



Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

Wafiyah Hasan Aljadidi¹, Najat Mohammed Ali¹, Dania Alsadiq Naser Losta¹, Fatema Ali Felo¹

¹ Faculty of Engineering, Sabratha University, Libya

Corresponding Author's Email: Wafiyah.aljadidi@sabu.edu.ly

Received: 15/8/2026 Revised: 17/8/2026 Accepted: 05/9/2026 Publication: 28/10/2026

Abstract

This study aims to prepare zinc oxide nanoparticles through green synthesis using moringa (*Moringa oleifera*) leaf extract, characterize the material, and evaluate the removal of methylene blue from an aqueous solution. An initial main experiment was conducted using 0.05 g of the material in 50 mL of dye solution at a concentration of 10 mg/L, with 30 minutes in the dark followed by exposure to light for up to 60 minutes. An additional, independent experimental series and control tests for photolysis and adsorption in the dark were also conducted.

The material was characterized using XRD, SEM, and FTIR. In the additional series, the mixture containing the catalyst was kept in the dark for 60 minutes and then exposed to light for up to 60 minutes, and the experiments were performed in three independent replicates. Absorbance was measured at 664 nm, and the calibration equation was used to calculate the concentration and removal percentage. Results from the additional series were reported as the mean $\pm$ standard deviation.

In the initial experiment, the final concentration reached 0.40 mg/L, and the recorded removal was 96.0%. The additional series yielded a mean final concentration of 0.35 mg/L with a standard deviation of 0.03, and a mean cumulative removal of 96.5% with a standard deviation of 0.8 percentage points. Photolysis without a catalyst was 2.1% with a standard deviation of 0.3 percentage points, while adsorption in the dark was 18.3% with a standard deviation of 1.1 percentage points after 60 minutes. XRD revealed a hexagonal wurtzite structure and a crystallite size of 22.5 nm, while SEM indicated a size range of 20–35 nm, and a Zn–O peak appeared at 435 cm^{-1} in FTIR. The results of the two experiments were presented separately because their protocols differed.

Keywords: moringa, zinc oxide nanoparticles, green synthesis, methylene blue, photocatalysis.

Chapter 1: Introduction and Study Framework

1.1 Introduction

Water is an essential resource for sustaining life and human activities, making the preservation of its quality and the reduction of pollution important concerns in environmental protection. Some industrial activities may generate wastewater containing various chemical compounds, including organic dyes used in multiple applications. When these dyes enter water, they may alter its color and reduce light penetration, making it important to identify suitable methods for treating dye-containing water.

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

Water treatment methods vary according to the type and concentration of the pollutant and the characteristics of the water. Photocatalysis is among the methods studied for removing certain organic compounds; it uses materials capable of reacting upon exposure to light. Some semiconductors, including zinc oxide, have been investigated in this field. When the material interacts with light, active charges may form and contribute to chemical reactions that affect pollutant molecules. Treatment success depends on several factors, including the amount of material used, the dye concentration, the duration of light exposure, and other experimental conditions.

Previous research has examined the use of zinc oxide to treat solutions containing various dyes, and some studies have tested zinc oxide nanoparticles prepared using plant extracts. Methylene blue is among the dyes used in laboratory research, as changes in its concentration can be monitored using a UV–Vis spectrophotometer. Measuring absorbance at an appropriate wavelength, with reference to a calibration curve, helps estimate the dye concentration in samples before and after treatment.

In recent years, interest has grown in the green synthesis of nanomaterials using plant extracts, which contain natural compounds that can participate in the preparation process and help stabilize the particles. Moringa (*Moringa oleifera*) is among the plants used to prepare nanomaterials, including zinc oxide, and the properties and various applications of these materials have also been studied. Using moringa leaf extract to prepare zinc oxide combines the use of a plant source with the study of a material that can be tested in an environmental application.

Against this background, this study examines the preparation of zinc oxide nanoparticles using moringa leaf extract and their subsequent testing for the removal of methylene blue from a laboratory-prepared aqueous solution. A specified amount of the prepared material was added to the dye solution, and the mixture was left in the dark for 30 minutes before being exposed to light, with samples collected at different times up to 60 minutes. The study monitors changes in dye concentration using a UV–Vis spectrophotometer at a wavelength of 664 nm, and the extent of removal is calculated using the calibration curve.

This study is limited to the experimental conditions and laboratory solution used. The results are therefore discussed within this context and do not, on their own, demonstrate the method's efficiency in treating actual industrial wastewater. Measuring changes in dye concentration indicates the extent of its reduction in solution, whereas demonstrating complete degradation or identifying degradation products requires additional analyses.

1.2 Research Problem

The removal of dyes from water is an important topic in environmental treatment, and methylene blue is used in laboratory experiments to monitor the efficiency of materials used to reduce pollutant concentrations. Zinc oxide is among the materials studied for this purpose and can also be prepared using plant extracts such as moringa leaf extract.

The research problem addressed in this study is the evaluation of the performance of zinc oxide particles prepared using moringa leaf extract in reducing the concentration of methylene blue under laboratory experimental conditions. The testing focuses on monitoring the dye concentration after mixing the dye with the prepared material in the dark and then exposing it to light, and on measuring changes in concentration over different time intervals. The study thus seeks to determine the extent of removal recorded during the experiment while distinguishing between the decrease that occurs in the dark and the change observed after illumination.

1.3 Main research question:

How efficient is the material prepared using moringa leaf extract in reducing the concentration of methylene blue under the experimental conditions used?

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

1.4 Significance of the Study

The significance of this study lies in its examination of the preparation of zinc oxide particles using moringa leaf extract and the evaluation of their efficiency in removing methylene blue from an aqueous solution. The study combines green synthesis with the application of nanomaterials to the treatment of organic pollutants, supporting interest in developing methods that can be investigated for water treatment.

The study also provides experimental data on changes in dye concentration during the dark and illumination stages and shows the extent of removal recorded under the experimental conditions adopted. Its significance also lies in the use of spectroscopic measurements and a calibration curve to estimate dye concentration and monitor its change over time.

