

The Removal of Sulfamonomethoxine (SMM) with Copper oxide/Zirconium dioxide (CuO/ZrO₂) Nanocomposites by Photocatalytic Degradation in Pharmaceutical Industry Wastewaters and the Evaluation of Microtox (*Aliivibrio fischeri*) and *Daphnia magna* Acute Toxicity Assays

ABSTRACT

In this study, Copper oxide/zirconium dioxide (CuO/ZrO₂) nanocomposites (NCs) as a photocatalyst was examined during photocatalytic degradation process (PDP) in the efficient removal of Sulfamonomethoxine (SMM) from pharmaceutical industry wastewater (PI ww) plant, İzmir, Turkey. Different pH values (3.0, 4.0, 7.0, 9.0, 10.0 and 12.0), increasing SMM concentrations (5, 10, 20 and 40 mg/l), increasing CuO/ZrO₂ NCs concentrations (100, 200, 400, 800 and 1000 mg/l), different CuO/ZrO₂ NCs mass ratios (1/2, 2/3, 3/4, 4/4, 4/3, 3/2, 2/1), increasing recycle times (1., 2., 3., 4., 5., 6. and 7.) was operated during PDP in the efficient removal of SMM in PI ww. The characteristics of the synthesized nanoparticles (NPs) were assessed using XRD, DRS, FESEM, EDX, FTIR, TEM and XPS analyses, respectively. The acute toxicity assays were operated with Microtox (*Aliivibrio fischeri* also called *Vibrio fischeri*) and *Daphnia magna* acute toxicity tests. The photocatalytic degradation mechanisms of CuO/ZrO₂ NCs and the reaction kinetics of SMM were evaluated in PI ww during PDP. Cost analysis was evaluated for SMM removal with CuO/ZrO₂ NCs by PDP in PI ww. ANOVA statistical analysis was used for all experimental samples. Maximum 99.6% SMM removal was obtained during PDP in PI ww, at 350 W UV-vis light irradiation power, at 147 mW/cm² light intensity, after 120 min, at pH=12.0 and at 25°C, respectively. Maximum 99.2% SMM removal was found with PDP in PI ww, at 5 mg/l SMM, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively. Maximum 99.5% SMM removal was measured to 800 mg/l CuO/ZrO₂ NCs with PDP in PI ww, at 5 mg/l SMM, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively. Maximum 99.4% SMM removal was measured at 4/3wt CuO/ZrO₂ NCs mass ratios, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively. Maximum 99.5% SMM recovery was measured in PI ww during PDP, after 1. recycle time, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 4/3wt CuO/ZrO₂ NCs mass ratio, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively. 97.31% maximum Microtox acute toxicity removal was found in SMM=20 mg/l after 180 min, and at 60°C. It was observed an inhibition effect of SMM=40 mg/l to Microtox after 180 min, and at 60°C. 93.27% maximum *Daphnia magna* acute toxicity removal was obtained in SMM=20 mg/l after 180 min, and at 60°C, respectively. It was obtained an inhibition effect of SMM=40 mg/l to *Daphnia magna* after 180 min and at 60°C. As a result, the CuO/ZrO₂ NCs during PDP in PI ww was stable in harsh environments such as acidic, alkaline, saline and then was still effective process. Finally, the combination of a simple, easy operation preparation process, excellent performance and cost effective, makes this CuO/ZrO₂ NCs a promising option during PDP in PI ww treatment.

Keywords: ANOVA statistical analysis; Antibiotics; Copper oxide/zirconium dioxide (CuO/ZrO₂) nanocomposites; Cost analysis; Diffuse reflectance UV-Vis spectra; Energy-dispersive X-ray; Field emission scanning electron microscopy; Fourier transform infrared spectroscopy; Hydroxyl (OH[•]) radicals; Microtox (*Aliivibrio fischeri* or *Vibrio fischeri*) and *Daphnia magna* acute toxicity tests; Nanoparticles; Pharmaceutical industry wastewater; Photocatalytic degradation mechanisms; Reaction kinetics; Sulfamonomethoxine;

Sulfonamide antibiotics; Transmission electron microscopy; Ultraviolet; X-ray diffraction; X-Ray photoelectron spectroscopy.

1. INTRODUCTION

Emerging contaminants (ECs), sometimes known as contaminants of emerging concern (CECs) can refer to a wide variety of artificial or naturally occurring chemicals or materials that are harmful to human health after long-term disclosure. ECs can be classified into several classes, including agricultural contaminants (pesticides and fertilizers), medicines and antidote drugs, industrial and consumer waste products, and personal care and household cleaning products [1,2]. Antibiotics are one of the ECs that have raised concerns in the previous two decades because they have been routinely and widely used in human and animal health care, resulting in widespread antibiotic residues discharged in surface, groundwater and wastewater.

Antibiotics, which are widely utilized in medicine, poultry farming and food processing [3,4], have attracted considerable attention due to their abuse and their harmful effects on human health and the ecological environment [5,6]. The misuse of antibiotics induces Deoxyribonucleic Acid (DNA) contamination and accelerates the generation of drug-resistant bacteria and super-bacteria [7-9]; thus, some diseases are more difficult to cure [10]. A number of studies have revealed that the level of antibiotics in the soil, air and surface water, and even in potable water, is excessive in many areas [11-13], which will ultimately accumulate in the human body via drinking water and then damage the body's nervous system, kidneys and blood system. Therefore, it is necessary to develop an efficient method to remove antibiotics present in PI ww.

The uncontrolled, ever-growing accumulation of antibiotics and their residues in the environment is an acute modern problem. Their presence in water and soil is a potential hazard to the environment, humans, and other living beings. Many therapeutic agents are not completely metabolized, which leads to the penetration of active drug molecules into the biological environment, the emergence of new contamination sources, the wide spread of bacteria and microorganisms with multidrug resistance [14-16]. Modern PI ww facilities do not allow efficient removal of antibiotic residues from the environment [17,18], which leads to their accumulation in ecological systems [19,20]. Global studies of river pollution with antibiotics have shown that 65% of surveyed rivers in 72 countries on 6 continents are contaminated with antibiotics [21]. According to the World Health Organization (WHO), surface and groundwater, as well as partially treated water, containing antibiotic residue and other pharmaceuticals, typically at < 100 ng/l, whereas treated water has < 50 ng/l, respectively [22]. The discovery of ECs in numerous natural freshwater sources worldwide is growing yearly. Several antibiotic residues have been reported to have been traced at concentrations greater than their ecotoxicity endpoints in the marine environment, specifically in Europe and Africa [23]. Thus, the European Union's Water Framework Directive enumerated certain antibiotics as priority contaminants [24-26]. In some rivers, the concentrations were so high that they posed a real danger to both the ecosystem and human health. The development of effective approaches to the removal of antibiotics from the aquatic environment is of great importance.

The removal of antibiotics and their residues from water and wastewater prior to their final release into the environment is of particular concern [27]. Modern purification methods can be roughly divided into the following three categories depending on the purification mechanism: biological treatment [28,29], chemical degradation [27,30], and physical removal. Each of these methods has its own advantages and disadvantages. For example, biological purification can remove most antibiotic residues, but the introduction of active organisms into the aquatic environment can upset the ecological balance. Various chemical

approaches (ozonation, chlorination, and Fenton oxidation) cannot provide complete purification and, in some cases, lead to the death of beneficial microorganisms due to low selectivity. Photocatalysis is widely used in new environmental control strategies [31-33]. This method has a number of key disadvantages, such as insufficient use of visible light, rapid annihilation of photogenerated carriers, and incomplete mineralization, which greatly limits its application [27].

Sulfonamide antibiotics (SAs) are synthetic antimicrobials derived from sulfanilic acid. Sulfamonomethoxine (SMM) is a SAs. The chemical structure of SAs is characterized by a common sulfanilamide group and a distinct 5- or 6-member heterocyclic ring [34]. The SAs have a wide spectrum of antimicrobial action against most gram-positive and numerous gram-negative microorganisms. They inhibit the proliferation of bacteria by acting as the competitive inhibitors of p-aminobenzoic acid in the folic acid metabolism cycle [35]. SAs are widely used in animal husbandry, and account for a high proportion of the total usage of antibiotics in animal therapy. SAs exhibit high excretion rates in the urine and feces of medicated animals as parent compounds or metabolites [36], and are transferred easily from contaminated sites to surrounding waters because of their low sorption to soil and sediments [35,37]. The lack of appropriate treatment for wastewater from farms for removing SA residues has resulted in the residues reaching the receiving waters surrounding the animal farms [38,39]. The SA residues have been detected in numerous aquatic environments including fish farms [40,41], their effluents and downstream waters [42-44], rivers [45,46], lakes, groundwater [47,48], and sewage wastewater [34,49]. The spread of SA residues in aquatic environments has generated great concern because of the potential risks such as enhancing microbial resistance through the prolonged exposure of microorganisms to SAs [50]. Certain SAs in aquatic environments are potentially toxic to aquatic organisms [51].

