimidazolone Yellow H3G	

The risks posed by the disposal of untreated azo dyes in the environment are mostly associated with their primary byproducts, aromatic amines, which result from cleavage of the main azo bonds. These have been classified as significant carcinogens, representing a great risk to human health [36]. Therefore, studies have focused on finding methods to treat industrial synthetic dye effluents, especially those containing azo dyes. The proposed solutions have included biological, chemical, and physical processes, with a particular focus on bioremediation, due to its relatively low cost and high effectiveness in decoloring azo dyes in contaminated environments. A number of different microorganisms, including bacteria, have been proposed as biotechnological solutions [37].

Azo dyes cause harmful effects on ecosystems, even in low concentrations [38]. In particular, dumping dyes directly into water bodies without prior treatment limits the transmittance of sunlight [39]. The resulting high BOD and chemical oxygen demand (COD) inhibits photosynthesis and affects plant growth. Synthetic azo dyes are recalcitrant, bioaccumulative, toxic, mutagenic, and carcinogenic [40].

1.3 Impacts on Crops and Plant Growth

Azo dyes are a subject of much concern because of the range of health and environmental risks they pose [41–43]. The contribution of each toxic component to the total toxicity in any environment varies based on its concentration and its dispersion through that environment, which then affects the diversity of that living environment [44]. Living organisms are exposed to possible genotoxic environmental agents at the cellular and molecular levels. Studies on genotoxic potential are vital in being able to predict the impact of specific agents on faunas and floras and, consequently, human beings [45,46]. Wang and Keturi reported that percentage seed germination and root:shoot ratios can be used as reliable, rapid, simple,

and reproducible techniques for investigating the damage caused by azo dyes and toxic compounds in polluted wastewater reservoirs [47,48]. Umesh et al. evaluated the toxicity of synthetic azo dyes on the germination and root/shoot elongation of five plant seeds [49]. Phytotoxicity bioassays can be used as efficient toxicity tests for synthetic azo dyes and also to monitor environmental contamination.

Dye- and mineral-uptake processes and accumulation by crops and other plants depend on certain factors, including the concentration of the dye in water, the metals available in the soil, their solubility indices, and the plant species [50]. Leafy vegetables show greater uptake and accumulation rates of heavy metals than other plants. This has been attributed to the higher rates of transpiration and translocation in leafy vegetables compared to non-leafy vegetables, with the movement of metals from root to stem and then to fruit in the latter taking more time and resulting in lower accumulation rates [51,52]. Oguntade et al. determined the percentage growth, dry matter, and heavy-metal uptake in potted *Amaranthus cruentus* L. for different azo-dye concentrations [53]. They found that at lower azo-dye concentrations, the nutrient components positively impacted the *Amaranthus* growth rate and yield, although the heavy-metal-content ratio and their degraded products accumulated in the edible shoots, whereas a high concentration of azo-dye solution retarded both the growth and yield rates. Bioaccumulated heavy metals and organic azo-dye molecules were found to be more concentrated in the edible shoots than in the roots, with concentrations of zinc (Zn), manganese (Mn), and (iron) Fe in the edible shoots being much higher than the international permissible limits for human consumption provided by the World Health Organization, the Nigerian Federal Environmental Protection Agency, and the UN Food and Agriculture Organization. These limits are based on the health risks attached to regularly consuming vegetables

contaminated with dye effluent. In addition, Savin and Butnaru found that the toxic nature of reactive azo dyes can kill soil microorganisms, damaging agricultural productivity [54]. Thus, industrial effluent pretreatment and detoxification are required when water for crop and vegetable production is taken directly from wastewater.

1.4 Impact on Water Ecosystems

Waste from azo dyes is one of the most important sources of water pollution and has been reported as one of the leading causes of environmental problems. Azo-dye effluent has been shown to cause significant physiological disorders (including intermittent fever, hypertension, renal damage, and cramps) when consumed by aquatic organisms [55]. The accumulation of organic and inorganic toxicants in aquatic environments depends on their persistence in the water ecosystem and the food chain, and their physiological properties. Aquatic vertebrates, such as fish and tadpoles, live in intimate contact with the aquatic ecosystem through their gills—the primary organs for respiration, acid–base balance, and osmoregulation—making them highly susceptible to aquatic pollutants. If the pollution reaches high levels, physiological malfunctions in these organs can cause homeostatic disorders that can result in poor overall life performance [56].

