



Book of The First Scientific Conference of the Faculty of Sciences

"Environmental Pollution and Challenges of Environmental Sustainability"

(Reconciliation with the Environment)

Sirte, October, 14, 2025



Edited by:

Prof. Abdussalam Salhin Mohamed.

Prof. Hisain Masoud Abomadina.

Dr. Ashraf Mustafa Abusenaina.

Dr. Fathia Abdulljawad Mosa

Publications of Research and Consultation Center– Sirte University

First Edition 2025

The First Scientific Conference of The Faculty of Science – Sirte University “Environmental Pollution and the Challenges of Environmental Sustainability”

EPESC October 14, 2025

Part One: Dedicated to papers
submitted in the English language

Conference Committees

Higher Committee

Prof. Dr. Sulayman M. Alshatir	University President
Prof. Dr. Eltayeb M. Elgobbi	University Vice President
Dr. Ashraf M. Abusenaina	Dean of the Faculty

Scientific Committee

Prof. Dr. Abdussalam Salhin Mohamed	Faculty of Science / Sirte University	Head
Prof. Dr. Hamid M. Younis Ahmed	Faculty of Science / Sirte University	Member
Prof. Dr. Ramadan Abdel-Mawla Al-Hindawi	Faculty of Science / Omar Al-Mukhtar	Member
Prof. Dr. Tahani Younis Omar	Faculty of Science / Sirte University	Member
Dr. Fathia A. Mosa	Faculty of Science / Sirte University	Member

Preparatory Committee

Ms. Zainab Ragab Al-Fitouri	Head
Mr. Ali M. Naji	Member
Mr. Ali A. Alghazali	Member
Dr. Souad A. Abumaryam	Member
Abdulhalim M. Alshatir	Member
Khaled J. Amhalhal	Member
Sofian S. Alshaali	Member
Ali M. Almaedani	Member
Yousuf M. Alkirrami	Member

Editorial Team:

Prof. Dr. Abdussalam Salhin Mohamed
Prof. Dr. Hussein Massoud Abu Madina
Dr. Ashraf M. Abusenaina
Dr. Fathia A. Mosa

Contact Email: epesc@su.edu.ly

Conference Website:

<https://su.edu.ly/research/index.php/conferences/science/sc1>

Contents

1. The Impact of Environmental Awareness on the Economy and Sustainability Development	1
2. Socio-Economic Impacts of Wastewater Pollution on the Agricultural Activities in Wadi El-Shees, Al-Bayda, Libya.....	8
3. Impacts of Renewable Energy Sources on Biological Diversity and Conservation, An Overview.....	12
4. A Comparative Study of The Presence of Different Pieces of Marine Litter on Five Different Beaches on Western Coast of Libya.....	31
5. A Review Study on Designing a Modern, Environmentally Friendly Landfill Using a Lining System.....	39
6. Ecological and Biological Impacts of Azo Compounds: A Concise Review of Toxicity, Degradation, and Sustainable Solutions	65
7. Oil pollution, its occurrence, origin on the Libyan coast, and its impact on the marine environment	83
8. <i>Effect of Extending Contact Time on Efficiency of Fava Beans Pods as Bioadsorbents to Remove Lead Ions from Aquatic Solutions.....</i>	<i>100</i>
9. Some growth traits of the Atlantic lizardfish <i>Synodus saurus</i> in the Southern Mediterranean Sea	108
10. Design of an Eco-Friendly Tandem Solar Cell Based on Lead-Free Perovskite (MASnI ₃)/Silicon with Enhanced Performance via SCAPS-1D	122
11. :Microplastic Pollution in the Coastal Sediments of Derna, Libya Environmental Assessment and Student Awareness	137

12. Investigation of Solvent Effects on Spectroscopic and Photochemical Properties of Ruthenium (II) Polypyridyl Complexes.....	145
13. <i>Highly Green Synthesis of Pure ZnO Nanoparticles as a Solid Oxidant or Enhanced Photodegradation of MB as Organic Contaminants.....</i>	158
14. Effect of seasonal variations on testes and serum protein expression of Desert Tortoise (<i>Testudo graeca cyrenaica</i> Linnaeus, 1758).....	166
15. Extraction and Spectrophotometric Analysis of Nicotine in Natural and Processed Products Available in the Libyan Market.....	171
16. The Potential of Using Wild Giant Common Reed Plant (<i>Phragmites australis</i>) as a Source for Biodegradable Plastic.....	179
17. Seasonal Environmental Variability Drives Physiological Adaptations in the Thick-Lipped Grey Mullet (<i>Chelon labrosus</i>) of the Southern Mediterranean Sea	188
18. <i>Green synthesis of AgNPs from Viburnum tinus plant and some biological applications on the extracts.....</i>	201
19. Urinary Tract Infections and Antimicrobial Resistance: Analysis of Pathogen Prevalence and Treatment Efficacy at Al Manar Laboratory in Derna City	212



The First Scientific Conference of the
Faculty of Science – Sirte University
Environmental Pollution and Challenges
of Environmental Sustainability (EPESC),
Oct. 14, 2025



Ecological and Biological Impacts of Azo Compounds: A Concise Review of Toxicity, Degradation, and Sustainable Solutions

Fathia A. Mosa*, and Warda H. Mohammed

Department of Chemistry, Faculty of science, Sirte University, Sirte, Libya.

 Corresponding Author Email: fathia@su.edu.ly

Article History:

Received: 23 April 2025

Revised: 22 May 2025

Published: 15 November 2025

Keywords:

Azo dyes toxicity;
biodegradation;
environmental
sustainability;
regulatory policies;
remediation
strategies.

Abstract: Azo compounds, distinguished by the $-N=N-$ group, are widely used in dyes, pharmaceuticals, and agrochemicals for their vibrant colors and versatility. However, their industrial use poses environmental and health risks due to degradation into carcinogenic aromatic amines. This review explores their toxicity, degradation pathways, and sustainable remediation strategies.

The study outlines acute and chronic health effects, including skin irritation and carcinogenicity, with benzidine and 2-naphthylamine as key hazards. Environmental persistence disrupts aquatic ecosystems and contaminates water resources. While regulations like EU REACH and FDA bans exist, enforcement gaps persist.

Emerging remediation technologies, including eco-remediation, plasma oxidation, and nanocellulose adsorbents, show promise for efficient dye removal, while green chemistry approaches and digital monitoring tools offer sustainable solutions. The review underscores the need for further research to optimize the safety and efficacy of azo compounds, balancing their industrial benefits with environmental and health protections. This work provides valuable insights for policymakers, researchers, and industries striving to address the challenges posed by azo dye pollution.

1. Introduction

Azo dyes are the most widely manufactured type of synthetic dyes, playing a pivotal role in the textile and printing industries. These dyes are synthesized through *diazotization* and *coupling* reactions, with various techniques employed to optimize color properties and dye particle size for enhanced dispersibility (Shankarling et al., 2017). Accounting for approximately **70% of global synthetic dye production**, azo dyes dominate

the market (Berradi et al., 2019). Commonly used in industries such as **food, textiles, leather, and polyamide fiber production**, azo dyes include variants like Acid Red 37 (AR-37) (Elshaarawy et al., 2017). Their defining feature is the **azo group (-N=N-)**, which connects symmetric or asymmetric alkyl or aryl radicals (Fig. 1). This unique structure enables a broad spectrum of colors and versatile industrial applications (Marinescu et al., 2022).

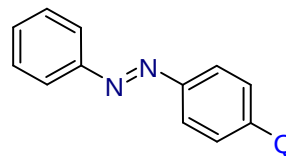


Figure 1. An azo compound
(Di Martino et al., 2022).

Azo compounds are vibrant chemical compounds synthesized through the **diazotization of primary aromatic amines**, followed by coupling with heterocyclic systems such as benzothiazole, pyrazole, thiazole, and thiophene (Maliyappa et al., 2019). These compounds are classified in the **Colour Index (C.I.)**, updated since 1924, based on their composition, provenance, color, and application. Representative azo dyes include **Methyl Orange, Acid Orange 7, Congo Red, Direct Blue 160, Amido Black, Remazol Brilliant Orange 3R, and Amaranth** (Rauf et al., 2011).