The impetus of choosing SMM as the target contaminant stems from the fact that SMM is one of the most popular-used pharmaceuticals used for personal care products (PPCPs) but can cause serious environmental pollutions [52-54]. The facile, effective, and environment-friendly degradation of SMM-contained wastewater by a UV (365 nm)/Oxone oxidative process is reported, with presenting detailed investigations on the effects of pH values, concentrations of reagents, and reaction time within the treatment process [55]. 96.78% removal was found at 5 mg/l SMM, at $\lambda=365$ nm, after 90 min, respectively [55]. 100% removal was measured at 0.06 mM SMM, at 2.5 mM Fe_3O_4 catalyst after 15 min, respectively [56]. 98.5% photodegradation and 99% COD removal were observed at 4.53 mg/l SMM, at 0.49 mmol/l H_2O_2 , at 19.51 $\mu\text{mol/l}$ Fe(II), at pH=4.0 after 120 min, respectively [57]. In a research by Zhu et al. [58], iron oxide magnetic nanoparticles (Fe_3O_4 MNPs) were used to activate persulfate anions ($\text{S}_2\text{O}_8^{2-}$) to produce sulfate free radicals ($\text{SO}_4^{\cdot -}$), which are a powerful oxidant with promising applications to degrade organic contaminants. The kinetics of SMM degradation was studied in the system of Fe_3O_4 MNPs and $\text{S}_2\text{O}_8^{2-}$. A complete removal of the added SMM (0.06 mM) within 15 min with the addition of 1.20 mM $\text{S}_2\text{O}_8^{2-}$ and 2.40 mM Fe_3O_4 MNPs [56]. There was an optimum concentration of Fe_3O_4 MNPs because Fe_3O_4 MNPs may also act as a $\text{SO}_4^{\cdot -}$ scavenger at higher concentrations. Degradation mechanism of SMM on the basis of identification of the degradation intermediates was studied with liquid chromatography combined with mass spectroscopy (LC-MS) [56].

Generally, the advanced oxidation processes (AOPs), such as the Fenton or Fenton-like reaction, ozonation or catalytic ozonation, photocatalytic oxidation, electrochemical oxidation, and ionizing radiation, have been widely used for antibiotics degradation in recent years [59-63]. One of the most promising techniques applied for efficient degradation of antibiotics are AOPs [61,64-67]. Nowadays, particular attention is paid to photocatalytic reactions, in which highly oxidizing species responsible for mineralization of organic pollutants are formed in-situ in the reaction media by means of light and a photocatalyst

[61,64,68,69]. The photocatalytic activity is closely related to the physicochemical properties but also to the morphology and texture of the materials studied, for this reason the synthesis techniques are often of great importance. Photocatalysis, which occurs under exposure to UV light, is also a common method for the environmental pollutant elimination [70]. The conventional photocatalysis utilizes mostly UV from sunlight, which accounts for only 4% solar energy. Through the introduction of catalysts, the utilization rate of sunlight can be effectively improved. To overcome the low-efficiency problem of the photocatalysis, the development of a more efficient catalyst system that would effectively improve the catalytic oxidation efficiency and overcome the existing limitations is important. The catalytic activity of the catalyst can be effectively improved by modulating its surface area, preparation method, and changing its properties and structures [71-74].

Copper oxide (CuO), a p-type semiconductor with a constrained band gap (1.2-2.0 eV) and a foundation for many high-temperature superconductors and giant magnetoresistance materials, has attracted a lot of attention in recent years [75,76]. One of the attractive features of CuONPs is that by controlling its shape and size, optical properties can be controlled. This feature is very important because it directly affects photocatalyst features. This gives importance to CuO synthesis methods because applied synthesis methods can lead to different shapes and sizes [77-79]. Optical properties play an important role in the photocatalyst process, so here the optical properties of CuONPs are investigated in more detail. CuO NPs have the unique ability to regulate the possible energy levels of conduction bands (CBs) and valence bands (VBs), as well as the bandgap, by adjusting the size and shape of CuO NPs. The band gap in CuO NPs is blue-shifted than bulk CuO NPs. As a result, for wider bandgap nanostructured CuO NPs samples exhibiting absorption in the UV field, CuO photocatalyst exhibits strong absorption in the visible spectrum with a little transparency [80-84]. Various techniques have been used to improve the photocatalytic properties of CuO NPs such as NCs formation, binary and ternary heterojunction formation, Z-scheme based photocatalytic system, introducing of different metal ions as dopants, and coupling with carbonaceous materials [85-90].

Zirconium dioxide (or Zirconia, ZrO_2) as broad bandgap (3.25–5.1 eV) semiconductor has been widely explored due to the presence of a large number of O_2 vacancies on the surface, significant ion exchange ability and physicochemical stability [91]. ZrO_2 NPs possess a high value of the dielectric constant, tensile strength, melting and boiling point, however, its wide band gap and low separation rate of photogenerated electron and hole hinder the wide-scale applications [92]. To overcome this, various enhancement strategies like doping, heterojunction formation incorporation of cocatalysts, etc., have been potentially explored. For instance, Co doped ZrO_2 NCs synthesized by facile-chemical precipitation showed greater degrading activity against methylene blue dye when exposed to visible light due to the improved dielectric properties [93]. Therefore, coupling ZrO_2 NPs with narrow band semiconductors like MoS_2 , TiO_2 and $g-C_3N_4$ also retards the photocarrier reassembly and augments its photocatalytic performance [94]. The optical properties of ZrO_2 NPs can be enhanced by introducing new electronic states by doping with rare earth metals. For example, the introduction of Ce (IV) ions in ZrO_2 NPs enhanced the photoresponse through the formation of midgap states and the Ce 4f empty states acted as an electron transfer bridge between VB and CB of ZrO_2 NPs by low energy photons [95]. Recent research has manifested that zirconia-based materials show high redox abilities, large surface area, and low thermal conductivity in PDP. In this regard, Aldeen et al. [92], comprehensively explored the altered ZrO_2 NPs based photocatalysts for organic pollutant degradation. The typical insights of inorganic pollutant degradation, specifically the heavy metal ions present in wastewater, are missing. Additionally, the importance of generating O_2 vacancies (OVs) in ZrO_2 NPs for the synergistic improvement in opto-electronic properties still needs further comprehension.

Hence, the present review article highlights the features of ZrO₂NPs semiconducting material for the photocatalytic removal of organic as well as inorganic pollutants present in wastewater. Starting from the optimal crystal structure of ZrO₂ NPs, various phases and the subsequent photocatalytic features are comprehensively reviewed. Significant methods to circumvent the inherent drawbacks of the bare ZrO₂ photocatalyst have been inclusively illustrated using different methods such as doping, heterojunction formation, morphology and structural modulation along with the formation of OVs. After this, the review introduces facile synthesis routes involving bottom-up (solvothermal, Morphology control, and doping) and top-down methods (ultrasonication and chemical reduction) to design ZrO₂-based photocatalytic materials. Then, in order to highlight the advancement of ZrO₂ as a photocatalyst, the mechanistic insights of wastewater treatment via photocatalysis of organic and inorganic pollutants have been broadly reviewed.

CuO/ZrO₂NCs is one of the most prominent member of CuO-based nanostructures which have been widely prepared and applied in the PDP. The physical and chemical properties of ZrO₂NPs lead to excellent photodegradation via CuO/ZrO₂NCs[96-98]. Nanda et al. [99]prepared CuO/ZrO₂ –MCM-41 NCs via integrating ZrO₂ into the MCM-41 (M-41) framework, then loading CuO NPs using the wetness impregnation process while maintaining a Si/Zr ratio of 10. For the photoreduction of Cr⁶⁺, CuO/ZrO₂–MCM-41 was found to be an effective photocatalyst. Within 30 min, CuO/ZrO₂–MCM-41 NCs achieved at99% reduction in Cr⁶⁺[100]. CuO/ZrO₂NCs were prepared via modified sol-gel and solid-state process. The structural and morphological properties of as-prepared samples were compared. Then, The photocatalytic H₂ evolution from oxalic acid solution under solar irradiation was used to examine the photocatalytic activity of CuO/ZrO₂NCs. It was reported that when CuO/ZrO₂NCs is made by sol-gel process and the mole ratio of CuO to ZrO₂ is 40%, the optimum activity of photocatalytic H₂ evolution (2.41 mmol/h.μ) is achieved [101].

In this study, CuO/ZrO₂NCs as a photocatalys was examined during PDP in the efficient removal of Sulfamonomethoxine (SMM) fromPI ww plant, İzmir, Turkey. Different pH values (3.0, 4.0, 7.0, 9.0, 10.0 and 12.0), increasing SMM concentrations (5, 10, 20 and 40 mg/l), increasing CuO/ZrO₂ NCs concentrations (100, 200, 400, 800 and 1000 mg/l), different CuO/ZrO₂ NCs mass ratios (1/2, 2/3, 3/4, 4/4, 4/3, 3/2, 2/1), increasing recycle times (1., 2., 3., 4., 5., 6. and 7.) was operated during PDPin the efficient removal of SMM in PI ww. The characteristics of the synthesized NPs were assessed using XRD, DRS, FESEM, EDX, FTIR, TEM andXPS analyses, respectively. The acute toxicity assays were operated with Microtox (*Aliivibrio fischeri*) and *Daphnia magna* acute toxicity tests. The PDP mechanisms of CuO/ZrO₂ NCs and the reaction kinetics of SMM were evaluated in PI ww during PDP. Cost analysis was evaluated for SMM removal with CuO/ZrO₂ NCs by PDP in PI ww. ANOVA statistical analysis was used for all experimental samples.