The study's results can contribute to knowledge of the use of zinc oxide prepared with moringa extract for dye removal and can be compared with the findings of related studies, taking into account differences in preparation and testing conditions across studies.

1.5 Study Objectives

This study aims to prepare zinc oxide using moringa leaf extract and evaluate its performance in removing methylene blue from an aqueous solution under the experimental conditions. The specific objectives are as follows:

- Prepare an aqueous extract of moringa leaves and use it to prepare zinc oxide.
- Test the prepared material using a methylene blue solution with an initial concentration of 10 mg/L.
- Monitor changes in dye concentration after mixing the dye with the prepared material in the dark and then exposing it to light.
- Measure the absorbance of dye samples at a wavelength of 664 nm using a UV–Vis spectrophotometer.
- Calculate the dye concentration and removal percentage at the different measurement times using the calibration curve.
- Compare the decrease in dye concentration during the dark and illumination phases, and discuss the results within the limitations of the laboratory experiment.

1.6 Research Questions

This study seeks to answer the following main question:

- How efficient are zinc oxide particles prepared using Moringa leaf extract at removing methylene blue under the conditions of the laboratory experiment?
- The following questions arise from this main question:
- How does the concentration of methylene blue change after it is mixed with the particles in the dark for 30 minutes?
- How does the dye concentration change during exposure of the mixture to light for up to 60 minutes?
- What removal percentage is recorded at each measurement time?
- What is the difference between the decrease in dye concentration during the dark phase and the decrease recorded during the illumination phase?
- How suitable is the calibration curve used to estimate the dye concentration in the samples?

1.7 Scope and Limitations of the Study

The experiments were conducted in September 2026 in private laboratories and a public laboratory in Tripoli, all of which specialize in conducting these experiments.

1.8 Study Methodology

The study used an experimental approach that involved preparing Moringa leaf extract, preparing zinc oxide, and then testing it for methylene blue removal.

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

1.9 Preparation of the Extract and Material

Fresh Moringa leaves were washed with distilled water, shade-dried, and then ground. A 10 g portion of the powder was mixed with 100 mL of distilled water, and the mixture was heated at 70–80 °C while stirring at 500 rpm for 30 minutes. After cooling, the extract was filtered and stored in a dark bottle at 4 °C.

To prepare zinc oxide, 2.195 g of zinc acetate dihydrate was dissolved in 100 mL of distilled water, and Moringa extract was then added gradually with stirring for 15 minutes. Sodium hydroxide at a concentration of 0.1 M was added until the pH reached 10, and the mixture was then heated at 60 °C with stirring for 60 minutes. The precipitate was separated by centrifugation at 4000 rpm for 15 minutes and washed three times with distilled water and once with ethanol. It was dried at 80 °C for 24 hours and then calcined at 400 °C for two hours.

1.10 Dye Removal Testing and Analysis

A methylene blue solution at a concentration of 10 mg/L was prepared, and standard solutions were prepared from it to construct the calibration curve. A 0.05 g portion of the prepared material was added to 50 mL of the dye solution, and the mixture was stirred in the dark for 30 minutes. It was then exposed to light with continued stirring, and samples were collected at the start of the experiment, after the dark phase, and after 10, 20, 30, 45, and 60 minutes of illumination. The particles were separated from the samples by centrifugation at 4000 rpm for 10 minutes, and absorbance was then measured at 664 nm using a UV–Vis spectrophotometer. The calibration equation recorded in the report was used:

$Abs = 0.0888 C + 0.0076$, where $R^2 = 0.9992$.

The remaining concentration was calculated as: $C = (Abs - 0.0076) \div 0.0888$, and the removal percentage as: $[(C_0 - C_t) \div C_0] \times 100$.

Chapter 2: Theoretical Framework and Previous Studies

2.1 Introduction

This chapter addresses the scientific concepts relevant to the study, beginning with water pollution by organic dyes, followed by an introduction to methylene blue and nanoscale zinc oxide, an explanation of green synthesis using Moringa extract, the principle of photocatalysis, and the measurement of dye concentration. It also reviews several previous studies relevant to the research topic.

2.2 Water Pollution by Organic Dyes

Dyes are used in various industrial activities, and some types may enter water through wastewater discharges. The presence of dyes in water is an environmental problem that requires treatment: coloration may affect light penetration, while the persistence of organic compounds in water may affect its properties. The treatment methods used vary depending on the type and concentration of the pollutant and the characteristics of the water. These methods include adsorption, filtration, chemical treatment, and photocatalysis.

Some dyes are used in laboratory experiments as model compounds to test materials proposed for water treatment. Using a solution of known concentration allows changes in the amount of dye to be monitored during treatment, but a distinction must be made between a decrease in its concentration in solution and evidence of its complete degradation or conversion into harmless products.

2.3 Methylene Blue

Methylene blue is a colored dye used in various applications and in laboratory studies to test the efficiency of treatment materials. Its concentration in solution can be monitored using an ultraviolet–visible (UV–Vis) spectrophotometer by measuring its absorbance at an appropriate wavelength.

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

The present experiment used a wavelength of 664 nm to measure absorbance. A calibration curve prepared from solutions of known concentrations is used to relate the absorbance reading to the dye concentration. The concentrations of samples collected during the experiment can then be estimated and compared with the initial concentration.

2.4 Nanoscale Zinc Oxide

Zinc oxide is an inorganic compound studied for various applications, including photocatalysis. When it is in the form of fine or nanoscale particles, its surface properties and the way it interacts with solution components may change. The properties of the resulting material are affected by preparation factors, such as precursor concentrations, pH, temperature, reaction time, and calcination.

It is important to distinguish between preparing a powder believed to be nanoscale and demonstrating that its size actually falls within the nanoscale range. A change in the color of the mixture or the formation of a precipitate alone is not sufficient to determine particle size or confirm composition. Appropriate characterization techniques are therefore used, such as X-ray diffraction (XRD) to study crystal structure, scanning electron microscopy (SEM) to examine morphology and size, and Fourier-transform infrared spectroscopy (FTIR) to study chemical groups.