Aromatic and heteroaromatic azo dyes are among the most diverse synthetic dyes, serving as colorants in textiles and industrial applications such as optical data storage, non-linear optics, and biomedical research (Pavlović et al., 2009). Their widespread use extends to textiles, leather, paper, food, cosmetics, solar cells, photodynamic therapy, and laser technology (Mohamed-Smati et al., 2020). First discovered by Peter Griess in 1858, azo dyes have evolved significantly—from the early adoption of Congo Red in 1884 to today's more heat- and light-stable synthetic variants (El-Sayed et al., 2024).

2. Classification of azo dyes based on molecular structure

Azo dyes are categorized according to the number of **azo (-N=N-) linkages** in their molecular structure, including:

- **Monoazo** (1 azo group)
- **Disazo** (2 azo groups)
- **Trisazo** (3 azo groups)
- **Polyazo** (4+ azo groups)
- **Azole dyes** (complex structures)

The **Color Index (C.I.) system**, developed by the Society of Dyers and Colourists (SDC) and the American Association of Textile Chemists and Colorists (AATCC), assigns unique identifiers (**C.I. numbers 11,000–39,999**) to azo dyes based on their chemical composition and structural features (Benkhaya et al., 2020). This system standardizes dye classification for industrial and research applications, ensuring consistent identification (Table 1).

Table 1. Classification of azo dyes in the color index.

Chemical Group	C.I. Number Range	Chemical Group	C.I. Number Range
Monoazo	11,000–19,999	Polyazo	35,000–36,999
Bisazo	20,000–29,999	Azoic	37,000–39,999
Trisazo	30,000–34,999		

The **number of azo groups** (Fig. 2) directly influences a dye's:

- **Color intensity**
- **Chemical stability**
- **Industrial suitability**

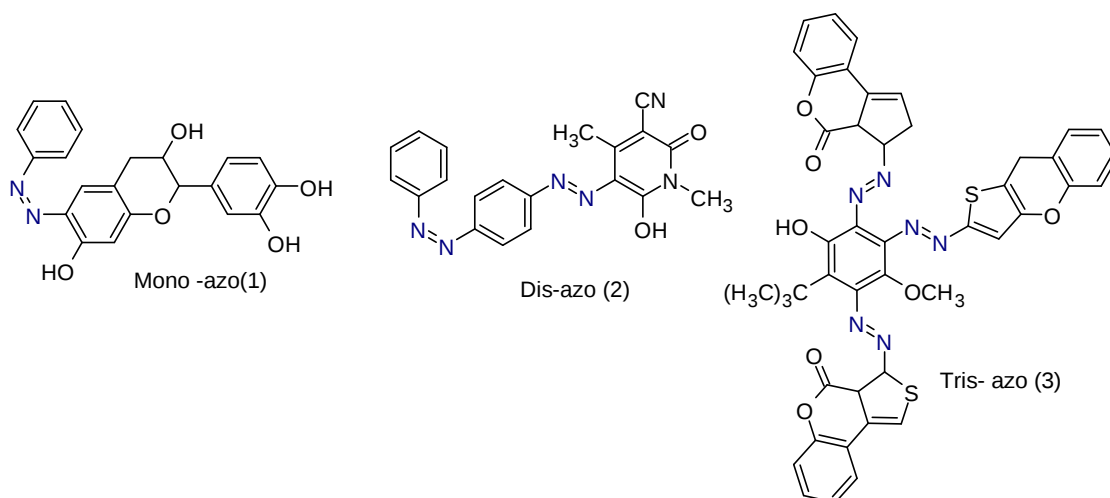


Figure 2. Molecular Structures of Monoazo (1), Disazo (2), and Trisazo (3) Dyes (Mezgebe & Mulugeta, 2022).

3. Analytical and Industrial Significance of Azo Compounds

Azo compounds are of exceptional significance in both analytical and industrial applications due to their strong complexation abilities with metal ions, high sensitivity as chromogenic reagents, and versatile use in spectrophotometric and extractive photometric analysis. These compounds are highly effective as indicators in complexometric titrations, owing to their ability to form stable complexes with metal ions. In spectrophotometry, azo compounds are particularly valuable because their vivid color production, comparable to that of many organic reagents, makes them ideal for detecting metal ions and separating transition metals, which tend to form stable chelates. Their broad applicability in these fields further emphasizes their importance in both research and industrial settings (Eltaboni et al., 2022).

4. Solubility Challenges and Solutions

Azo-metal complexes often face solubility challenges due to their limited aqueous solubility. To overcome this, these complexes typically require organic solvent systems for effective extraction. Additionally, specialized techniques such as co-precipitation and solid-phase extraction are employed to enhance their solubility and improve their analytical utility. These methods play a crucial role in both qualitative and quantitative metal ion determination, significantly expanding the applications of azo compounds in analytical chemistry (Baliza et al., 2009).

5. Pharmaceutical and Food Applications

Azo dyes play a crucial role in pharmaceutical formulations by improving the visual appeal and product identification of drugs, enhancing the stability of drug preparations, and serving as excipients in various dosage forms such as tablets, soft gelatin capsules, ointments, and liquid solutions (Dan et al., 2018). Despite their utility, studies have shown that certain food dyes can interfere with biochemical analyses and alter the histological appearance of liver

and kidney tissues, underscoring the need for comprehensive safety evaluations of these compounds. The multifunctionality of azo compounds extends beyond pharmaceuticals to fields such as analytical chemistry, where they are used for metal ion detection, and food technology, where they enhance color. The multifunctionality of azo compounds spans:

1. **Analytical chemistry** (metal ion detection)
2. **Pharmaceutical industry** (drug formulation)
3. **Food technology** (color enhancement)

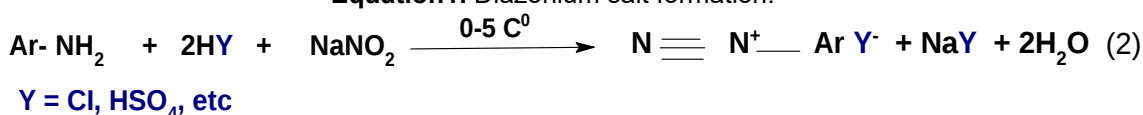
Their unique properties and wide-ranging applications continue to drive innovation across multiple scientific and industrial domains.

6. Synthesis

To make azo compounds, several steps must be taken. These reactions can be divided into several groups, including azo group synthesis, diazotization, and coupling, nitro compound condensation with amines, nitro compound reduction, and amino compound oxidation.

6.1 Diazotization and Coupling: Primary aromatic amines are diazotized by reacting with nitrites, particularly sodium nitrite, in an aqueous mineral acid solution at temperatures that are very close to absolute zero. The amine is converted into the equivalent diazonium salt in this reaction (Equation 1).

Equation 1. Diazonium salt formation.



6.2 Diazonium Coupling: When amine diazonium ions react with extremely reactive aromatic compounds like phenols and tertiary arylamines, they form azole molecules, also known as diazo coupling reactions (Fig. 3).

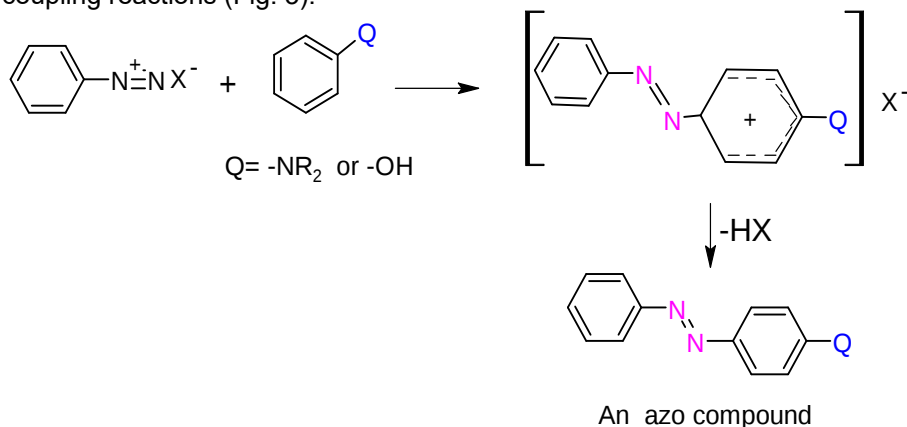
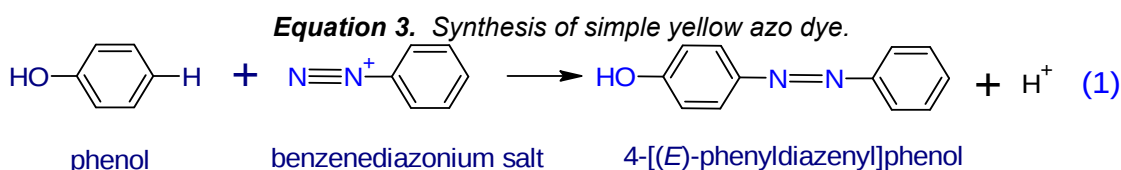


Figure 3. General reaction of azo coupling (Çakmak, 2023).