2. MATERIALS AND METHODS

2.1. Characterization of Pharmaceutical Industry Wastewater

Characterization of the biological aerobic activated sludge proses from a PI wwplant, İzmir, Turkey was performed(Table 1).

Table 1: Characterization of Pharmaceutical Industry Wastewater (n=3, mean value)

Parameters	Unit	Concentrations
Chemical oxygen demand-total (COD _{total})	(mg/l)	4000
Chemical oxygen demand-dissolved (COD _{dissolved})	(mg/l)	3200
Biological oxygen demand-5 days (BOD ₅)	(mg/l)	1500
BOD ₅ / COD _{dissolved}		0.5
Total organic carbons (TOC)	(mg/l)	1800

Dissolved organic carbons (DOC)	(mg/l)	1100
pH		8.3
Salinity as Electrical conductivity (EC)	(mS/cm)	1552
Total alkalinity as CaCO ₃	(mg/l)	750
Total volatile acids (TVA)	(mg/l)	380
Turbidity (Nephelometric Turbidity unit, NTU)	NTU	7.2
Color	1/m	50
Total suspended solids (TSS)	(mg/l)	250
Volatile suspended solids (VSS)	(mg/l)	187
Total dissolved solids (TDS)	(mg/l)	825
Nitride (NO ₂ ⁻)	(mg/l)	1.7
Nitrate (NO ₃ ⁻)	(mg/l)	1.91
Ammonium (NH ₄ ⁺)	(mg/l)	2.3
Total Nitrogen (Total-N)	(mg/l)	3.2
Sulfite (SO ₃ ⁻²)	(mg/l)	21.4
Sulfate (SO ₄ ⁻²)	(mg/l)	29.3
Chloride (Cl ⁻)	(mg/l)	37.4
Bicarbonate (HCO ₃ ⁻)	(mg/l)	161
Phosphate (PO ₄ ⁻³)	(mg/l)	16
Total Phosphorus (Total-P)	(mg/l)	40
Total Phenols	(mg/l)	70
Oil&Grease	(mg/l)	220
Cobalt (Co ⁺³)	(mg/l)	0.2
Lead (Pb ⁺²)	(mg/l)	0.4
Potassium (K ⁺)	(mg/l)	17
Iron(Fe ⁺²)	(mg/l)	0.42
Chromium (Cr ⁺²)	(mg/l)	0.44
Mercury (Hg ⁺²)	(mg/l)	0.35
Zinc (Zn ⁺²)	(mg/l)	0.11

2.2. Preparation of CuO NPs

CuO NPs were prepared as the following: First, copper sulfate (CuSO₄) and ammonia (NH₃) were applied as reactants. The stock solution of 0.1 M CuSO₄ was prepared in 100 ml deionized water. To this stock solution, aqueous NH₃ was added under continuous stirring to get the reactants at pH=9.0. The solution is next transferred into Teflon lined sealed stainless steel autoclaves and maintained at 200°C for 6 h. It was then let to cool to 35°C. The precipitate so obtained is placed in a furnace and calcined for 2 h at 500°C.

2.3. Synthesis of ZrO₂NPs

In a conventional synthesis, in 100 ml distilled water 0.1 M ZrOCl₂.8H₂O was dissolved with effective stirring. After a few minutes, 0.2 M KOH is added to the above solution. Afterward, the solution formed is transferred into a stainless steel Teflon lined sterilized capacity of 100 ml and kept in an oven at 180°C for 16 h. To remove the soluble impurities and depress agglomeration, the resulting precipitates are cleaned with distilled water and absolute ethanol (C₂H₆O). The final product was dried for 3 h in a vacuum at 80°C.

2.4. Preparation of CuO/ZrO₂NPs

The as-prepared CuO and ZrO₂ nanostructures were dispersed in deionized water under vigorous stirring separately. After that, the CuO NPs dispersion was added to ZrO₂NPs

dispersion under ultrasonic for 60 min at 25°C. The final CuO/ZrO₂NCs was filtered and dried at 60°C for 10 h.

2.5. Photocatalytic Degradation Experiments

The photocatalytic activity of CuO/ZrO₂NCs was studied against the photodegradation of SMM antibiotic under ultraviolet (UV) and visible irradiation. To attain the adsorption-desorption equilibrium, 0.03 g of CuO/ZrO₂NCs powder was dispersed into a 50 ml solution of SMM with the concentration of 1 mg/l, then stirred in the dark for 30 min. After then, the as-obtained suspension was exposed to the light of various wavelengths. For providing UV-Vis test, 5 ml suspensions were taken at various periods and filtered to remove the CuO/ZrO₂NCs.

2.6. Photocatalytic Degradation Reactor

A 2 liter cylinder quartz glass reactor was used for the photodegradation experiments in **PI ww** at different operational conditions. 1000 ml **PI ww** was filled for experimental studies and the photocatalyst were added to the cylinder quartz glass reactors. The UV-A lamps (light intensity=147 mW/cm²) were placed to the outside of the photo-reactor with a distance of 3 mm. The photocatalytic reactor was operated with constant stirring (1.5 rpm) during the **PDP**. In a typical experiment, 25 mg sample was added to 50 ml target pollutant solution. Prior to irradiation, the suspensions were stirred in dark for 100 min to reach adsorption/desorption equilibrium. 10 ml reacting solution were sampled and centrifugated at 10000 rpm, at different time intervals. The UV irradiation treatments were created using UV-A lamp emitting in $\lambda=350-400$ nm range ($\lambda_{\max}=368$ nm; FWHM=17 nm; Actinic BL TL-D 18W, Philips). Seven 50 W UV-A lamps (Total: 350 W UV-A lamps) were used during experimental conditions for this study. The residual concentration of organic solution in the filtrate was analyzed by using UV-vis spectrophotometer.

2.7. Characterization

2.7.1. X-Ray Diffraction (XRD) Analysis

Powder XRD patterns were recorded on a Shimadzu XRD-7000, Japan diffractometer using Cu K α radiation ($\lambda=1.5418$ Å, 40 kV, 40 mA) at a scanning speed of 1°/min in the 10-80°2 θ range. Raman spectrum was collected with a Horiba Jobin Yvon-Labram HR UV-Visible NIR (200-1600 nm) Raman microscope spectrometer, using a laser with $\lambda=512$ nm. The spectrum was collected from 10 scans at a resolution of 2 /cm. The zeta potential was measured with a SurPASS Electrokinetic Analyzer (Austria) with a clamping cell at 300 mbar.

2.7.2. Field Emission Scanning Electron Microscopy (FESEM) Analysis

The morphological features and structure of the synthesized catalyst were investigated by a FESEM (FESEM, Hitachi S-4700), equipped with an EDX spectrometry device (TESCAN Co., Model III MIRA) to investigate the composition of the elements present in the synthesized catalyst.

2.7.3. Energy Dispersive X-Ray (EDX) Spectroscopy Analysis

The morphological features and structure of the synthesized catalyst were researched by an EDX spectrometry device (TESCAN Co., Model III MIRA) to investigate the composition of the elements present in the synthesized catalyst.

2.7.4. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectra of samples was recorded using the FT-NIR spectroscope (RAYLEIGH, WQF-510).

2.7.5. Transmission Electron Microscopy (TEM) Analysis

The structure of the samples were analysed TEM analysis. TEM analysis was recorded in a JEOL JEM 2100F, Japan under 200 kV accelerating voltage. Samples were prepared by applying one drop of the suspended material in ethanol onto a carbon-coated copper TEM grid, and allowing them to dry at 25°C.

2.7.6. Diffuse Reflectance UV-Vis Spectra (DRS) Analysis

DRS Analysis in $\lambda=200-800$ nm were recorded on a Cary 5000 UV-Vis Spectrophotometer from Varian.

2.7.7. X-Ray Photoelectron Spectroscopy (XPS) Analysis

The valence state of NPs was investigated and was analyzed using XPS (ESCALAB 250Xi, England). XPS used an Al K α source and surface chemical composition and reduction state analyses was done, with the core levels recorded using a pass energy of 30 eV (resolution ≈ 0.10 eV). The peak fitting of the individual core-levels was done using XPS-peak 41 software, achieving better fitting and component identification. All binding energies were calibrated to the C 1s peak originating from C-H or C-C groups at 284.6 eV.

2.8. Analytical Procedures

COD_{total}, COD_{dissolved}, Total-P, PO₄³⁻-P, Total-N, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, BOD₅, pH, T[(°C)], TSS, TVSS, TOC, Oil, Cl⁻, total phenol, TVA, DOC, total alkalinity, turbidity, TDS, color, SO₃⁻², SO₄⁻², HCO₃⁻, salinity, Co⁺³, Pb⁺², K⁺, Fe⁺², Cr⁺², Hg⁺² and Zn⁺² were measured according to the Standard Methods (2022) 5220B, 5220D, 4500-P, 4500-PO₄⁻³, 4500-N, 4500-NH₄⁺, 4500-NO₃⁻, 4500-NO₂⁻, 5210B, 4500-H⁺, 2320, 2540D, 2540E, 5310, 5520, 4500-Cl⁻, 5530, 5560B, 5310B, 2320, 2130, 2540E, 2120, 4500-SO₃⁻², 4500-SO₄⁻², 5320, 2520, 3500-Co⁺³, 3500-Pb⁺², 3500-K⁺, 3500-Fe⁺², 3500-Cr⁺², 3500-Hg⁺², 3500-Zn⁺², respectively [102].