2.5 Green Synthesis Using Moringa Extract

Green synthesis refers to the use of plant-derived materials or extracts in the preparation of a material, with the aim of utilizing compounds present in the plant and reducing reliance on some conventional chemicals. Moringa (*Moringa oleifera*) leaves contain plant compounds, such as phenols, flavonoids, and other compounds. A review by Perumalsamy and colleagues (2024) discussed the use of different parts of Moringa in the preparation of nanomaterials and their applications.

In the preparation of zinc oxide, components of the plant extract can interact with the chemical species present in the preparation mixture and influence the formation or stability of the material. The outcome is affected by the preparation conditions; therefore, quantities, temperature, pH, stirring duration, washing, drying, and calcination should be documented precisely.

Describing a method as “green” does not automatically mean that the final product is nontoxic or that the process has no environmental impact. These depend on the materials used and the manufacturing conditions, and assessing safety and environmental impacts requires independent testing.

2.6 Principle of Photocatalysis

Photocatalysis involves using a material that interacts with light to help induce chemical changes in other substances. Theoretical studies explain that the absorption of suitable light energy by a semiconductor material may generate electrons and positive holes. These reactive species can participate in surface reactions with water and oxygen, forming oxidizing species that contribute to changes in some organic compounds.

Whether this process can occur depends on the properties of the material, the energy of the light reaching it, and the conditions of the solution. Dye concentration may also decrease as a result of adsorption onto the material surface before or during illumination. When interpreting the results, it is therefore important to distinguish between adsorption in the dark and the change that occurs after the sample is exposed to light.

In the present study, a decrease in dye concentration was recorded after the mixture had been kept in the dark for 30 minutes, and the concentration continued to decrease during illumination. These measurements support a description of the change in dye concentration under the experimental conditions, but they do not, on their own, establish the chemical mechanism or the formation of reactive radical species; establishing the mechanism requires additional experiments and analyses.

2.7 Absorbance Measurement and Calculation of Removal Percentage

A UV–Vis instrument measures the amount of light absorbed by a sample at a specific wavelength. Measurements of standard solutions are used to construct a calibration curve relating absorbance to concentration, and the equation of this curve is then used to estimate the concentrations of unknown samples.

The experimental report provided the following calibration curve:

$$A = 0.0888 C + 0.0076$$

where A represents the absorbance reading and C represents the dye concentration in mg/L.

The removal percentage is calculated using the following equation:

$$\text{Removal (\%)} = [(C_0 - C_t) / C_0] \times 100$$

where C_0 represents the initial dye concentration and C_t represents the remaining concentration at the time of measurement. This equation reflects the extent of the decrease in dye concentration in the solution. However, confirming complete degradation or identifying degradation products requires other analyses and cannot be established from color or absorbance measurements alone.

2.8 Previous Studies

Various studies have investigated the preparation of zinc oxide using *Moringa oleifera* leaf extract and tested its use in removing dyes from aqueous solutions. Pal et al. (2018) studied the preparation of zinc oxide using *Moringa oleifera* leaf extract and tested it on Titan Yellow dye, while also investigating its antibacterial activity. Adam et al. (2021) studied the effect of calcination temperature on the properties of the material and its activity toward methylene blue. Matinise et al. (2017) also investigated the physical properties and formation mechanism of zinc oxide prepared using *Moringa oleifera* extract. Zahro et al. (2025) focused on characterizing the structure and morphological properties of particles prepared using the plant extract. Parven et al. (2025) tested the oxidation of erythrosine dye using zinc oxide prepared with *Moringa oleifera* under sunlight, while Natrayan et al. (2025) examined photocatalysis and antibacterial applications.

These studies share with the present research the use of *Moringa oleifera* to prepare a zinc oxide-based material and the testing of its application to a dye in aqueous solution. The present study, however, focuses on monitoring changes in methylene blue concentration after the dark phase and then at the illumination times recorded in the experiment, using UV–Vis measurements and the calibration curve.

In other studies, Irfan et al. (2021) used *Moringa oleifera* gum to prepare zinc oxide particles and tested their antibacterial activity. Shashikala et al. (2024) also investigated the synthesis and characterization of zinc oxide using *Moringa oleifera* leaves, while Sarwar et al. (2025) studied its antibacterial and antioxidant properties. These studies provide background on synthesis methods, characterization, and biological properties, although their objectives differ from methylene blue removal in the present research.

Chapter 3: Materials and Methods

3.1 Study Location and Period

The experiments were conducted during September 2026 in private laboratories and a public laboratory in Tripoli, all of which specialize in conducting these experiments.

3.2 Materials and Equipment

Dried and ground *Moringa oleifera* leaves, zinc acetate dihydrate, sodium hydroxide, methylene blue dye, distilled water, and absolute ethanol were used. The equipment and supplies included an analytical balance, a magnetic stirrer with a hot plate, a pH meter, a centrifuge, a drying oven, a calcination furnace, and a UV–Vis spectrophotometer, along with glassware and filter paper.

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

3.3 Preparation of Moringa oleifera Extract

Fresh Moringa oleifera leaves were washed with distilled water to remove dust and impurities, then shade-dried and ground to obtain a fine powder. A 10 g portion of the powder was weighed and added to 100 mL of distilled water. The mixture was heated at 70–80 °C and stirred at 500 rpm for 30 minutes. It was then allowed to cool and filtered using filter paper. The extract was stored in a dark bottle in a refrigerator at 4 °C until use.

3.4 Preparation of the Zinc Oxide Material

Zinc acetate dihydrate (2.195 g) was dissolved in 100 mL of distilled water to obtain a 0.1 M solution. Moringa oleifera extract was gradually added to the zinc solution with stirring for 15 minutes. A 0.1 M sodium hydroxide solution was then added gradually while monitoring the pH until it reached 10.

The mixture was heated at 60 °C with continued stirring for 60 minutes. The precipitate was then separated by centrifugation at 4000 rpm for 15 minutes. It was washed three times with distilled water and once with absolute ethanol. The precipitate was dried at 80 °C for 24 hours and then calcined at 400 °C for two hours.