7. Azo-compounds Coupling Reactions:

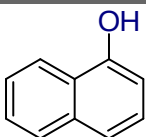
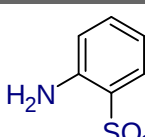
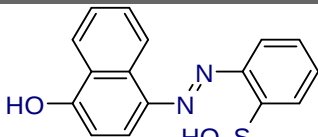
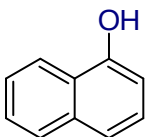
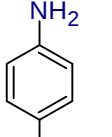
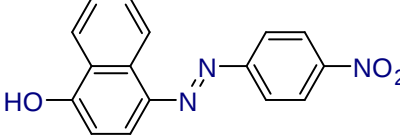
Alkaline azo dyes are synthesized through electrophilic aromatic substitution reactions between benzene diazonium salts and activated aromatic compounds. The reaction mechanism involves two key components: a benzene diazonium salt, which serves as a weak

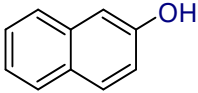
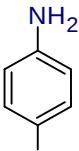
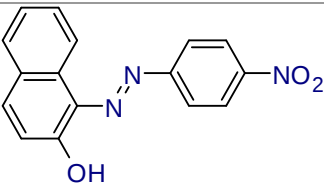
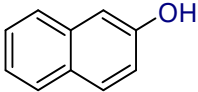
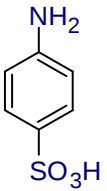
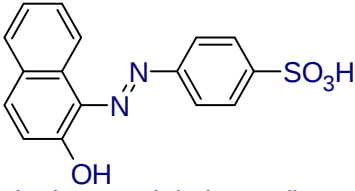
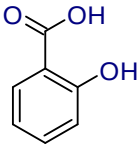
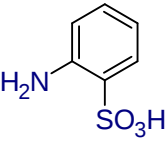
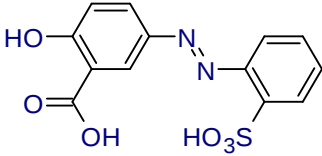
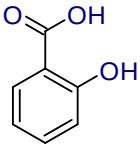
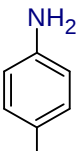
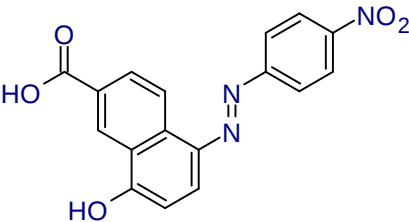
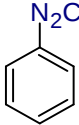
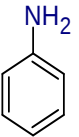
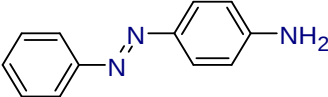
electrophile, and an alkaline solution of phenol or aromatic amine (such as phenylamine), which acts as the nucleophile. For the reaction to occur efficiently, the aromatic ring must be activated by electron-donating groups like -OH or -NH₂. These groups increase the electron density at the ortho and para positions of the ring, facilitating the electrophilic attack by the diazonium cation (Aljamali, 2015). The reaction specificity for phenol coupling typically favors substitution at the para position; however, if this position is already occupied, substitution shifts to the ortho position. In acidic conditions, such as in aniline coupling, 4-(phenyldiazenyl)aniline, a simple yellow azo dye, is formed (*Equation 2*). This dye has historical significance as one of the earliest known azo dyes but has limited industrial use due to its high solubility in acidic media, which makes it difficult to isolate and use in some textile or printing applications.



These reactions demonstrate the critical role of activating groups and reaction conditions in determining azo dye properties and applications. Table 2 outlines several examples of azo compounds, along with the corresponding substrates and coupling compounds used in their synthesis. The azo compounds (column 3 in Table 2) which can easily prepare from the substrate (column 2) and coupling compound (column 1).

Table 2. Examples of the azo compounds with their substrates.

Coupling Compound (Electron-Donor)	Substrate (Diazonium Source)	Resulting Azo Compound
 naphthalen-1-ol	 2-aminobenzene-1-sulfonic acid	 2-[(4-hydroxynaphthalen-1-yl)diazenyl]benzene-1-sulfonic acid
 naphthalen-1-ol	 4-nitroaniline	 4-[(4-methylphenyl)diazenyl]naphthalen-1-ol

 naphthalen-2-ol	 4-nitroaniline	 1-[(4-nitrophenyl)diazenyl] naphthalen-2-ol
 naphthalen-2-ol	 4-aminobenzenesulfonic acid	 4-[(2-hydroxynaphthalen-1-yl) diazenyl]benzenesulfonic acid
 Salicylic acid	 2-aminobenzenesulfonic acid	 2-hydroxy-5-[(2-sulfophenyl)diazenyl] benzoic acid
 Salicylic acid	 4-nitroaniline	 8-hydroxy-5-[(4-nitrophenyl)diazenyl] naphthalene-2-carboxylic acid
 benzenediazonium chloride	 Aniline	 4-(phenyldiazenyl)aniline
Synthetic Note: The azo compounds in Column 3 form readily when the activated coupling compound (Column 1) reacts with the diazonium derivative of the substrate (Column 2) under alkaline conditions.		

8. Toxicity of Azo Dyes

8.1 Hazards of Aromatic Amines: Azo dyes present significant health and environmental risks primarily through their degradation into aromatic amines, which are classified as potent carcinogens (IARC, 2018). Approximately 6% of azo dyes undergo cleavage of the -N=N- bond (Fig. 4A & 4B), releasing amines associated with hepatic, pancreatic, and bladder cancers.