Total-N, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, Total-P, PO₄³⁻-P, total phenol, Co⁺³, Pb⁺², K⁺, Fe⁺², Cr⁺², Hg⁺², Zn⁺², SO₃⁻², and SO₄⁻² were measured with cell test spectroquant kits (Merck, Germany) at a spectroquant NOVA 60 (Merck, Germany) spectrophotometer (2003).

The measurement of color was carried out following the methods described by Olthof and Eckenfelder [103] and Eckenfelder [104]. According these methods, the color content was determined by measuring the absorbance at three wavelengths (445, 540 and 660 nm), and taking the sum of the absorbances at these wavelengths. In order to identify the color in 25 ml PI ww was acidified at pH=2.0 with a few drops of 6 N HCl and extracted three times with 25 ml ethyl acetate. The pooled organic phases were dehydrated on sodium sulphate, filtered and dried under vacuum. The residue was silylated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in dimethylformamide and analyzed by gas chromatograph (GC) (Agilent Technology model 6890N) equipped with a mass selective (MS) detector (Agilent 5973 inert MSD) gas chromatography-mass spectrometry (GC-MS). Mass spectra were recorded using a VGTS 250 spectrometer equipped with a capillary SE 52 column (HP5-MS 30 m, 0.25 mm ID, 0.25 μ m) at 220°C with an isothermal program for 10 min. The initial oven temperature was kept at 50°C for 1 min, then raised to 220°C at 25°C/min and from 200 to 300°C at 8°C/min, and was then maintained for 5.5 min. High purity He (g) was used as the carrier gas at constant flow mode (1.5 ml/min, 45 cm/s linear velocity).

The total phenol was monitored as follows: 40 ml PI ww was acidified to pH=2.0 by the addition of concentrated HCl. Total phenol was then extracted with ethyl acetate. The organic phase was concentrated at 40°C to about 1 ml and silylated by the addition of N,O-bis(trimethylsilyl) acetamide (BSA). The resulting trimethylsilyl derivatives were analysed by GC-MS (Hewlett-Packard 6980/HP5973MSD).

Methyl tertiary butyl ether (MTBE) was used to extract oil from the water and NPs. GC-MS analysis was performed on an Agilent GC system. Oil concentration was measured using a UV-vis spectroscopy fluorescence spectroscopy and a GC-MS (Hewlett-Packard 6980/HP5973MSD). UV-vis absorbance was measured on a UV-vis spectrophotometer and oil concentration was calculated using a calibration plot which was obtained with known oil concentration samples.

2.9. Acute Toxicity Assays

2.9.1. Microtox Acute Toxicity Test

Toxicity to the bioluminescent organism *Aliivibrio fischeri* (also called *Vibrio fischeri* or *V. fischeri*) was assayed using the Microtox measuring system according to DIN 38412L34, L341, (EPS 1/ RM/24 1992). Microtox testing was performed according to the standard procedure recommended by the manufacturer [105]. A specific strain of the marine bacterium, *V. fischeri*-Microtox LCK 491 kit [105], was used for the Microtox acute toxicity assay. Dr. LANGE LUMIX-mini type luminometer was used for the Microtox toxicity assay [106].

2.9.2. *Daphnia magna* Acute Toxicity Test

To test toxicity, 24-h born *Daphnia magna* were used as described in Standard Methods sections 8711A, 8711B, 8711C, 8711D and 8711E, respectively [107]. After preparing the test solution, experiments were carried out using 5 or 10 *Daphnia magna* introduced into the test vessels. These vessels had 100 ml of effective volume at pH=7.0–8.0, providing a minimum DO=6 mg/l, at an ambient T=20–25°C. Young *Daphnia magna* were used in the test (≤ 24 h old); 24–48 h exposure is generally accepted as standard for a *Daphnia magna* acute toxicity test. The results were expressed as mortality percentage of the *Daphnia magna*. Immobile animals were reported as dead *Daphnia magna*.

2.10. Statistical Analysis

ANOVA analysis of variance between experimental data was performed to detect F and P values. The ANOVA test was used to test the differences between dependent and independent groups, [108]. Comparison between the actual variation of the experimental data averages and standard deviation is expressed in terms of F ratio. F is equal (found variation of the date averages/expected variation of the date averages). P reports the significance level, and d.f indicates the number of degrees of freedom. Regression analysis was applied to the experimental data in order to determine the regression coefficient R^2 , [109]. The aforementioned test was performed using Microsoft Excel Program.

All experiments were carried out three times and the results are given as the means of triplicate samplings. The data relevant to the individual pollutant parameters are given as the mean with standard deviation (SD) values.

3. RESULTS AND DISCUSSIONS

3.1. Characterizations of CuO/ZrO₂ NCs

3.1.1. The Results of X-Ray Diffraction (XRD) Analysis

The results of XRD analysis was observed to pure CuO NPs and pure ZrO₂ NPs, respectively, in PI ww with PDP for SMM removal (Figure 1). The characterization peaks of pure CuO NPs were observed at 2θ values and corresponding of 30.05° (110), 33.19° (002), 37.18° (111), 39.75° (202), 48.11° (020), 59.07° (202), 62.58° (113), 67.80° (311) and 75.12° (220), respectively, in PI ww with PDP for SMM removal (Figure 1a). The characterization peaks of pure ZrO₂ NPs were obtained at 2θ values and corresponding of 30.04° (110), 32.79° (011), 43.52° (110), 50.59° (111), 59.17° (002), 61.78° (200), 65.24° (211) and 74.37° (220), respectively, in PI ww with PDP for SMM removal (Figure 1b).

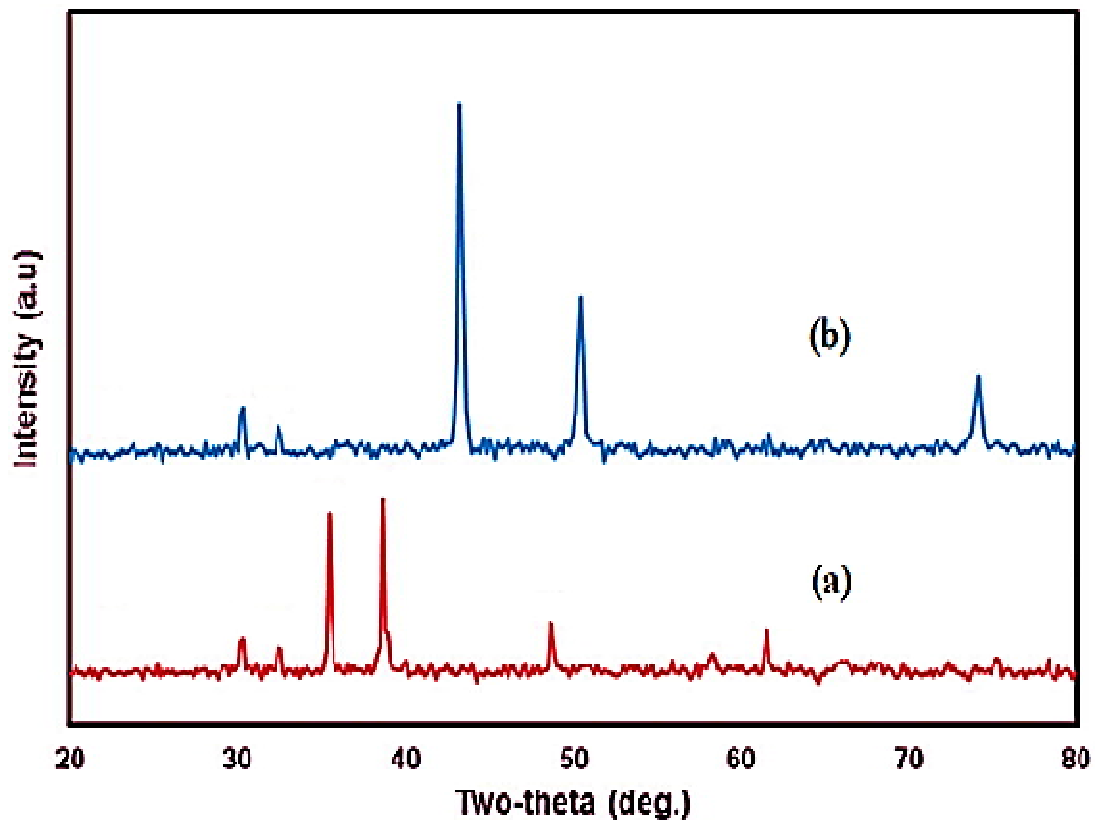


Figure 1:The XRD patterns of (a) pure CuO NPs (red pattern) and (b) pure ZrO₂ NPs (blue pattern), respectively, in PI ww with PDP for SMM removal.