3.5 Preparation of the Dye Solution and Calibration Curve

A 10 mg/L methylene blue solution was prepared by dissolving 0.01 g of the dye in one liter of distilled water. Standard solutions at concentrations of 2, 4, 6, 8, and 10 mg/L were also prepared, and their absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 664 nm. The measurements of the standard solutions were used to derive the calibration curve equation.

3.6 First Main Experiment for Methylene Blue Removal

A 50 mL aliquot of the 10 mg/L methylene blue solution was taken, and 0.05 g of the prepared material was added. The vessel was covered, and the mixture was kept in the dark with stirring for 30 minutes. The cover was then removed, and the mixture was exposed to light with continued stirring. Samples were collected at the start of the experiment, after the dark phase, and then at 10, 20, 30, 45, and 60 minutes of illumination.

The solid material was separated from each sample by centrifugation at 4000 rpm for 10 minutes, and the absorbance of the liquid was then measured at 664 nm using a UV–Vis spectrophotometer.

3.7 Data Processing and Calculations

The calibration equation recorded in the experiment was used:

$$A = 0.0888 C + 0.0076$$

The remaining concentration was calculated using the equation:

$$C_t (\text{mg/L}) = (A - 0.0076) / 0.0888$$

The removal percentage was then calculated as follows:

$$\text{Removal (\%)} = [(C_0 - C_t) / C_0] \times 100$$

where A represents the absorbance reading, C_0 the initial dye concentration, and C_t the remaining concentration at the time of measurement.

3.8 Additional Experiments and Characterization Tests

The protocol for the first main experiment in Section 3.6 and its results were retained as an independent series. Alongside it, a separate additional series was conducted with three independent replicates, together with the following two control experiments and characterization tests.

3.8.1 Additional Experimental Series

Zinc oxide (0.05 g) was added to 50 mL of a 10 mg/L methylene blue solution. The mixture was kept in the dark with stirring for up to 60 minutes, and samples were collected at 30 and 60 minutes. The mixture was then exposed to light with continued stirring, and samples were collected at 10, 20, 30, 45, and 60 minutes after the start of illumination. The particles were separated by centrifugation at 4000 rpm for 10 minutes, and the absorbance of the liquid was measured at 664 nm.

3.8.2 Direct Photolysis Experiment

A 50 mL volume of 10 mg/L methylene blue solution was tested without the addition of zinc oxide particles. The solution was exposed to the light source used in the experiment, with the illumination and stirring conditions kept constant as far as possible, and samples were collected at the specified times up to 60 minutes. Absorbance was measured at 664 nm to estimate the change caused by light in the absence of the catalyst.

3.8.3 Adsorption Experiment in the Dark

Zinc oxide particles (0.05 g) were added to 50 mL of a 10 mg/L methylene blue solution. The vessel was covered to exclude light, and the mixture was stirred in the dark for up to 60 minutes. Samples were collected periodically, including the 30- and 60-minute samples used to present the results. The particles were separated by centrifugation at 4000 rpm for 10 minutes, after which absorbance was measured at 664 nm and the removal percentage was calculated.

3.8.4 Material characterization

XRD, SEM, and FTIR analyses of the zinc oxide samples were performed at specialized laboratories. Details of the instrument models and measurement conditions were not available in the current textual data; these details will therefore be provided when transcribed from the original laboratory reports, and images of the spectra and diffraction patterns will be included in the appendix.

The additional experimental series and control tests were conducted in three independent replicates ($n = 3$), and the results are presented as means $\pm$ standard deviations based on the recorded values. The result of the first experiment remains separate, and no mean or standard deviation is calculated for it without its original replicate readings.

Chapter 4: Results and Discussion

This chapter presents the methylene blue measurements across the experimental stages, starting with the initial concentration, followed by the dark stage and then the light exposure stages. Concentrations were calculated from absorbance readings using the calibration curve equation provided in the experimental report, and removal percentages were calculated relative to the initial concentration.

4.1 Methylene blue calibration curve

The calibration curve was constructed using methylene blue standard solutions, with absorbance measured at a wavelength of 664 nm. The regression equation recorded in the report was as follows:

$$A = 0.0888 C + 0.0076$$

The coefficient of determination was $R^2 = 0.9992$, indicating high linearity between absorbance and concentration within the range of standard solutions used. The equation was used to calculate the dye concentration in the samples.

4.2 Changes in dye concentration and removal percentage

Table 4.1 presents the absorbance readings and calculated concentrations, while Table 4.2 presents the cumulative removal percentages relative to the initial concentration. Table 4.3 summarizes the change between the end of the dark stage and the end of the illumination stage. All values were calculated from the readings recorded during the experiment.

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

Table 4.1: Absorbance and remaining concentration across the experimental stages

Residual concentration (mg/L)	Absorbance at 664 nm	Stage
10.00	0.895	Initial
8.53	0.765	After 30 minutes in the dark
6.61	0.595	After 10 minutes of illumination
4.70	0.425	After 20 minutes of illumination
2.79	0.255	After 30 minutes of illumination
1.36	0.128	After 45 minutes of illumination
0.40	0.043	After 60 minutes of illumination

Table 4.2: Cumulative dye removal percentage

Cumulative removal relative to C_0 (%)	Stage
0.0	Initial
14.7	After 30 minutes in the dark
33.9	After 10 minutes of illumination
53.0	After 20 minutes of illumination
72.1	After 30 minutes of illumination
86.4	After 45 minutes of illumination
96.0	After 60 minutes of illumination

Table 4.3: Summary of the concentration change between the dark and illumination stages

Cumulative removal (%)	Magnitude of decrease (mg/L)	C at the end	C at the start	Stage
14.7	1.47	8.53	10.00	Dark stage (30 minutes)
96.0	8.13	0.40	8.53	Illumination (60 minutes)

4.3 Changes in dye concentration across the experimental stages

The dye concentration decreased from 10.00 mg/L at the start of the experiment to 8.53 mg/L after 30 minutes in the dark. After illumination began, the concentration continued to decrease, reaching 6.61 mg/L after 10 minutes and 0.40 mg/L after 60 minutes. Tables 4.1–4.3 present the readings and calculated values and summarize the changes during the two stages, while Figure 4.1 shows the remaining concentration by experimental stage.