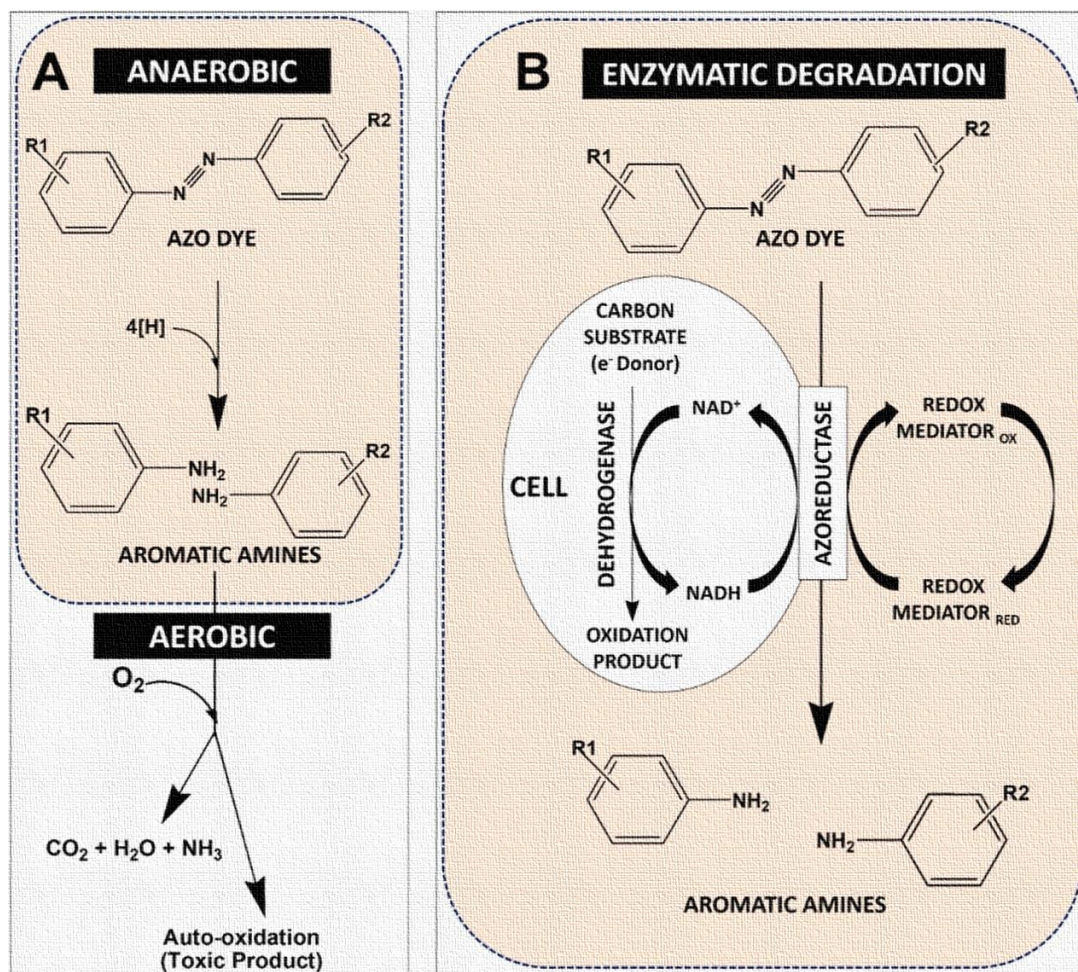


Figure 4. The cleavage of azo bonds (Singh et al., 2024).

As shown in Fig. 5, primary exposure pathways to azo dyes include occupational, consumer, and environmental routes. Occupational exposure typically occurs through inhalation and dermal contact, particularly in textile manufacturing settings. Consumers may be exposed via percutaneous absorption from dyed textiles, with absorption rates reaching up to 2.8 $\mu\text{g}/\text{cm}^2/\text{hr}$ under sweating conditions (Ahlström et al., 2005). Environmental exposure arises

primarily through ingestion of contaminated water, especially in regions where effluent-impacted aquifers contain dye concentrations exceeding 1.5 ppm.

Among the notable carcinogens associated with azo dyes are benzidine and 2-naphthylamine, both classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC), with clear evidence of carcinogenicity. Occupational exposure to these substances has been linked to elevated rates of bladder cancer, reaching 18.7 cases per 1000 workers (Carreón et al., 2014). Additionally, tobacco smoke acts synergistically with these exposures, further increasing the risk of **cancer in exposed populations**.

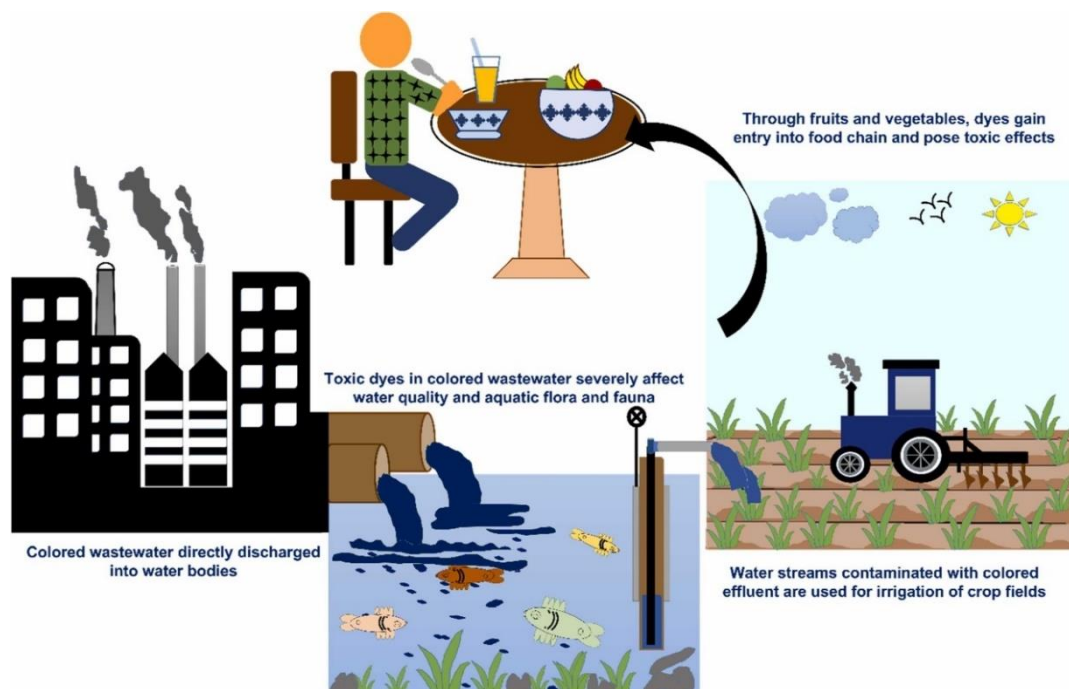


Figure 5. Primary exposure pathways of azo dyes (Singh et al., 2024)

8.2 Acute and Chronic Health Effects

Exposure to aromatic amines, commonly found in azo dye derivatives, can result in a range of acute and chronic health effects. Acute exposure may lead to immediate symptoms such as skin irritation, blindness, vertigo, vomiting, and respiratory distress (Gudelj et al., 2011). Chronic exposure poses more serious long-term risks. Benzidine derivatives, for example, exhibit carcinogenicity by forming DNA adducts through N-acetyltransferase-mediated pathways (Habil et al., 2020). Additionally, p-phenylenediamine is associated with mutagenicity and has been implicated in severe systemic reactions, including kidney failure. Although the mutagenic potential of Sunset Yellow metabolites remains disputed, some studies suggest possible genetic toxicity (Feng et al., 2012). These toxicological concerns are summarized in Table 3, which outlines the health impacts associated with various azo dye derivatives.

Table 3. Toxicological profile of azo dye derivatives

Compound	Acute Effects (LOAEL)	Chronic Effects	Reference
Benzidine	25 mg/kg (rat, oral)	Bladder carcinoma (0.1 ppm/yr)	NTP (2021)
2-Naphthylamine	50 mg/kg	Hepatocellular adenomas	IARC (2018)
p-Phenylenediamine	100 mg/m ³ (inhalation)	Contact dermatitis (15% prevalence)	SCCS (2022)

8.3 Pathological mechanisms:

Aromatic amine exposure induces a range of pathological mechanisms that contribute to both acute toxicity and chronic disease outcomes. One such mechanism is **methemoglobinemia**, which occurs through the inhibition of NADH-dependent cytochrome b5 reductase, an enzyme essential for maintaining hemoglobin in its oxygen-carrying ferrous state (Fig. 6). Inhibition of this enzyme, particularly by benzidine derivatives, leads to impaired oxygen transport and symptoms such as hypoxia, cyanosis, and lethargy. Notably, 28% of exposed workers exhibited cyanosis as a result of this mechanism (Kaushik & Chauhan, 2019).