3.1.2. The Results of Diffuse reflectance UV-Vis Spectra (DRS) Analysis

The absorption spectra of SMM was observed in DRS Analysis (Figure 2). First, the absorption spectra of SMM were obtained at maximum 1600 mg/l in the $\lambda=200-600$ nm using diffuse reflectance UV-Vis spectra (Figure 2). Absorption peaks were observed at $\lambda=225$ nm for CuO/ZrO₂ NCs, respectively, in PI ww with PDP for SMM removal (Figure 2).

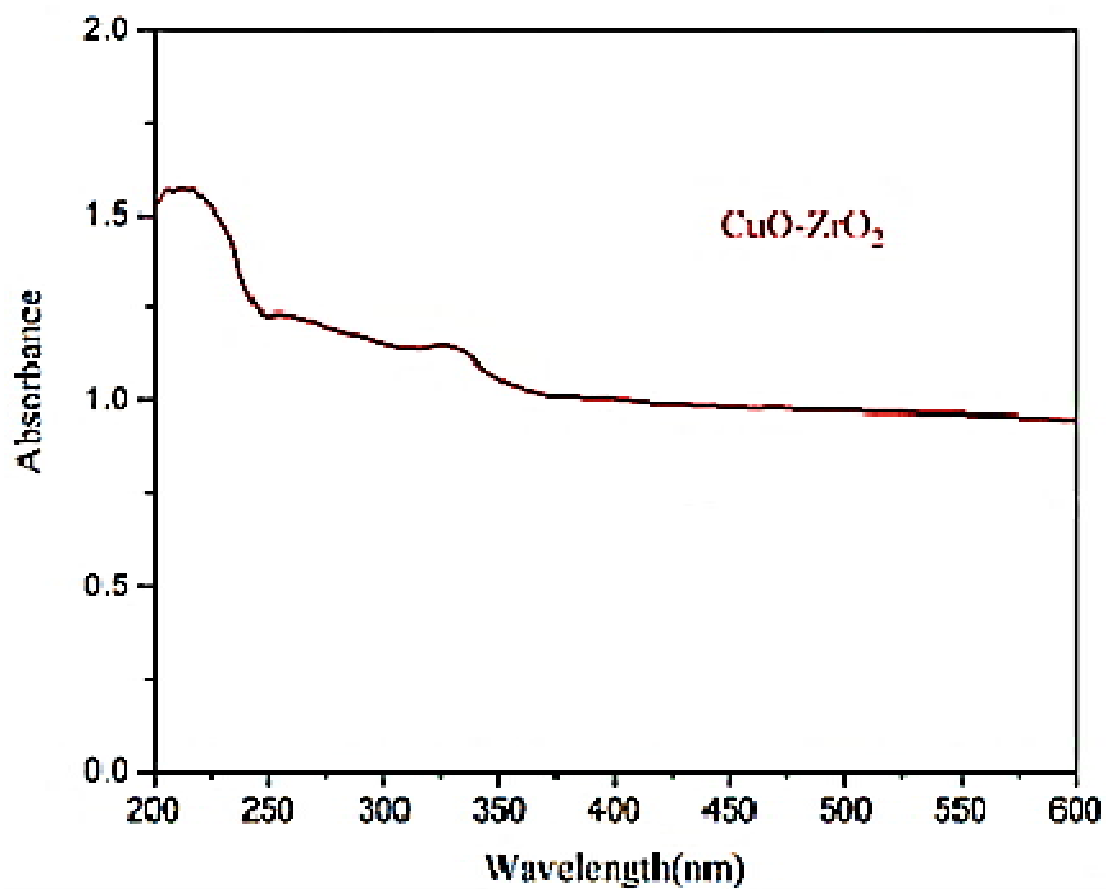


Figure 2:The DRS patterns of CuO/ZrO₂ NCs in PI ww with PDP for SMM removal.

3.1.3. The Results of Field Emission Scanning Electron Microscopy (FESEM) Analysis

The morphological features of pure CuO NPs, pure ZrO₂ NPs and CuO/ZrO₂ NCs were characterized through FESEM images (Figure 3). The FESEM images of pure CuO NPs (Figure 3a), pure ZrO₂ NPs (Figure 3b) and CuO/ZrO₂ NCs (Figure 3c) were characterized in PI ww with PDP for SMM removal.

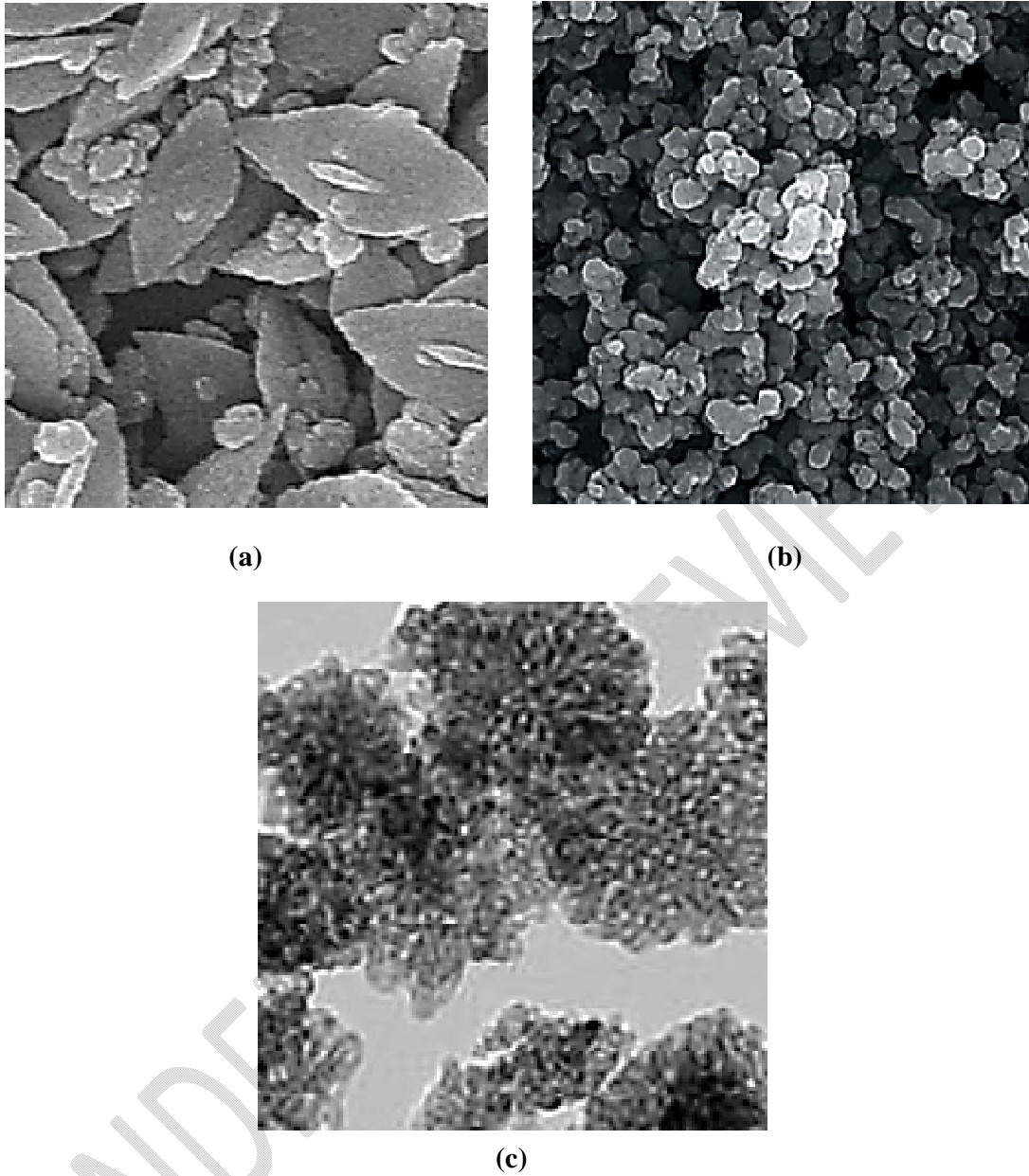


Figure 3:FESEM images of (a)pure CuO NPs, (b)pure ZrO₂NPs and (c)CuO/ZrO₂NCs, respectively,in PI ww with PDP for SMM removal. (FESEM images scale: 200 nm)

3.1.4. The Results of Energy Dispersive X-Ray (EDX) Spectroscopy Analysis

The EDX analysis was also performed to investigate the composition ofCuO/ZrO₂ NCs in PI ww with PDP for SMM removal(Figure 4).