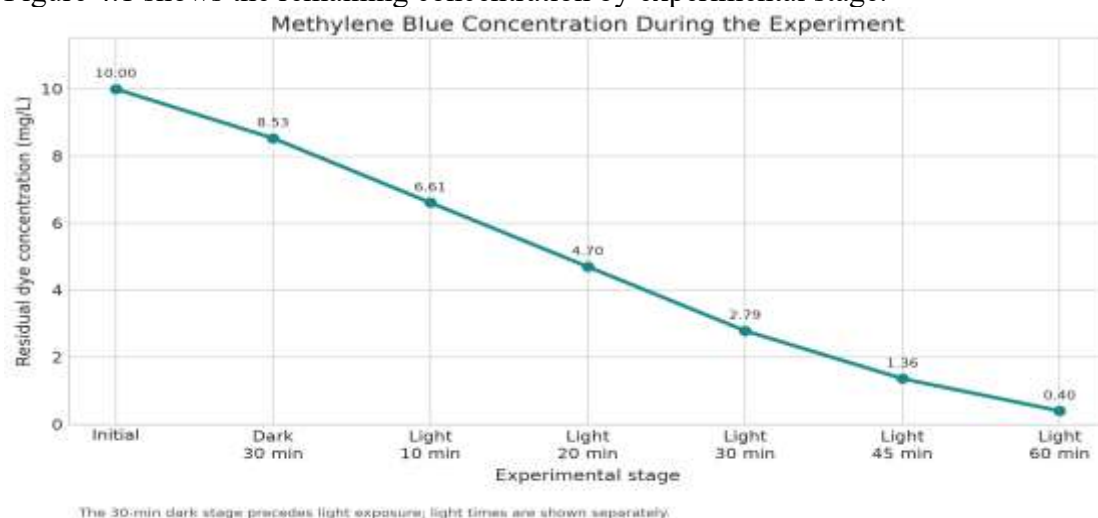


Figure 4.1: Remaining methylene blue concentration across the experimental stages.

4.4 Dye removal percentage

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

The overall removal percentage increased from 14.7% after the dark stage to 96.0% at the end of 60 minutes of illumination, relative to the initial concentration. Figure 4.2 shows this change. It should be noted that the final value includes the decrease that occurred during the dark stage before illumination.

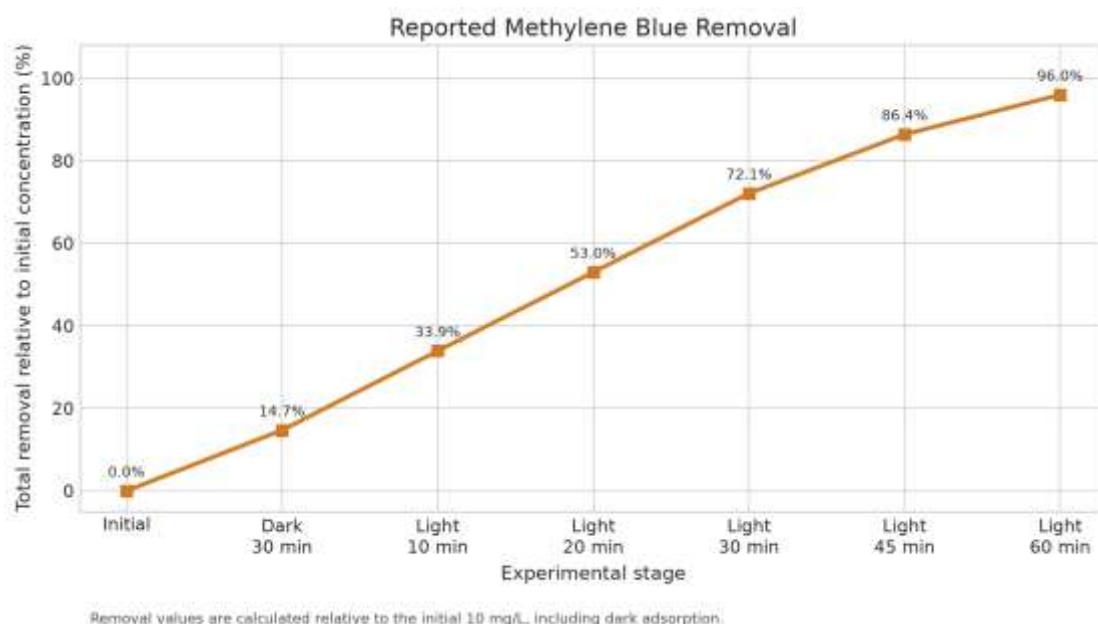


Figure 4.2: Overall dye removal percentage calculated relative to the initial concentration.

4.5 Discussion of the results

In the first main experiment, the dye concentration decreased from 10.00 to 8.53 mg/L after 30 minutes in the dark. It then continued to decrease during illumination, reaching 0.40 mg/L after 60 minutes, corresponding to a recorded overall removal of 96.0%. This series remains independent of the additional tests presented in Sections 4.7–4.11; its values are therefore not combined with the means of the new replicates.

The results of the first experiment are presented as measurements specific to that experiment, without an associated standard deviation, because independent replicate readings were not available for this series. The additional series includes three replicates and a 60-minute dark stage; it is therefore presented in separate tables and figures and is not treated as an identical replication of the first experiment.

The coefficient of determination for the calibration curve was $R^2 = 0.9992$, which describes the linearity of the calibration within its range but does not, by itself, establish the accuracy of the sample measurements. Likewise, a decrease in absorbance establishes a decrease in the measured concentration, but does not, by itself, establish complete mineralization or the safety of the degradation products.

The results indicate that the prepared material reduced the methylene blue concentration under the test conditions used. This result is interpreted in light of the amount of material, the initial concentration, the dark stage, and the recorded illumination times, without directly generalizing it to real industrial wastewater.