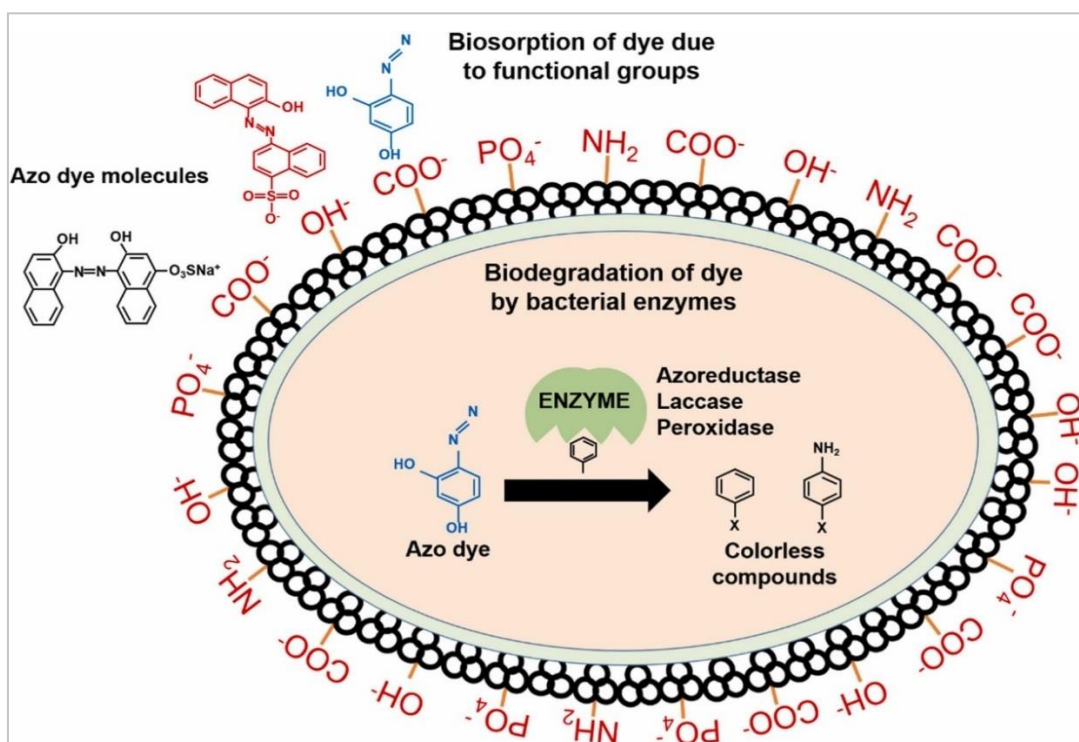


Figure 6. Biosorption of azo dye due to functional groups of protein, lipids, and other biochemical molecules (Singh et al., 2024).

8.4 Toxicity Data

Azo dyes generally exhibit low acute toxicity, with most compounds demonstrating LD₅₀ values greater than 5000 mg/kg in rats, indicating minimal immediate health hazards. Only about 1% of azo dyes fall within the highly toxic range (LD₅₀ between 100–250 mg/kg). Notably, Reactive Black 5 has been reported to possess exceptionally low acute toxicity, with an LD₅₀ exceeding 14,000 mg/kg (Hunger & Jung, 1991). When compared to other dye classes, anthraquinone and phthalocyanine dyes are also relatively low in toxicity, with LD₅₀ values ranging from 5000 to 15,000 mg/kg. In contrast, certain aromatic amines, which may be present as precursors or degradation products, exhibit higher toxicity, with LD₅₀ values between 500 and 5000 mg/kg (Saini & Choudhary, 2025).

Regarding developmental toxicity, zebrafish embryo studies (McGrath & Li, 2008) have shown that food dyes such as tartrazine and Sunset Yellow have Teratogenic Index (TI) values ranging from 1.10 to 1.31, suggesting a measurable risk of developmental effects. At a concentration of 50 mM, 87% of embryos developed yolk sac edema, while exposure to 100 mM resulted in complete embryonic lethality. The LC₅₀ values for these dyes range from 38.93 to 47.42 mM, levels far exceeding typical human exposures, yet they underscore the potential developmental risks posed by high concentrations of such compounds.

9. Environmental Distribution and Ecological Impacts

9.1 Textile Industry Pollution: Scale and Mechanisms

The textile industry generates 20% of global wastewater, with dyeing processes releasing complex effluents containing heavy metals (e.g., chromium, lead), salts, and 10–15% unfixed dyes (Chequer et al., 2013). A single mill discharges 200–350 m³ of wastewater per ton of fabric, often at 40–50°C above ambient water temperatures, exacerbating thermal pollution (Khan et al., 2019).

One plain example is the Buriganga River in Bangladesh, where over 700 textile mills in Dhaka collectively discharge an estimated 1.5 million liters of untreated wastewater daily. The ecological impacts are profound, with dissolved oxygen levels dropping below 2 mg/L—well beneath the 5 mg/L necessary to sustain aquatic life—and a 60% reduction in native fish populations (Sarkar et al., 2020). Comparative data (Table 4) from textile production hubs such as Bangladesh and China reveal consistently high levels of biological oxygen demand (BOD), chemical oxygen demand (COD), and heavy metal contamination, indicating a pervasive global pattern (Islam et al., 2018; Wang et al., 2011).

Table 4. Effluent characteristics in global textile hubs

Region	BOD (mg/L)	COD (mg/L)	Heavy Metals	Reference
Bangladesh	380 ± 42	1240 ± 180	Cr: 8.7 ppm	Islam et al. (2018)
China	210 ± 35	980 ± 120	Pb: 2.1 ppm	Wang et al. (2011)

9.2 Ecological Impacts: From Molecules to Ecosystems

Aquatic Systems: The environmental impacts of textile dyes extend beyond aquatic systems. Azo dyes, one of the most commonly used dye classes, impede light penetration even at concentrations as low as 1 mg/L, causing photosynthesis in aquatic ecosystems to decline by up to 70%. Under anaerobic conditions, these dyes degrade into carcinogenic compounds

like benzidine, with environmental half-lives of 5–10 years. When combined with heavy metals such as hexavalent chromium, the mutagenicity of these compounds can increase threefold (Selvaraj et al., 2021). The toxicity of azo dyes extends across multiple levels of aquatic ecosystems. Microbial and algal communities exhibit significant sensitivity, with algae such as *Chlorella vulgaris* showing up to a 70% reduction in photosynthetic capacity (Khataee et al., 2013). Invertebrates like *Daphnia magna* are acutely vulnerable, exhibiting lethal concentration (LC₅₀) values between 10–100 mg/L and chronic reproductive impairments (Andrés, et al., 2025). Fish species, including *Danio rerio* and *Oreochromis niloticus*, suffer DNA damage and liver necrosis, highlighting the bioaccumulative and genotoxic nature of these pollutants (El-Sheekh et al., 2009; Ramamurthy et al., 2024).

Terrestrial Contamination: Terrestrial ecosystems are similarly affected. Irrigation with dye-contaminated wastewater results in the accumulation of aromatic amines in agricultural soils, with concentrations ranging from 4–12 mg/kg in crops. This not only reduces rice yields by 30–40% but also diminishes soil microbial diversity by 40–60%, disrupting essential nutrient cycles (Banat et al., 1996; Pandey et al., 2007; Kurade et al., 2016). Plant studies have documented inhibited seed germination and bioaccumulation of dyes in crops like wheat (*Triticum aestivum*), raising concerns about food safety (Copaciu et al., 2013; Lade et al., 2015). Moreover, certain dye metabolites, such as Sudan I, have been shown to mimic estrogen, inducing reproductive dysfunction in aquatic organisms (Khandelwal et al., 2024).

Fish consumed from polluted ponds have dye residues up to 4.7 mg/kg—47 times higher than fish from uncontaminated waters. Nearly 90% of residents in such areas exceed safe intake levels for aromatic amines (Khan & Malik, 2014). These exposures have measurable health impacts. For instance, dye industry workers face a six-fold increase in the risk of bladder cancer, a malignancy linked to Group 1 carcinogens. Miscarriage rates in communities relying on contaminated water sources reach as high as 22% (Hao et al., 2000).

These data collectively underscore the extensive ecological and human health consequences of textile dye pollution, highlighting the urgency for stringent regulation, effective remediation, and sustainable industry practices.

10. Regulatory and Remediation Strategies

The environmental and health impacts of azo dyes have prompted significant regulatory attention globally. The European Union's REACH Annex XVII regulation prohibits the use of 22 amine-releasing azo dyes in textiles and leather, setting stringent detection limits below 30 ppm. Similarly, China's "Water Ten Plan" mandates zero liquid discharge (ZLD) in industrial zones, aiming to eliminate wastewater release. Despite such initiatives, regulatory implementation remains uneven. In the United States, the Food and Drug Administration (FDA) has banned or restricted numerous azo dyes, particularly in food, drugs, and cosmetics, under regulations codified in 21 CFR Parts 70–82 (Table 5). These include Sudan I, II, III, and IV, Para Red, Orange II, and Butter Yellow—all prohibited due to their carcinogenic or toxic degradation products (FDA, 2019; NTP, 2021).

The EU extends similar restrictions to dyes such as Disperse Blue 85 and Direct Red 28, which release known carcinogens like benzidine and o-anisidine upon breakdown (Table 7). Comparable standards are upheld in China (GB 18401-2010), India (BIS Standards), and by the Oeko-Tex certification system, which collectively aim to phase out dyes associated with

cancer, allergenic reactions, and persistent aquatic toxicity (Chung, 2016). Below is a list of keys **banned azo dyes** under FDA, EU (REACH), and other global regulations.