Azo dyes in water ecosystems have an inhibitory effect on the aquatic biota and affect the photosynthesis of green biome species. Despite being nondegradable in nature, synthetic azo dyes are broken down anaerobically in sediments, producing toxic amines [41,56,57]. Karthikeyan et al. examined the impact of synthetic azo effluents on a proteinous freshwater fish, *Mastacembelus armatus*, by monitoring changes in the ionic regulation of selected tissues (liver, kidney, and muscle) before and after treatment with sublethal concentrations of Acid Blue 92, a reactive dye, for 35 days [58]. It was reported that the concentrations of sodium (Na^+) and

chloride (Cl^-) ions declined, while the concentrations of potassium (K^+) and calcium (Ca^{2+}) ions increased significantly, with magnesium (Mg^{2+}) ion concentrations increasing slightly. These results are evidence that textile effluents affect ionic regulation in fish.

A toxicological study comparing the impact of treated and untreated textile-dye wastewater on the freshwater fish *Gambusia affinis* was carried out by Soni et al. [59]. It was found that treatment markedly reduced mortality rates and the cytotoxic effects on red blood cells, including a reduction in the incidence and extent of poikilocytosis, in which red blood cells develop abnormal shapes. Following on from this work, Selvaraj et al. studied the toxicological and histopathological impacts of synthetic textile-dye effluent on teleost fish (*Poecilia reticulata*) [60]. The dye effluent caused abnormal behaviors, including rapid opercular movement, hyperexcitation, and erratic swimming, as well as a thickening of the mucus covering. Histopathological changes were also detected in the study, including enlargement of the primary gill bar, detachment of the secondary gill bar, deterioration of the intestinal villi, and significant infiltration of the hemocytes inside the internal lumen.

Spirulina platensis is a filamentous cyanobacterium that is sometimes used as a food supplement due to its high nutritional value. Cyanobacteria are particularly sensitive to contaminants. Azo dyes significantly affect several of their biochemical parameters, such as growth, their protein, mineral, and pigment contents, and other nutrients. However, different types of azo dyes have significantly different effects on cyanobacteria, depending on their toxicity, reactivity, and functional moiety. De Sousa et al. evaluated the effect of simulated textile azo-dye effluent (specifically, the dye Remazol Red Brilliant) on phytoplankton using Winogradsky columns containing a micro-ecosystem consisting of soil (taken from a river bed), water, and necessary nutrients

[61]. The presence of Remazol Red Brilliant dye was found to impede photosynthesis and cause ecological imbalance in the food chain. Consequently, water containing high concentrations of industrial azo dyes has a negative impact on phytoplankton, as well as other microorganisms in aquatic ecosystems.

1.5 Environmental Impact of Synthetic Azo Dyes

It is a commonly agreed fact that environmental pollution is one of the greater challenges faced by humanity; the textile industry is a significant polluter due to the high amounts of effluent pollutants discharged into water bodies. The persistent colour and the extreme biological oxygen demand (BOD) have been proven to be environmentally unappealing and aesthetically unattractive.

The largest producer of colored wastewater is the textile industry [62]. Approximately 20% of the synthetic dye used in dyeing textile fibers is not fixed to the fibers and is disposed of in wastewater [63], resulting in large amounts of pollution [63], as the remaining azo dyes which are unbound to fibers or fabrics ultimately find their way into wastewater streams. The biodegradation of azo dyes is incredibly tough because of their complex structure and synthetic nature.

Azo dyes in general have toxic effects, including mutagenicity (capacity to induce mutations), cytotoxicity (the quality a substance has of being toxic or poisonous), and carcinogenicity (capacity to cause cancer). However, perhaps the greatest long-term human health risk of textile dyes is their genotoxicity (capacity to cause DNA or chromosomal damage).