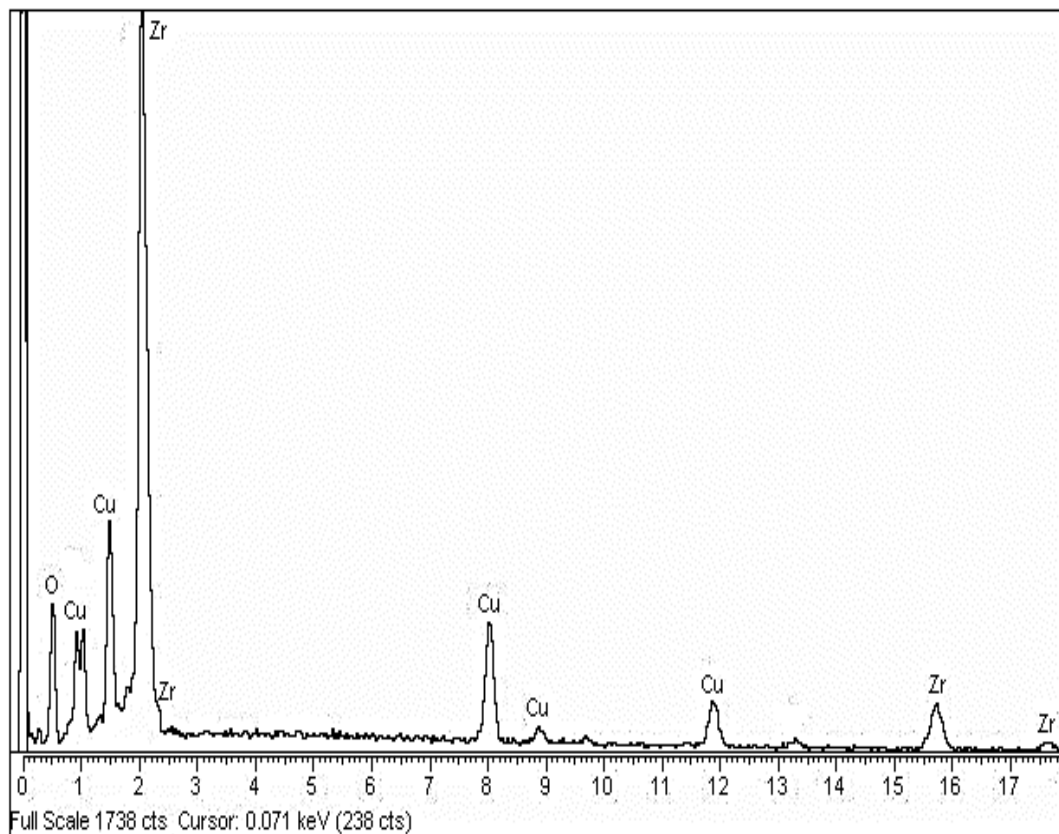


Figure 4:EDX spectrum of CuO/ZrO₂ NCs, respectively, in **PI ww** with **PDP** for SMM removal.

3.1.5. The Results of Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectrum of CuO/ZrO₂ NCs in **PI ww** with **PDP** for SMM removal (**Figure 5**). The main peaks of FTIR spectrum for CuO/ZrO₂ NCs was observed at 3475 cm⁻¹, 1051 cm⁻¹, 627 cm⁻¹ and 512 cm⁻¹ wavenumber, respectively (**Figure 5**).

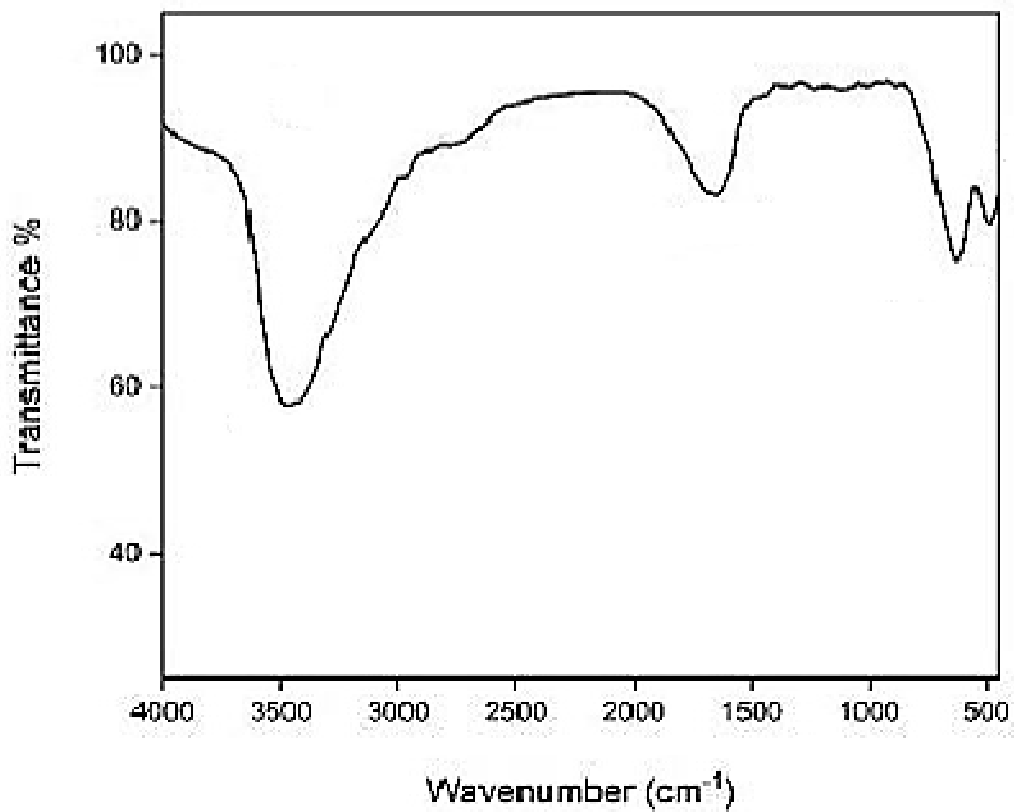


Figure 5: FTIR spectrum of CuO/ZrO₂ NCs in PI ww with PDP for SMM removal.

3.1.6. The Results of Transmission Electron Microscopy(TEM) Analysis

The TEM images of CuO/ZrO₂ NCs was observed in micromorphological structure level in PI ww with PDP for SMM removal (**Figure 6**).

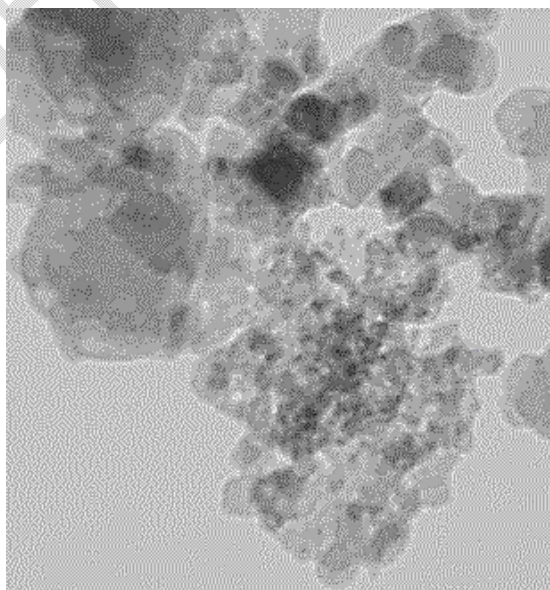


Figure 6: TEM images of CuO/ZrO₂ NCs in micromorphological structure level in PI ww with PDP for SMM removal. (TEM images scale: 200 nm)

3.1.7. X-Ray Photoelectron Spectroscopy (XPS) Analysis

The XPS analysis of CuO/ZrO₂ NCs in micromorphological structure level in PI ww with PDP for SMM removal (Figure 7).

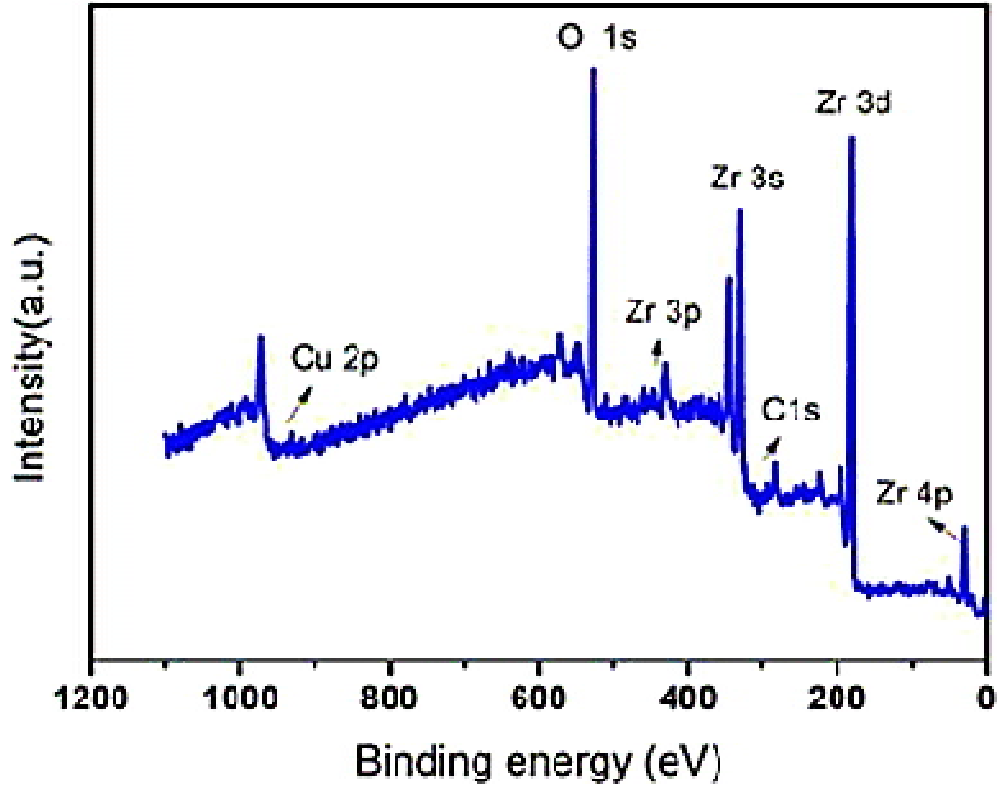


Figure 7: XPS spectra of CuO/ZrO₂ NCs in micromorphological structure level in PI ww with PDP for SMM removal.