Table 5. Azo dyes banned by the FDA.

Azo Dye name	Azo Dyes Banned by the FDA
Sudan I (CI 12055)	Banned in food due to carcinogenicity (FDA, 2019).
Sudan II, III, IV	Prohibited in food products (FDA, 2019).
Para Red (CI 12070)	Illegal in food due to toxic breakdown products.
Orange II (CI 15510)	Not permitted in food or drugs.
Butter Yellow (Dimethylaminoazobenzene, CI 11020)	Banned due to liver carcinogenicity (NTP, 2021).
(Note: The FDA allows some azo dyes, like FD&C Red No. 40, but with strict purity requirements.)	

Table 6. Azo Dyes Banned in the EU (REACH Regulation).

Azo Dye (CI Name/Number)	Carcinogenic Amine Released
Disperse Blue 85 (CI 61125)	3,3'-Dichlorobenzidine
Disperse Orange 1 (CI 11080)	4-Aminoazobenzene
Disperse Yellow 3 (CI 11855)	4-Aminoazobenzene
Direct Black 38 (CI 30235)	Benidine
Direct Red 28 (CI 22120, Congo Red)	Benidine
Basic Red 46 (CI 110825)	o-Anisidine

Recent technological advances offer promising alternatives to conventional wastewater treatment. Mycoremediation, using fungi such as *Pleurotus ostreatus*, can achieve 95% dye removal within 72 hours at a cost-effective rate of \$0.20/m³, making it attractive for small-scale industries. Plasma oxidation, while delivering up to 99% dye removal in just 15 minutes, remains limited by high operational costs (\$4.50/m³). Electrocoagulation presents a balanced solution, achieving 98% chemical oxygen demand (COD) reduction at \$1.50/m³. Additionally, nanocellulose-based adsorbents have demonstrated high adsorption capacities (900 mg/g) and excellent reusability across multiple cycles, aligning well with circular economy principles (Panwar et al., 2022; Crini, 2006).

11. Innovative Remediation strategies for azo dyes

11.1 Emerging Remediation Technologies

Recent advances in dye wastewater treatment have provided promising solutions to mitigate environmental contamination (Fig. 7). Mycoremediation, using fungal species such as *Pleurotus ostreatus*, achieves a remarkable 95% dye removal within 72 hours at a low cost of \$0.20/m³, making it an attractive option for small-scale industries (Asgher et al., 2008). Plasma oxidation is highly efficient, offering a 99% dye removal rate within just 15 minutes, but its high operational cost of \$4.50/m³ limits its scalability (Jiang et al., 2013). Electrocoagulation strikes a balance between efficiency and cost, removing 98% of chemical oxygen demand (COD) for \$1.50/m³ (Mollah et al., 2001). Lastly, nanocellulose adsorbents offer exceptional adsorption capacity (900 mg/g) and reusability for up to 10 cycles, making them ideal for circular economy approaches (Mohammed et al., 2018). These methods (Table

7) collectively provide a range of options with varying efficiency and cost profiles, contributing to more sustainable dye wastewater management.

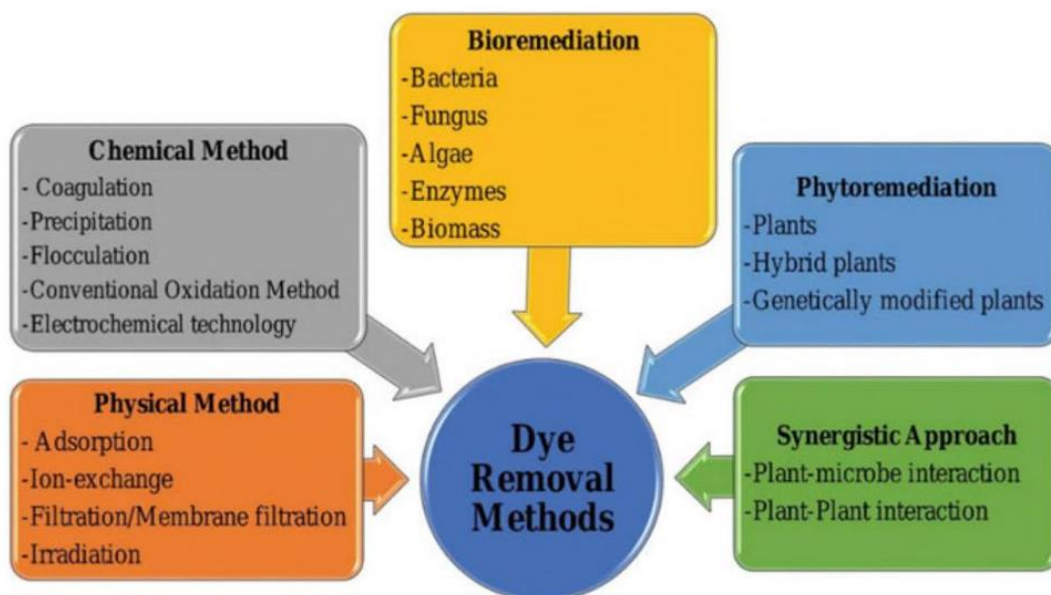


Figure 7. various approaches of remediation of waste water of azo dyes (Panwar et al., 2022).

Table 7. Comparative performance of remediation technologies

Technology	Removal Efficiency	Cost (USD/m ³)	Scalability
Mycoremediation	95% @ 72h	0.20	Medium
Plasma oxidation	99% @ 15min	4.50	Low
Electrocoagulation	98% COD reduction	1.50	High

Industry-Led Innovations further complement these remediation strategies. Waterless dyeing technologies, particularly **supercritical CO₂-based systems** adopted by global brands like Nike and Adidas, have successfully reduced water usage by up to 90%. Vietnam's "Zero Discharge" initiative exemplifies circular production by reclaiming and reusing 85% of dye inputs, yielding both environmental and economic benefits. Looking ahead, the adoption of green chemistry approaches—such as enzymatic treatment using laccase—has shown a 99% reduction in mutagenicity, offering a sustainable alternative to conventional chemical treatments. Digital tools, including blockchain-based effluent tracking systems piloted in Bangladesh, have improved compliance monitoring by 40%. Machine learning models with predictive accuracies up to $R^2 = 0.94$ now facilitate rapid toxicity assessment of novel dye compounds, enhancing regulatory oversight and risk evaluation (Ayed et al., 2011).

11.2 Future Directions

Looking ahead, the adoption of green chemistry approaches, such as enzymatic treatment using laccase has shown a 99% reduction in mutagenicity, offering a sustainable alternative to conventional chemical treatments. Digital tools, including blockchain-based effluent tracking systems piloted in Bangladesh, have improved compliance monitoring by 40%.

Machine learning models with predictive accuracies up to $R^2 = 0.94$ now facilitate rapid toxicity assessment of novel dye compounds, enhancing regulatory oversight and risk evaluation. Azo dyes exhibit high environmental persistence due to their stable aromatic structures, rendering them resistant to conventional biodegradation (Ali, 2010). Under anaerobic conditions, microbial azo reductases cleave azo bonds, releasing aromatic amines such as benzidine—compounds often more toxic than their parent dyes (Pandey et al., 2007; Yagub et al., 2014). This transformation not only perpetuates long-term ecological risks but also complicates remediation efforts. The persistent nature of these metabolites exacerbates bioaccumulation and hampers the recovery of affected ecosystems (Lellis et al., 2019).

12. Environmental Persistence and Ecological Toxicity

12.1 Degradation Mechanisms

Azo dyes resist biodegradation due to their stable aromatic structures (Yagub et al., 2014). Under anaerobic conditions, microbial azo reductases cleave azo bonds, generating carcinogenic aromatic amines (Pandey et al., 2007). These metabolites often exhibit higher toxicity than parent compounds, exacerbating ecological risks (Lellis et al., 2019).

13. Limitations and Future Perspectives:

While azo dyes demonstrate antimicrobial potential, challenges remain, including cytotoxicity and environmental concerns related to dye degradation. Future research should focus on developing more environmentally friendly synthesis routes and improving selectivity for microbial cells over mammalian cells.