Not all textile processing plants treat their wastewater to break down the azo dyes, with the consequence that significant amounts of untreated effluents are directly discharged into numerous water bodies, thereby “posing serious ecotoxicological threats as well as toxic effects on living organisms”. Additionally, dyes, heavy

metals and electrolytes are not easily removed by conventional wastewater systems.

The discharge of untreated textile effluents is an aesthetic nuisance. However, more significantly it leaves a trail of environmental issues: it reduces the reoxygenation capacity of water bodies, it compromises the ability of sunlight to penetrate water thereby disrupting the photosynthetic capacity of aquatic plants, it increases oxygen-demanding substances (both chemical and biological) and solid particles in water bodies, and finally, under the appropriate conditions it leads to the generation of aromatic amines which are considered to be carcinogenic agents. Some of the human health risks to from “sporadic and excessive exposure to coloured effluents” as immunological, neurological and circulatory disorders, irritation to lung edema, infections of eye and skin and allergy problems are summarized (summary of research from Anliker’s *Toxic hazard assessment of chemicals*, 1986).

However, the environmental damage does not depend only on the quantity of the disposed dye; it also depends on the composition of the dye mixture, with various common constituents having toxic characteristics [64]. Colored wastewater from synthetic dyes sometimes contains dye that is visible to the naked eye (<1 ppm) [65,66]. When discharged into surface water or groundwater bodies, it causes a decrease in the concentration of dissolved oxygen in the water and an increase in the values of physicochemical and biological parameters, such as COD [67], BOD, total dissolved solids, total N, total phosphorus (P), and nonbiodegradable organic compounds. Such wastewater can possess a wide range of pH values and concentrations of heavy metals, such as chromium (Cr), arsenic (As), and Zn [68].

In general, synthetic azo dyes are not biodegradable due to their chemical properties and structure, and most being recalcitrant, carcinogenic, and toxic, resulting in long-lasting adverse effects on

the environment [69]. The adverse impacts of dyes can be biomagnified, resulting in high contamination rates at high trophic levels. However, the toxicity of each synthetic dye must be assessed individually because the damage they can cause depends on the structure and exposure concentration [70]. Some synthetic dyes can last for a long time (50 years or more) in the environment [15]. Their persistence is directly related to their chemical reactivity. Unsaturated compounds are less persistent than saturated ones due to their reactivity. The persistence of aromatic compounds significantly increases as the number of halogen and chemical substitutions increases; the same is true of the persistence of synthetic azo dyes [68]. Most synthetic dyes in use belong to the azo, anthraquinone, or triarylmethane classes [68,71].

The uses and negative effects of synthetic azo dyes have been widely studied. Between 60% and 70% are toxic, carcinogenic, and resistant to conventional physicochemical treatments [68]. Their toxicity is due to the way they chemically degrade, forming aromatic amines, such as dimethoxybenzidine, benzidine, and dimethylbenzidine. The toxicity of aromatic amines is associated with their metabolic oxidation, which generates electrophilic reductive intermediaries (diazonium salts) that bond covalently to DNA. These compounds are mutagenic and can cause diseases such as cancer. A variation in this mechanism is caused by the chemical reduction of some of the azo bonds to their corresponding toxic aromatic mono-azo amines [72].

When azo-ionic dyes are discarded into surface water or wastewater, they can bind to suspended organic matter by electrostatic interactions and adhere to sediments or wastewater sludge, thereby increasing their persistence [73]. Colored water and contaminated sludge can be taken up by aquatic animals, transferring the hazardous compounds through the food chain to humans, causing various health disorders, such as hypertension, cramps, nausea, bleeding, and ulceration of the skin or

mucous membranes. Exposure to azo dyes may cause significant damage to the kidneys, reproductive system, liver, brain, and central nervous system [72]. Parrot et al. assessed the effects of different concentrations of azo dyes on the fat-headed fish *Pimephales promelas* in the embryonic (larval) stage. They found that 25.4 and 16.7 mg L⁻¹ concentrations of the synthetic azo dyes Disperse Yellow 7 and Sudan Red G, respectively, decreased larvae survival, with death occurring between four and 10 days after hatching [74].