4.6 Summary of the first main experiment

A gradual decrease in methylene blue concentration from 10.00 to 0.40 mg/L was recorded across the experimental stages, and the calculated overall removal percentage reached 96.0% after the dark stage followed by 60 minutes of illumination. This result is presented as a measured change in dye concentration under the experimental conditions, distinguishing the change that occurred in the dark from the subsequent decrease during illumination.

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

4.7 Characterization results for the prepared material

The analyses were performed on the prepared zinc oxide sample, and the available results are summarized in Table 4.4. These indicators support the description of the sample as zinc oxide with a crystalline structure and a size in the nanoscale range, although the original laboratory reports must be attached.

Table 4.4: Summary of zinc oxide characterization results

Value/interpretation	Observed result	Analysis
Average crystallite size calculated using the Scherrer equation: 22.5 nm.	The reported peaks matched the hexagonal wurtzite structure of ZnO (JCPDS 36-1451).	XRD
Reported size range: 20–35 nm.	Quasi-spherical particles/agglomerates were observed, and their distribution was described as uniform.	SEM
Supports the detection of the Zn–O bond; does not, on its own, establish the reduction or stabilization mechanism.	An absorption peak at 435 cm ⁻¹ attributed to Zn–O vibration, with bands associated with plant-derived groups.	FTIR

The crystallite size inferred from XRD is not necessarily equal to the size of the particles or agglomerates observed by SEM; the two values are therefore presented using their correct designations. Nor are FTIR peaks alone interpreted as direct evidence of the reduction or stabilization mechanism.

4.8 Results of the additional control experiments

The dye was tested under light without a catalyst, and a separate adsorption test was conducted in the dark in the presence of zinc oxide. The results are summarized in Table 4.5, with values presented as mean $\pm$ standard deviation for three independent replicates.

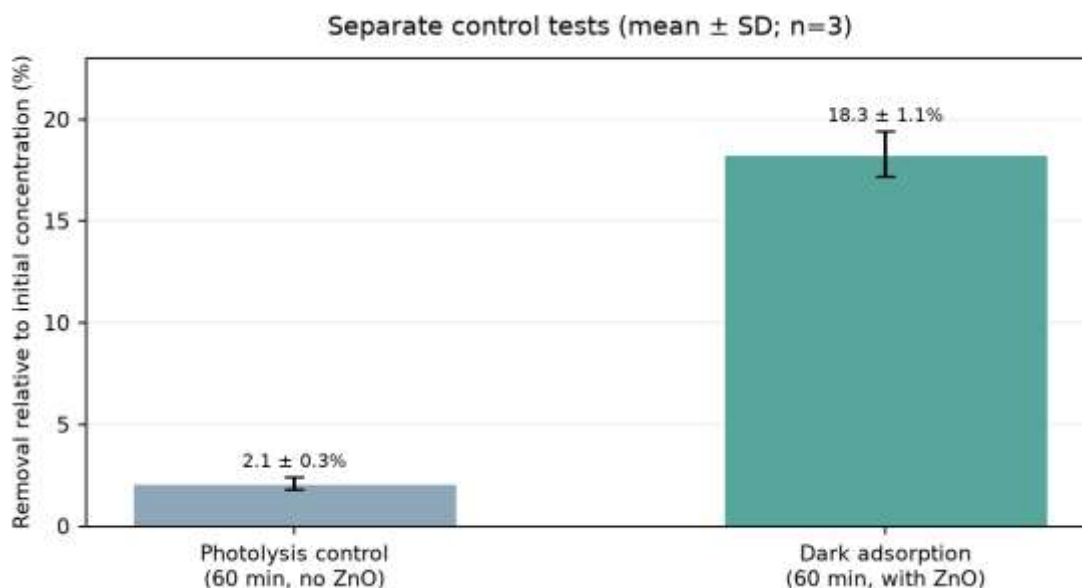
Table 4.5: Endpoint results of the control experiments (Mean $\pm$ SD; n = 3)

Note	Removal (%)	Measurement point	Control experiment
Limited photolysis in the absence of the catalyst.	2.1 $\pm$ 0.3	60 minutes of illumination	Photolysis without ZnO
Initial decrease in dye concentration.	14.7 $\pm$ 0.9	30 minutes in the dark	Dark adsorption with ZnO
Reported final removal value at equilibrium.	18.3 $\pm$ 1.1	60 minutes in the dark	Dark adsorption with ZnO

Mean photolytic removal without a catalyst was 2.1%, with a standard deviation of 0.3 percentage points, after 60 minutes, whereas mean removal in the presence of the catalyst in the dark was 18.3%, with a standard deviation of 1.1 percentage points, at 60 minutes. The 30- and 60-minute data points indicate a slower increase in the second half of the dark period, but the details of the curve between these two points cannot be inferred without intermediate measurements.

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

Figure 4.3: Final percentage recorded in the photolysis and dark adsorption tests.



4.9 Results of the additional experimental series (three replicates)

This series differs from the first main experiment in that its dark stage lasted 60 minutes before illumination began. The absorbance, concentration, and removal values recorded in the replicate data are presented in Table 4.6. These results are not combined with the data from the first experiment because the dark-stage duration differs between the protocols.

Table 4.6: Results of the additional series during the dark and illumination stages (Mean $\pm$ SD; n = 3)

Cumulative removal (%)	Concentration (mg/L)	Absorbance at 664 nm	Time (min)	Stage
0.0 $\pm$ 0.0	10.00 $\pm$ 0.00	0.895 $\pm$ 0.005	0	Initial
14.7 $\pm$ 0.9	8.53 $\pm$ 0.09	0.765 $\pm$ 0.008	30	Dark
18.3 $\pm$ 1.1	8.17 $\pm$ 0.11	0.733 $\pm$ 0.010	60	Dark
33.9 $\pm$ 0.8	6.61 $\pm$ 0.08	0.595 $\pm$ 0.007	10	Illumination
53.0 $\pm$ 0.7	4.70 $\pm$ 0.07	0.425 $\pm$ 0.006	20	Illumination
72.1 $\pm$ 0.6	2.79 $\pm$ 0.06	0.255 $\pm$ 0.005	30	Illumination
86.4 $\pm$ 0.5	1.36 $\pm$ 0.04	0.128 $\pm$ 0.004	45	Illumination
96.5 $\pm$ 0.8	0.35 $\pm$ 0.03	0.039 $\pm$ 0.003	60	Illumination

In this series, the mean concentration was 8.17 mg/L, with a standard deviation of 0.11, after 60 minutes in the dark. After 60 minutes of illumination, the mean concentration was 0.35 mg/L, with a standard deviation of 0.03, and mean cumulative removal was 96.5%, with a standard deviation of 0.8 percentage points, relative to the initial concentration. Cumulative removal includes the decrease that occurred before illumination.