14. Conclusions:

The antimicrobial properties of azo dyes continue to be an exciting field of study. Advances in synthesis, and structural modifications have enhanced their efficacy against various pathogens. With further optimization, azo dyes hold promise as multifunctional agents for biomedical and textile applications. Azo compounds exhibit significant antimicrobial properties, primarily through oxidative stress, membrane disruption, and DNA damage. Future research should focus on optimizing the safety and effectiveness of these compounds for therapeutic applications.

Conflicts of Interest: There are no conflicts of interest declared by the authors.

References

- Ahlström, L., Eskilsson, C. S., & Björklund, E. (2005). Determination of banned azo dyes in consumer goods. *TrAC Trends in Analytical Chemistry*, 24(1), 49–56. <https://doi.org/10.1016/j.trac.2004.09.004>
- Ali, H. (2010). Biodegradation of synthetic dyes—A review. *Water, Air, & Soil Pollution*, 213(1-4), 251–273. <https://doi.org/10.1007/s11270-010-0382-4>
- Aljamali, N. M. (2015). Review in Azo Compounds and its Biological Activity. *Biochemistry & Analytical Biochemistry*, 04(02). <https://doi.org/10.4172/2161-1009.1000169>
- Andrés, E. R., Ignacio, M. B. K., & Maribel, M. T. (2025). Aromatic amines in the environment: ecotoxicological effects in vertebrates and invertebrates: a review. *Chemistry and Ecology*, 41(8), 1115-1149.

- Asgher, M., Bhatti, H. N., Ashraf, M., & Legge, R. L. (2008). Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. *Biodegradation*, 19(6), 771–783. <https://doi.org/10.1007/s10532-008-9185-3>
- Ayed, L., Mahdhi, A., Cheref, A., & Bakhrouf, A. (2011). Decolorization and degradation of azo dye Methyl Red by an isolated *Sphingomonas paucimobilis*: biotoxicity and metabolites characterization. *Desalination*, 274(1-3), 272–277.
- Baliza, P. X., Ferreira, S. L. C., & Teixeira, L. S. G. (2009). Application of pyridylazo and thiazolylazo reagents in flow injection preconcentration systems for determination of metals. *Talanta*, 79(1), 2–9. <https://doi.org/10.1016/j.talanta.2009.02.055>
- Banat, I. M., Nigam, P., Singh, D., & Marchant, R. (1996). Microbial decolorization of textile-dyecontaining effluents: A review. *Bioresource Technology*, 58(3), 217–227. [https://doi.org/10.1016/s0960-8524\(96\)00113-7](https://doi.org/10.1016/s0960-8524(96)00113-7)
- Benkhaya, S., M'rabet, S., & Harfi, A. E. (2020). Classifications, properties, recent synthesis and applications of azo dyes. *Heliyon*, 6(1), e03271. <https://doi.org/10.1016/j.heliyon.2020.e03271>
- Berradi, M., Hsissou, R., Khudhair, M., Assouag, M., Cherkaoui, O., Bachiri, A. E., & Harfi, A. E. (2019). Textile finishing dyes and their impact on aquatic enviroins. *Heliyon*, 5(11), e02711. <https://doi.org/10.1016/j.heliyon.2019.e02711>
- Çakmak, B. (2023). Synthesis Of Azo Containing Compounds and Their Photoisomerization with Polarized Light [M.S. - Master of Science]. Middle East Technical University. <https://open.metu.edu.tr/handle/11511/102065>
- Carreón, T., Hein, M. J., Hanley, K. W., Viet, S. M., & Ruder, A. M. (2014). Bladder cancer incidence among workers exposed too-toluidine, aniline and nitrobenzene at a rubber chemical manufacturing plant. *Occupational and Environmental Medicine*, 71(3), 175–182. <https://doi.org/10.1136/oemed-2013-101873>
- Chung, K. (2016). Azo dyes and human health: A review. *Journal of Environmental Science and Health Part C*, 34(4), 233–261. <https://doi.org/10.1080/10590501.2016.1236602>
- Chequer, F. D., de Oliveira, G. A., Ferraz, E. R., Cardoso, J. C., Zanoni, M. V. B., & de Oliveira, D. P. (2013). Textile Dyes: Dyeing Process and Environmental Impact. In *Eco-Friendly Textile Dyeing and Finishing*. IntechOpen. <https://doi.org/10.5772/53659>
- Copaciu, F., Opreș, O., Coman, V., Ristoiu, D., Niinemets, Ü., & 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, & Soil Pollution*, 224, 1–11.
- Crini, G. (2006). Non-conventional low-cost adsorbents for dye removal: A review. *Bioresource Technology*, 97(9), 1061–1085. <https://doi.org/10.1016/j.biortech.2005.05.001>
- Dan, O. D., Vicaș, L., Marian, E (2018). Impact of Azo Dyes on the Body and Their Use in the Food Industry and Pharmaceutics. University of Oradea, Faculty of Medicine and Pharmacy, Pta 1 Decembrie, Oradea, Romania. Retrieve From https://protmed.uroadea.ro/facultate/publicatii/protectia_mediului/2018B/miscellaneous/03.%20Dan%20Olimpia.pdf
- El-Sayed, E., El-Aziz, E. A., Othman, H., & Hassabo, A. (2024). Azo dyes: Synthesis, Classification and Utilisation in Textile Industry. *Egyptian Journal of Chemistry*, 67, 87 – 97. DOI: 10.21608/ejchem.2024.257952.9057

- Elshaarawy, R. F., Sayed, T. M., Khalifa, H. M., & El-Sawi, E. A. (2017). A mild and convenient protocol for the conversion of toxic acid red 37 into pharmacological (antibiotic and anticancer) nominees: Organopalladium architectures. *Comptes Rendus Chimie*, 20(9–10), 934–941. <https://doi.org/10.1016/j.crci.2017.07.003>
- El-Sheekh, M. M., Gharieb, M. M., & Abou-El-Souod, G. W. (2009). Biodegradation of azo dyes by algae: A review. *International Biodeterioration & Biodegradation*, 63(6), 699–704.
- Eltaboni, F., Bader, N., El-Kailany, R., Elsharif, N., & Ahmida, A. (2022). Chemistry and Applications of Azo Dyes: A Comprehensive Review. *Journal of Chemical Reviews*, 4(4), 313–330. <https://doi.org/10.22034/jcr.2022.349827.1177>
- Feng, J., Cerniglia, C. E., & Chen, H. (2012). Toxicological significance of azo dye metabolism by human intestinal microbiota. *Frontiers in Bioscience-Elite*, E4(2), 568–586. <https://doi.org/10.2741/e400>
- Gudelj, I., Hrenović, J., Dragičević, T., Delaš, F., Šoljan, V., & Gudelj, H. (2011). Azo dyes, their environmental effects, and defining a strategy for their biodegradation and detoxification. *Archives of Industrial Hygiene and Toxicology*, 62(1), 91–101. <https://doi.org/10.2478/10004-1254-62-2011-2063>
- Hao, O. J., Kim, H., & Chiang, P. (2000). Decolorization of wastewater. *Critical Reviews in Environmental Science and Technology*, 30(4), 449–505. <https://doi.org/10.1080/10643380091184237>
- Hunger, K., & Jung, R. (1991). On the toxicology and ecology of organic colorants. *Chimia*, 45(10), 297–297.
- International Agency for Research on Cancer (IARC). (2019). Some Aromatic Amines and Related Compounds. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 127*. Lyon, France: World Health Organization.
- Islam, M. S., Hossain, M. B., Matin, A., & Sarker, M. S. I. (2018). Assessment of heavy metal pollution, distribution and source apportionment in the sediment from Feni River estuary, Bangladesh. *Chemosphere*, 202, 25–32. <https://doi.org/10.1016/j.chemosphere.2018.03.077>
- Jiang, B., Zheng, J., Qiu, S., Wu, M., Zhang, Q., Yan, Z., & Xue, Q. (2013). Review on electrical discharge plasma technology for wastewater remediation. *Chemical Engineering Journal*, 236, 348–368. <https://doi.org/10.1016/j.cej.2013.09.090>
- Kaushik, P., & Chauhan, P. (2019). Azo Dyes: Their Environmental Impact and Degradation. In *Environmental Chemistry for a Sustainable World* (pp. 145–171). Springer, Cham.
- Khan, S., Anas, M., & Malik, A. (2019). Mutagenicity and genotoxicity evaluation of textile industry wastewater using bacterial and plant bioassays. *Toxicology Reports*, 6, 193–201. <https://doi.org/10.1016/j.toxrep.2019.02.002>
- Khan, S., & Malik, A. (2014). Environmental and health effects of textile industry wastewater. In *Springer eBooks* (pp. 55–71). https://doi.org/10.1007/978-94-007-7890-0_4
- Khandelwal, D., Rana, I., Mishra, V., Ranjan, K. R., & Singh, P. (2024). Unveiling the impact of dyes on aquatic ecosystems through zebrafish—a comprehensive review. *Environmental Research*, 119684.
- Khataee, A. R., Movafeghi, A., Vafaei, F., Salehi Lisar, S. Y., & Zarei, M. (2013). Potential of the aquatic fern *Azolla filiculoides* in biodegradation of an azo dye: modeling of experimental results by artificial neural networks. *International Journal of Phytoremediation*, 15(8), 729–742.