1.6 Legislation on the Use and Disposal of Dyes

In the European Union, there are strict regulations on the use of azo dyes in consumer products, from cosmetics, clothing, and toys to food. The EU regulation “Registration, Evaluation, Authorization, and Restriction of Chemicals” (REACH) lists 24 different types of aromatic amines considered to be hazardous and toxic to human life and forbids the use of synthetic azo dyes that produce 30 mg kg⁻¹ or more of these amines in products that may have direct and prolonged contact with human skin. The REACH regulation also lists other colorants that are prohibited for use beyond concentrations of 0.1% of the product’s total weight.

European Norm 71 deals with this class of colorants in toys. The regulations (EC) 1223/2009 and (EU) 10/2011 deal with the use of benzidine and o-dianisidine in cosmetic products, and in plastic products that come into contact with food components—the release of primary aromatic amines into the food must not exceed the limit of 0.01 mg kg⁻¹. Several countries such as Germany, the Czech Republic, and Switzerland [75], have regulations that place limits on the levels of azo dyes and released aromatic amines allowed.

In the USA, there is no specific law restricting the use of azo dyes. However, there are restrictions on the aromatic amines

released from azo dyes. Several states have their own laws restricting the use of hazardous chemicals in certain products. Some laws that place limits on aromatic amines from azo dyes are Washington's Children's Safe Product Act, California's Proposition 65, and Vermont's Act 188 [76].

In Asia, India was the first country to regulate chemicals, including azo dyes, in 1997, with regulations covering 112 synthetic dyes, including azo dyes. The list of prohibited aromatic amines is the same as the list in REACH Regulation sheet 1907/2006. Other countries have issued their own regulations, which include restrictions on azo dyes and the accepted levels of aromatic amines, including China (2005), South Korea (2010), Taiwan (2011), and Egypt (2012). In 2014, Japan joined this list when it designated azo dyes as hazardous substances, restricting 24 aromatic amines originating from azo dyes in all industries, including textiles, leather, and fur manufacturing, to concentrations below 30 mg kg⁻¹. These concentration limits were also applied to 22 aromatic amines in the regulations of Vietnam [77–80].

Other countries, such as Australia, Canada, France, Turkey, Brazil, Pakistan, Malaysia, and Morocco, have also regulated the impacts of industrial synthetic dyes [81].

1.7 Synthetic Azo-Dye Regulations and Toxicological Prospects

The toxic character and adverse effects of synthetic azo dyes and pigments have been considered by scientific committees worldwide, which have concluded that these effects arise not only from the toxicity of the dyes themselves, but also from certain mordants that have to be used with them [82]. There are no rigid legal restrictions on synthetic dyes in any country. Some bodies, such as the International Association for Research and Testing in the Field of Textile and Leather Ecology—a union of 18 independent textile research centers and testing institutes in Europe and Japan and

their worldwide representative offices [83]—provide textile manufacturers with certification of textile products “from the thread to the end product” by including tests for prohibited azo dyes among a suite of tests in their certification process. Other authorities responsible for defining and maintaining specifications and safety assessments for different applications include the European Commission in the EU, the Food and Drug Administration in the US, the Ministry of Health, Labor, and Welfare in Japan, and the Government of Canada [83]. The European Commission has formulated a working document concerning limitations on the preparation, marketing, and use of hazardous substances, including synthetic azo dyes. Following recommendations by the European Parliament and the European Council, there are 22 aromatic amines prohibited by Directive 2002/61/EC [84]. In addition, REACH gives detailed explanatory notes relating to dyes, based on several reports using specified testing methods [85].

The European Integrated Pollution Prevention and Control Bureau has estimated that the textile industry releases more than 0.2 million tonnes of salts into the environment annually [86]. Several synthetic azo dyes and their intermediates have been reported by the International Agency for Research on Cancer as being categorized as known, probable, and possible (Groups 1, 2A, and 2B, respectively) human carcinogens [87,88].

The Government of India restricted the handling of 42 benzidine-based dyes in 1993, and the Ministry of Environment and Forests prohibited the use of more than 70 synthetic azo dyes in 1997 under the provisions of the Environment Protection Act 1986 [89,90].