Figure 4.4: Change in dye concentration in the additional series; error bars represent SD.

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

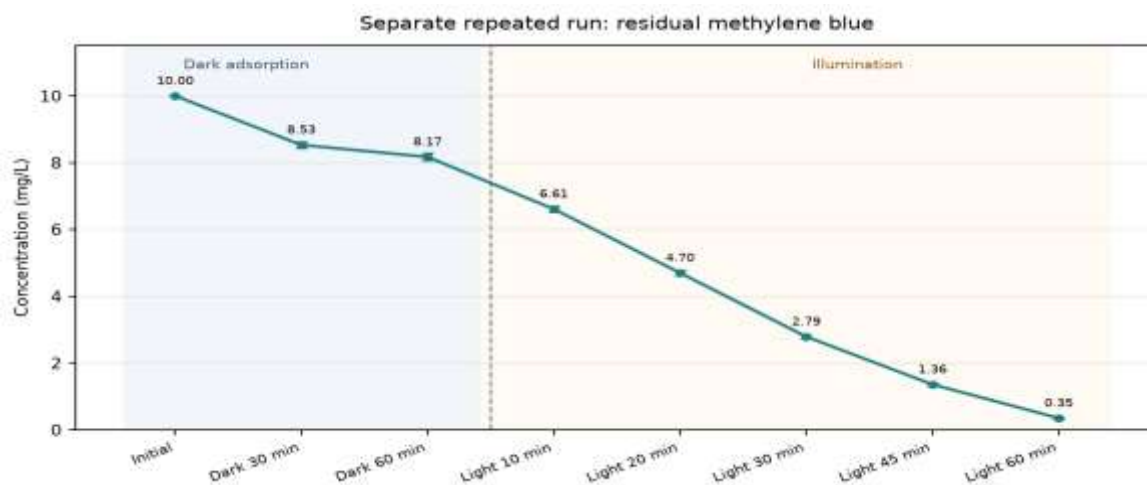
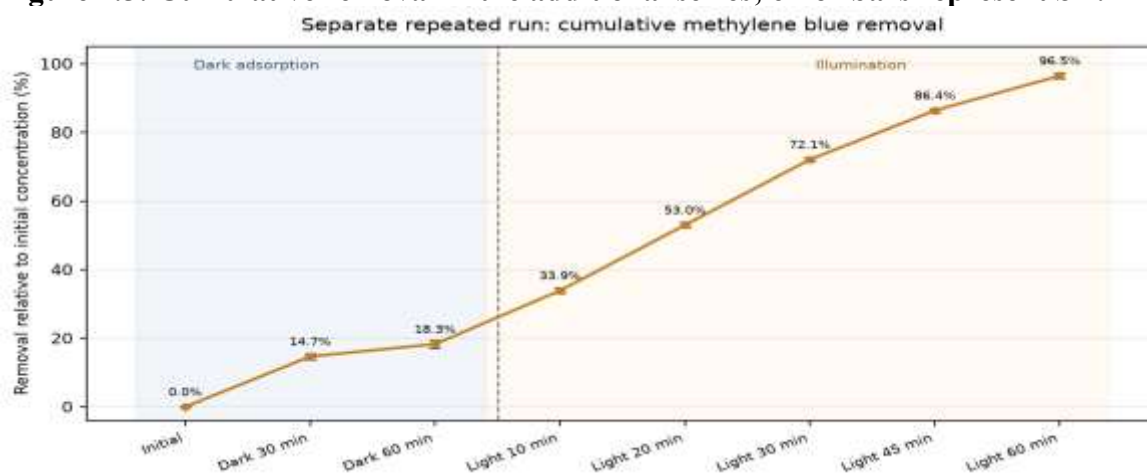


Figure 4.5: Cumulative removal in the additional series; error bars represent SD.



4.10 Comparison between the first experiment and the additional series

The final values are presented descriptively in Table 4.7. The dark-stage duration differs between the two experiments (30 minutes in the first and 60 minutes in the additional series). The additional series is therefore not considered an identical replication of the first, and the two values are neither combined into a single mean nor compared statistically.

Table 4.7: Descriptive comparison of the results of the two independent experiments

Note	Removal (%)	Final concentration (mg/L)	Timing conditions	Series
Reading from the first experiment as previously reported.	96.0	0.40	30 min in the dark + 60 min under illumination	First experiment main
Mean $\pm$ SD; the dark-period duration differs between protocols.	96.5 $\pm$ 0.8	0.35 $\pm$ 0.03	60 min in the dark + 60 min under illumination	Additional series (n=3)

The two results are similar in the magnitude of the final measured decrease. However, the difference in dark adsorption time and the availability of replicate data only for the second series preclude pooling them statistically. The laboratory notebooks must be consulted to verify the record of each experiment, the lighting conditions, and the individual replicate readings.

4.11 Summary of the additional findings

The supplied characterization results showed a hexagonal wurtzite structure, an average crystallite size of 22.5 nm, a size range of 20–35 nm in SEM, and a Zn–O peak at 435 cm⁻¹ in FTIR. Mean photolysis without a catalyst was 2.1%, with a standard deviation of 0.3 percentage points, and dark adsorption was 18.3%, with a standard deviation of 1.1 percentage points, after 60 minutes. In the additional series with three replicates, the mean final concentration was 0.35 mg/L, with a standard deviation of 0.03, and mean cumulative removal was 96.5%, with a standard deviation of 0.8 percentage points. These results remain separate from the first main experiment, in which the final concentration was 0.40 mg/L and removal was 96.0%.