- Kurade, M. B., Waghmode, T. R., Kagalkar, A. N., & Govindwar, S. P. (2016). Decolorization of textile industry effluent containing disperse dye Scarlet RR by a newly developed bacterial-yeast consortium BL-GG. *Chemical Engineering Journal*, 184, 33–41. <https://doi.org/10.1016/j.cej.2011.12.058>
- Lade, H. S., Waghmode, T. R., Kadam, A. A., & Govindwar, S. P. (2015). Enhanced biodegradation and detoxification of disperse azo dye Rubine GFL and textile industry effluent by defined fungal-bacterial consortium. *International Biodeterioration & Biodegradation*, 72, 94–107.
- Lellis, B., Fávaro-Polonio, C. Z., Pamphile, J. A., & Polonio, J. C. (2019). Effects of textile dyes on health and the environment and bioremediation potential of living organisms. *Biotechnology Research and Innovation*, 3(2), 275–290. <https://doi.org/10.1016/j.biori.2019.09.001>
- McGrath, P., & Li, C. (2008). Zebrafish: a predictive model for assessing drug-induced toxicity. *Drug Discovery Today*, 13(9–10), 394–401. <https://doi.org/10.1016/j.drudis.2008.03.002>
- Maliyappa, K., J., Mahanthappa, M., Shivaraj, Y., & Basavarajappa, K. (2019). 6-Substituted benzothiazole based dispersed azo dyes having pyrazole moiety: Synthesis, characterization, electrochemical and DFT studies. *Journal of Molecular Structure*, 1199, 126959. <https://doi.org/10.1016/j.molstruc.2019.126959>
- Marinescu, M., Popa, C. V., Tănase, M. A., Soare, A. C., Tablet, C., Bala, D., Cinteza, L. O., Dițu, L. M., Gifu, I. C., & Petcu, C. (2022). Synthesis, Characterization, DFT Study and Antifungal Activities of Some Novel 2-(Phenyldiazenyl)phenol Based Azo Dyes. *Materials*, 15(22), 8162. <https://doi.org/10.3390/ma15228162>
- Mezgebe, K., & Mulugeta, E. (2022). Synthesis and pharmacological activities of azo dye derivatives incorporating heterocyclic scaffolds: a review. *RSC Advances*, 12(40), 25932–25946. <https://doi.org/10.1039/d2ra04934a>
- Mohamed-Smati, S. B., Faraj, F. L., Becheker, I., Berredjem, H., Bideau, F. L., Hamdi, M., Dumas, F., & Rachedi, Y. (2020). Synthesis, characterization and antimicrobial activity of some new azo dyes derived from 4-hydroxy-6-methyl-2H-pyran-2-one and its dihydro derivative. *Dyes and Pigments*, 188, 109073. <https://doi.org/10.1016/j.dyepig.2020.109073>
- Mohammed, N., Grishkewich, N., & Tam, K. C. (2018). Cellulose nanomaterials: promising sustainable nanomaterials for application in water/wastewater treatment processes. *Environmental Science Nano*, 5(3), 623–658. <https://doi.org/10.1039/c7en01029j>
- Mollah, M. A., Schennach, R., Parga, J. R., & Cocke, D. L. (2001). Electrocoagulation (EC) — science and applications. *Journal of Hazardous Materials*, 84(1), 29–41. [https://doi.org/10.1016/s0304-3894\(01\)00176-5](https://doi.org/10.1016/s0304-3894(01)00176-5)
- National Toxicology Program (NTP). (2021). *Report on Carcinogens, Fifteenth Edition*. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service.
- Pandey, A., Singh, P., & Iyengar, L. (2007). Bacterial decolorization and degradation of azo dyes. *International Biodeterioration & Biodegradation*, 59(2), 73–84. <https://doi.org/10.1016/j.ibiod.2006.08.006>
- Pavlović, G., Racané, L., Čičak, H., & Tralić-Kulenović, V. (2009). The synthesis and structural study of two benzothiazolyl azo dyes: X-ray crystallographic and computational study of azo-hydrazone tautomerism. *Dyes and Pigments*, 83(3), 354–362. <https://doi.org/10.1016/j.dyepig.2009.06.002>
- Ramamurthy, K., Priya, P. S., Murugan, R., & Arockiaraj, J. (2024). Hues of risk: investigating genotoxicity and environmental impacts of azo textile dyes. *Environmental Science and Pollution Research*, 31(23), 33190–33211.

- Panwar, P., Mahajan, P., & Kaushal, J. (2022). Approaches in Dye Removal. *Biological Approaches in Dye-Containing Wastewater: Volume 2*, 129.
- Rauf, M. A., & Ashraf, S. S. (2009). Fundamental principles and application of heterogeneous photocatalytic degradation of dyes in solution. *Chemical Engineering Journal*, 151(1-3), 10–18. <https://doi.org/10.1016/j.cej.2009.02.026>
- Rauf, M., Meetani, M., & Hisaindee, S. (2011). An overview on the photocatalytic degradation of azo dyes in the presence of TiO₂ doped with selective transition metals. *Desalination*, 276(1–3), 13–27. <https://doi.org/10.1016/j.desal.2011.03.071>
- Saini, R., & Choudhary, K. (2025). Toxic potential of Azo dyes: A broader Understanding. In *Hazardous Chemicals* (pp. 469–481). Academic Press.
- Selvaraj, V., Karthika, T. S., Mansiya, C., & Alagar, M. (2021). An over review on recently developed techniques, mechanisms and intermediate involved in the advanced azo dye degradation for industrial applications. *Journal of Molecular Structure*, 1224, 129195. <https://doi.org/10.1016/j.molstruc.2020.129195>
- Sarkar, M., Islam, J. B., & Akter, S. (2016). Pollution and ecological risk assessment for the environmentally impacted Buriganga River, Bangladesh. *J. Mater. Environ. Sci.* 7(7), 2295–2304.
- Scientific Committee on Consumer Safety (SCCS). (2022). *Opinion on p-Phenylenediamine (PPD) - SCCS/1643/22*. European Commission, Health & Consumer Directorate-General.
- Shankarling, G. S., Deshmukh, P. P., & Joglekar, A. R. (2017). Process intensification in azo dyes. *Journal of Environmental Chemical Engineering*, 5(4), 3302–3308. <https://doi.org/10.1016/j.jece.2017.05.057>
- Singh, G. B., Vinayak, A., Mudgal, G., & Kesari, K. K. (2024). Azo Dye Bioremediation: an Interdisciplinary Path to Sustainable fashion. *Environmental Technology & Innovation*, 103832. <https://doi.org/10.1016/j.eti.2024.103832>
- U.S. Food and Drug Administration (FDA). (2019). Code of Federal Regulations Title 21, Parts 70–82: Color Additives. U.S. Government Publishing Office.
- Wang, Z., Xue, M., Huang, K., & Liu, Z. (2011). Textile dyeing wastewater treatment. *Advances in treating textile effluent*, 5, 91–116.
- Yagub, M. T., Sen, T. K., Afroze, S., & Ang, H. M. (2014). Dye and its removal from aqueous solution by adsorption: A review. *Advances in Colloid and Interface Science*, 209, 172–184. <https://doi.org/10.1016/j.cis.2014.04.002>