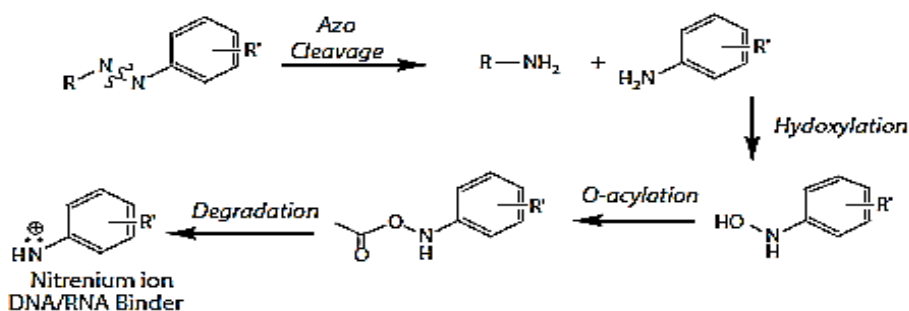
Azo dyes are the most important textile colorants and represent the greatest volume of dye chemistry, in large part because of their solubility. Upon degradation, azo dyes produce aromatic amines and their amino counterparts. It is well known that when soluble azo dyes enter the human body, they

split into their corresponding aromatic amines via azo-bond breakdown by enzymes in the internal organs and by intestinal microbes [91,92].

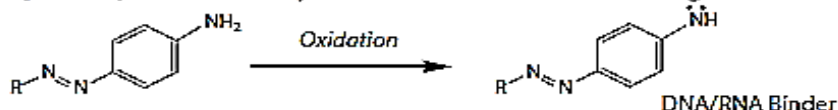
Some synthetic azo dyes can produce arylamines, which are suspected of having carcinogenic effects. As illustrated in Scheme 1, aromatic amines are formed,

followed by nitrenium ions are formed through the oxidation of a free aromatic amine group that is part of the azo dye structure. The resulting species have been found to have enough capacity to bind covalently to DNA or RNA, and thus can cause mutagenic reactions and severe toxicity [93,94].

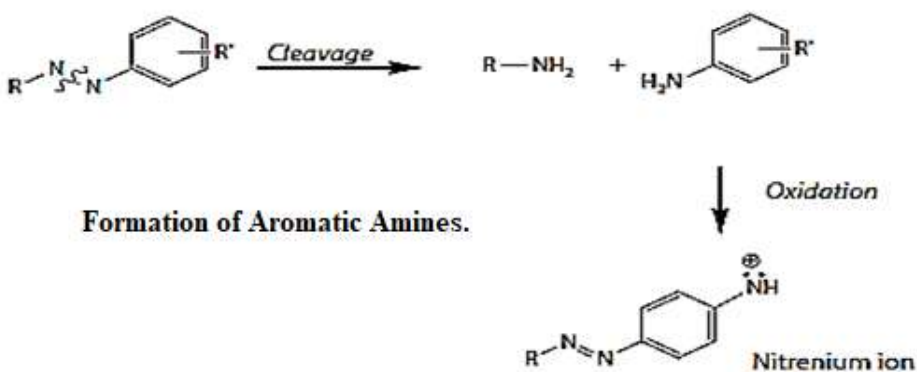
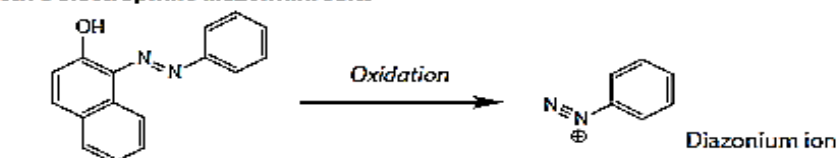
Pathway-I: formation of aromatic amines followed by nitrenium ion



Pathway-II: formation of reactive electrophilic species through oxidation of a free aromatic amine group that is part of the azo dye structure



Pathway-III: Activation of the azo dyes via direct oxidation of the azo linkage to highly reactive electrophilic diazonium salts



Scheme 1: Representation of azo-bond cleavage through the metabolic activation of azo dyes.