Chapter 5: Conclusions and Recommendations

5.1 Conclusions

- Zinc oxide particles were prepared by green synthesis using *Moringa oleifera* extract, and the supplied XRD, SEM, and FTIR results indicated a nanoscale structure and properties.
- The coefficient of determination for the calibration curve was $R^2 = 0.9992$, and the equation was used to estimate the dye concentration in both series.
- In the first main experiment, the concentration decreased to 0.40 mg/L, and the recorded removal was 96.0% after 30 minutes in the dark and 60 minutes of illumination.
- In the additional series (n=3), the mean final concentration was 0.35 mg/L, with a standard deviation of 0.03, and mean cumulative removal was 96.5%, with a standard deviation of 0.8 percentage points, after 60 minutes in the dark and 60 minutes of illumination.
- The controls showed a mean direct photolytic removal of 2.1%, with a standard deviation of 0.3 percentage points, and dark adsorption of 18.3%, with a standard deviation of 1.1 percentage points, after 60 minutes; the final result in the additional series includes both stages.
- The first experiment and the additional series must remain separate in presentation and analysis because the dark periods differed and replicates were available only for the additional series.

5.2 Recommendations

- Include the original XRD and SEM reports and images and FTIR spectra, along with instrument details, measurement conditions, and the source of the JCPDS card.
- Retain the individual readings for the three replicates and conduct control experiments with durations matching those of the main illumination test to strengthen the comparison.
- Document the type, power, wavelength, and intensity of the light source and its distance from the sample, keeping these conditions constant in comparative experiments.
- Complete the intermediate measurement points in the controls and investigate the effects of pH, material dosage, dye concentration, and illumination time.
- Examine the feasibility of recovering and reusing the material, and assess any changes in its performance after successive cycles.
- Conduct additional analyses if the aim is to demonstrate chemical degradation or identify degradation products, then test the method on real water samples after verifying that it can be applied safely.

Green Synthesis of Zinc Oxide Nanoparticles Using Moringa Leaf Extract and Evaluation of Their Efficiency in Removing Methylene Blue from an Aqueous Solution

References

- Adam, F., Himawan, A., Aswad, M., et al. (2021). Green synthesis of zinc oxide nanoparticles using *Moringa oleifera* L. water extract and its photocatalytic evaluation. *Journal of Physics: Conference Series*, 1763, 012002. [doi:10.1088/1742-6596/1763/1/012002](https://doi.org/10.1088/1742-6596/1763/1/012002)
- Irfan, M., Munir, H., & Ismail, H. (2021). *Moringa oleifera* gum based silver and zinc oxide nanoparticles: Green synthesis, characterization and their antibacterial potential against MRSA. *Biomaterials Research*, 25(1). [doi:10.1186/s40824-021-00219-5](https://doi.org/10.1186/s40824-021-00219-5)
- Matinise, N., Fuku, X. G., Kaviyarasu, K., Mayedwa, N., & Maaza, M. (2017). ZnO nanoparticles via *Moringa oleifera* green synthesis: Physical properties & mechanism of formation. *Applied Surface Science*, 406, 339–347. [Publisher page](#)
- Natrayan, L., Janardhan, G., Nadh, V. S., et al. (2025). Eco-friendly zinc oxide nanoparticles from *Moringa oleifera* leaf extract for photocatalytic and antibacterial applications. *Clean Technologies and Environmental Policy*, 27, 5761–5773. [doi:10.1007/s10098-024-02814-1](https://doi.org/10.1007/s10098-024-02814-1)
- Pal, S., Mondal, S., Maity, J., & Mukherjee, R. (2018). Synthesis and characterization of ZnO nanoparticles using *Moringa oleifera* leaf extract: Investigation of photocatalytic and antibacterial activity. *International Journal of Nanoscience and Nanotechnology*, 14(2), 111–119. [Publisher page](#)
- Parven, N., Almani, K. F., Bhatti, M. A., Tahira, A., Omm-e-Hany, Shah, A. A., Nafady, A., Tonezzer, M., & Ibupoto, Z. H. (2025). *Moringa oleifera* leaves extract-mediated synthesis of ZnO nanostructures for the enhanced photocatalytic oxidation of erythrosine. *RSC Advances*, 15(4), 2810–2824. [doi:10.1039/D4RA08782H](https://doi.org/10.1039/D4RA08782H)
- Perumalsamy, H., Balusamy, S. R., Sukweenadhi, J., et al. (2024). A comprehensive review on *Moringa oleifera* nanoparticles: Importance of polyphenols in nanoparticle synthesis, nanoparticle efficacy and their applications. *Journal of Nanobiotechnology*, 22, Article 71. [doi:10.1186/s12951-024-02332-8](https://doi.org/10.1186/s12951-024-02332-8)
- Sarwar, K., Nazli, Z. H., Munir, H., et al. (2025). Biosynthesis of zinc oxide nanoparticles using *Moringa oleifera* leaf extract, probing antibacterial and antioxidant activities. *Scientific Reports*, 15, Article 20413. [doi:10.1038/s41598-025-08839-w](https://doi.org/10.1038/s41598-025-08839-w)
- Shashikala, B. S., Ashwini, K. R., Lavanya, D. R., et al. (2024). *Moringa oleifera* leaf-based zinc oxide nanoparticles: Green synthesis, characterization, and their antibacterial investigations. *Inorganic Chemistry Communications*, 170, Article 113355. [doi:10.1016/j.inoche.2024.113355](https://doi.org/10.1016/j.inoche.2024.113355)
- Zahro, A. A., Trivida, E., Lailiyah, Q., Sugihartono, S., Dahnum, D., & Bakti, A. N. (2025). Structural characterization of zinc oxide nanoparticles using *Moringa oleifera* leaf extract. *E3S Web of Conferences*, 655, Article 04003. [doi:10.1051/e3sconf/202565504003](https://doi.org/10.1051/e3sconf/202565504003)