3.2. The Reaction Kinetics of SMM Antibiotic

The reaction kinetics SMM were investigated using the Langmuir–Hinshelwood first-order kinetic model, expressed by Eddy et al. [110], as following Equation (1):

$$r_o = -\frac{dc}{dt} = kC(1)$$

where; r_o : denotes the initial photocatalytic degradation reaction rate (mg/l.min), and k : denotes the rate constant of a first-order reaction. At the beginning of the reaction, $t = 0$, $C_t = C_0$, the equation can be obtained after integration as following Equation (2):

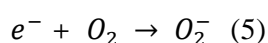
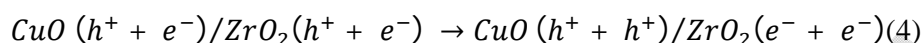
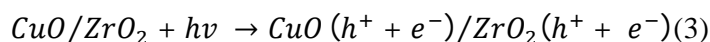
$$\ln \frac{c}{c_0} = -kt \tag{2}$$

where; C_0 and C : are the initial and final concentration (mg/l) of SMM; the solution at t (min) and k (1/min) are the rate constant.

The correlation coefficients had R^2 values greater than 0.9, as a result, the first-order kinetic model fit the experimental data well. The first-order rate constants (k) were determined from the slope of the linear plots.

3.3. Photocatalytic Degradation Mechanisms

The photocatalytic performance of the catalyst in the degradation of SMM is determined by photons. The degradation mechanism of SMM by hydroxyl radicals (OH^\bullet) radicals concerning CuO/ZrO_2 NCs as following equations (**Equation 3, Equation 4, Equation 5, Equation 6, Equation 7, Equation 8, Equation 9 and Equation 10**):



CuO/ZrO_2 NCs absorbs photons with energies greater than the photocatalyst bandgap. As a result, the electron in the VB jumps to the CB, leaving a hole in the CB. The electrons present in the CB and VB will react with O_2 and H_2O molecules which are absorbed by the photocatalyst and lead to the formation of OH^\bullet radicals which react with SMM. OH^\bullet radicals are produced when the photocatalyst surface is illuminated with photons, and OH^\bullet radicals are strong oxidising species, with an oxidation potential of approximately 2.8 V [as opposed to Normal hydrogen electrode (NHE)], which may increase total pollutant mineralisation. Normally, the higher the rate of formation of OH^\bullet radicals, the greater the separation efficiency of electron-hole pairs. In this way, there is a correlation between the increased photocatalytic activity and the rate of formation of OH^\bullet radicals. The OH^\bullet radicals generation of CuO/ZrO_2 NCs was extremely high, indicating that the sample has a high electron and hole separation rate.

3.4. Effect of Increasing pH values for SMM Removal in PI ww during PDP

Increasing pH values (pH=3.0, pH=4.0, pH=7.0, pH=9.0, pH=10.0 and pH=12.0, respectively) was examined during PDP in PI ww for SMM removal, at 350 W UV-vis, at 147 mW/cm^2 , after 120 min and at 25°C, respectively (**Figure 8**). 52.2%, 75.7%, 80.4%, 87.5% and 95.8% SMM removal was measured at pH=3.0, pH=4.0, pH=7.0, pH=9.0 and pH=10.0, respectively, at 350 W UV-vis, at 147 mW/cm^2 , after 120 min and at 25°C, respectively (**Figure 8**). The maximum 99.6% SMM removal was obtained during PDP in PI ww, at 350 W UV-vis, at 147 mW/cm^2 , after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 8**).

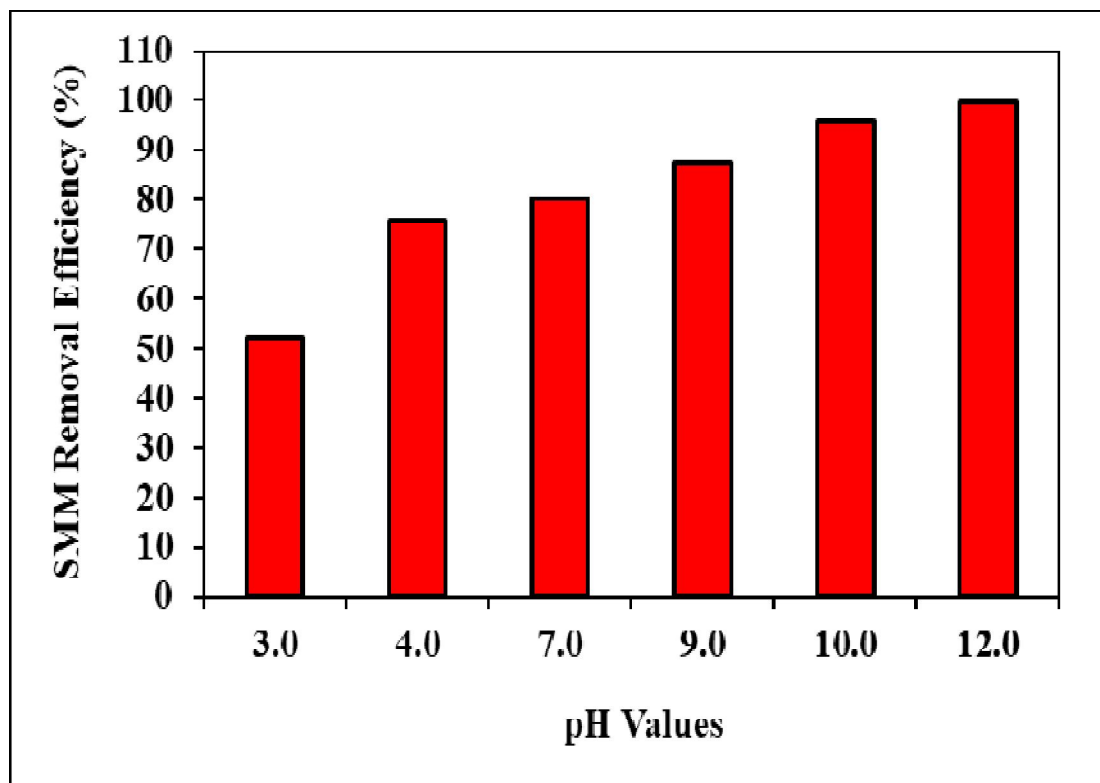


Figure 8:Effect of increasing pH values for SMM removal in **PI ww** during **PDP**, at 350 W UV-vis, at 147 mW/cm², after 120 min and at 25°C, respectively.

3.5. Effect of Increasing SMM Concentrations for SMM Removal in **PI ww** during **PDP**

Increasing SMM concentrations (5, 10, 20 and 40 mg/l) were operated at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 9**). 90.3%, 82.1% and 59.7% SMM removals were obtained to 10, 20 and 40 mg/l SMM concentrations, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 9**). The maximum 99.2% SMM removal was found with **PDP** in **PI ww**, at 5 mg/l SMM, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 9**).

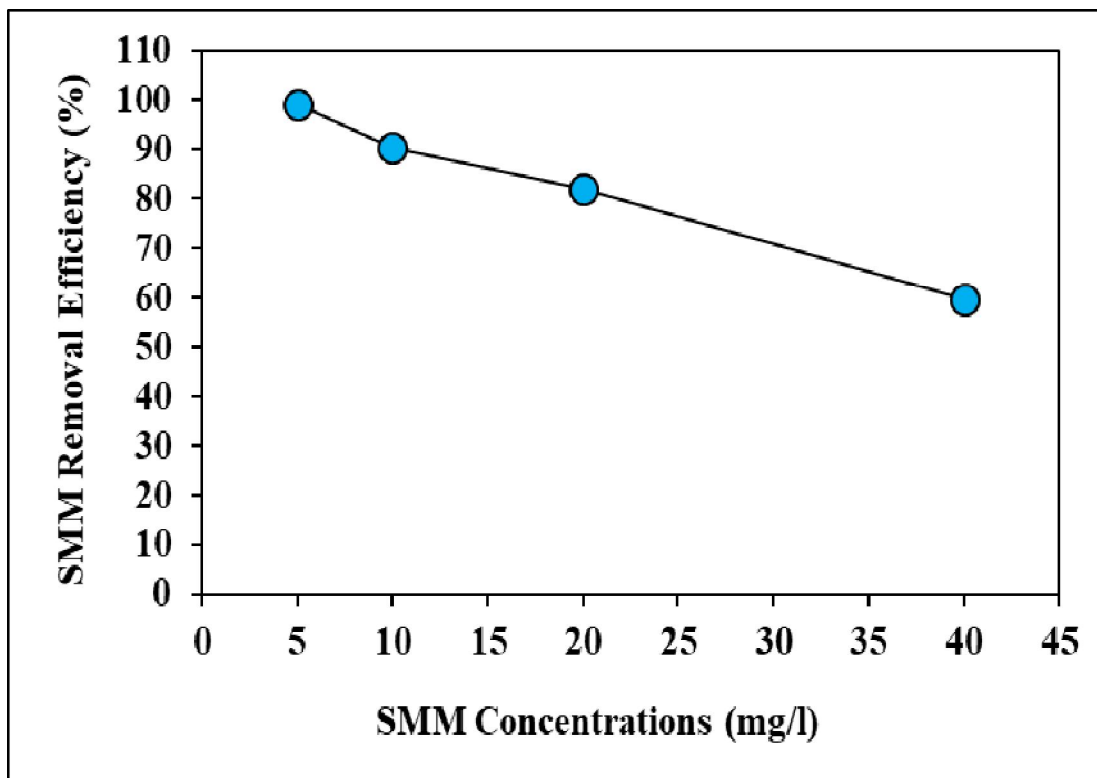


Figure 9: Effect of increasing SMM concentrations for SMM removal in **PI ww** during **PDP**, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively.