1.8 Azo-dye Removal Methods

Different treatment methods have been demonstrated for the removal of azo dyes because of their toxicity to aquatic life and carcinogenic impacts to humans, including

chemical, physical, biological, and hybrid approaches [96]. However, chemical and physical treatments have the disadvantage of generating sludge that is also difficult and expensive to handle, and requires large

treatment facilities [95]. By contrast, the biological degradation of synthetic dyes has been shown to be cheap and environmentally friendly, and it generates less sludge [98,99]. Microorganisms such as fungi, bacteria, yeasts, and algae can degrade and even completely mineralize azo dyes [100,101]. White-rot fungi are the most efficient at breaking down synthetic dyes because they produce enzymes that catalyze dye removal and degradation reactions [102]. Azo-dye removal efficiency is controlled by pH, nutrient load, treatment time, aeration, the carbon (C):N ratio, the biomass morphology, the inoculum concentration, co-substrate additions, and the presence of salts [97,103]. The production of toxic byproducts after primary cleavage should be considered because some phenolic compounds inhibit fungal and bacterial growth during bioremediation processes [104]. Enzymes are an alternative method that could reduce industrial azo-dye pollution and its environmental impacts. Any recommended methods for the degradation of azo dyes need to meet the international guidelines on the concentration of azo dyes and their byproducts in the environment.

Physico-chemical treatment methods

Physico-chemical treatment of wastewater is relatively easy to perform; however, it is associated with numerous drawbacks such as high cost, low efficiency, limited versatility, and even interference by other wastewater elements. Physico-chemical methods are not single methods but rather multistage treatment processes with lengthy retention times. The high quantity of by-products and sludge generated by the processes necessitates greater energy demand.

While physical methods can remove colour effectively, the dyes themselves are not fully broken down, and essentially become concentrated and in need of further disposal. Chemical methods on the other hand may remove the dyes through coagulation as well as flocculation techniques but they lead to

the generation of concentrated sludge. It is also possible, indeed likely, that other environmental problems may arise due to the interaction of numerous chemicals. Unfortunately chemical processes are limited by issues such as “dewatering, pH modification, high levels of residues associated in the supernatant, cost towards precipitation, sludge generation and its disposal” [87].

Biological treatment methods

Biological methods of treatment involve the breaking down of dyes through biological methods and have a number of advantages over physico-chemical methods. They are based primarily on adsorption by microbial biomass (living or dead) or application of enzymes for biodegradation of dyes. They are relatively easy to utilize, they use fewer chemical mixtures, they generate less sludge and are less energy-intensive. Additionally, the by-products of the bioremediation process are less toxic inorganic compounds. The main objective of biological treatment approaches is to transform objectionable organic dyes into non-toxic compounds.

The catabolism of the azo dyes takes place in two steps, firstly the dyes underwent through the breaking of the azo bonds forming the amines, and secondly the aromatic amines are further catabolized to small nontoxic molecules under aerobic environment. The idea is to rely on the ability of bacteria to operate in both aerobic and anaerobic conditions to complete the breakdown of azo bonds formed within the dyes. The products of aerobic treatment are biomass, carbon dioxide, and water while anaerobic treatment generates methane as a by-product. Point out that adsorption and degradation are the two main processes used for dye decolorization treatment of textile wastewater, and these processes occur under either aerobic or anaerobic conditions. [105]

CONCLUSIONS

The advent of synthetic dyes has revolutionized the color industry, and due to their low cost, high intensity, and color

fastness, their use has spread to many industrial sectors. However, azo dyes result in harmful environmental effects and contribute considerably to climate change phenomena even at low concentrations. It is known that dumping azo dyes into water bodies decreases the transmittance of sunlight and increases the BOD and COD, which prevents photosynthesis and inhibits plant growth. The dumping of untreated azo dyes into the environment results in the presence of aromatic amines (through the cleavage of their central azo bonds), which are recalcitrant, bioaccumulative, toxic, mutagenic, and carcinogenic. Hybrid chemical, physical, and biological treatment approaches should be employed to degrade azo dyes and their byproducts according to the legislation and guidelines published by governments and international organizations, such as the European Commission in the EU, the Food and Drug Administration in the USA, the Ministry of Health, Labor, and Welfare in Japan, and the Government of Canada.

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