3.6. Effect of Increasing CuO/ZrO₂ NCs Concentrations for SMM Removals in **PI ww** during **PDP**

Increasing CuO/ZrO₂ NCs concentrations (100, 200, 400, 800 and 1000 mg/l) were operated at 5 mg/l SMM, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 10**). 60.5%, 70.1%, 78.8% and 80.1% SMM removals were obtained to 100, 200, 400 and 1000 mg/l CuO/ZrO₂ NCs concentrations, respectively, at 5 mg/l SMM, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 10**). The maximum 99.5% SMM removal was measured to 800 mg/l CuO/ZrO₂ NCs with **PDP** in **PI ww**, at 5 mg/l SMM, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 10**).

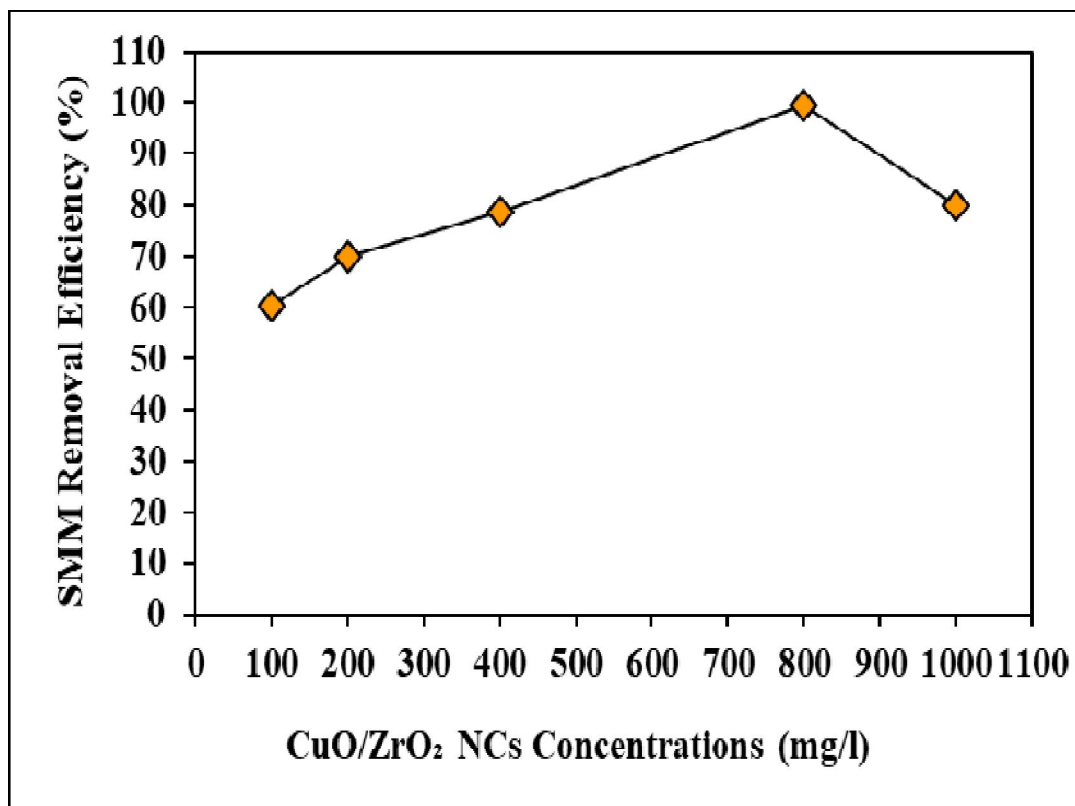


Figure 10: Effect of increasing CuO/ZrO₂NCs concentrations for SMM removal in **PI ww** during **PDP**, at 5 mg/l SMM, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively.

3.7. Effect of Different CuO/ZrO₂ NCs Mass Ratios for SMM Removals in **PI ww during **PDP****

Different CuO/ZrO₂ mass ratios (1/2wt, 2/3wt, 3/4wt, 4/4wt, 4/3wt, 3/2 and 2/1wt, respectively) were examined for SMM removal in **PI ww** during **PDP**, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 11**). 47.3%, 53.6%, 68.9%, 84.7%, 73.1% and 59.2% SMM removals were measured at 1/2wt, 2/3 wt, 3/4wt, 4/4wt, 3/2 and 2/1wt CuO/ZrO₂ NCs mass ratios, respectively, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 11**). The maximum 99.4% SMM removal was measured at 4/3wt CuO/ZrO₂ NCs mass ratios, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (**Figure 11**).

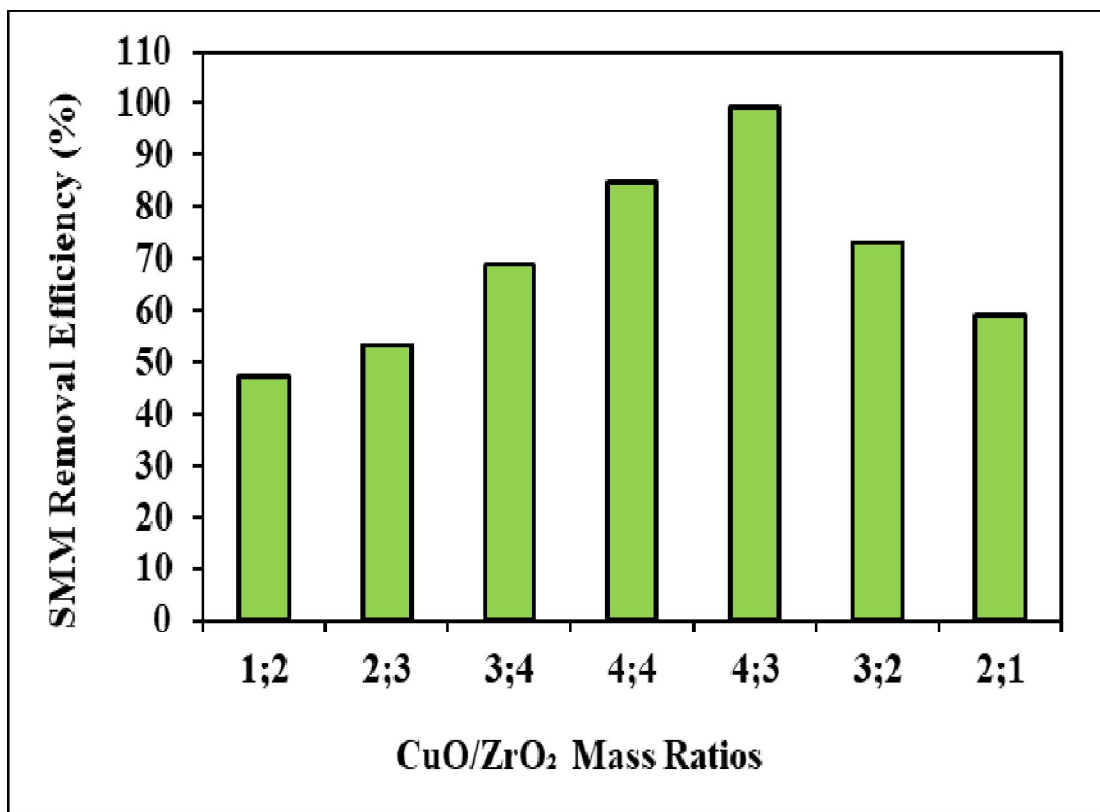


Figure 11: Effect of different CuO/ZrO₂ NCs mass ratios for SMM removal in PI ww during PDP, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively.

3.8. Effect of Different Recycle Times for SMM Removals in PI ww during PDP

Different recycle times (1., 2., 3., 4., 5., 6. and 7.) were operated for SMM removals in PI ww during PDP, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 4/3wt CuO/ZrO₂ NCs mass ratio, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (Figure 12). 92.6%, 85.2%, 77.8%, 73.2%, 68.6% and 64.1% SMM recovery yields were measured after 2. recycle time, 3. recycle time, 4. recycle time, 5. recycle time, 6. recycle time and 7. recycle time, respectively, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 4/3wt CuO/ZrO₂ NCs mass ratio, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (Figure 12). The maximum 99.5% SMM recovery was measured in PI ww during PDP, after 1. recycle time, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 4/3wt CuO/ZrO₂ NCs mass ratio, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively (Figure 12).

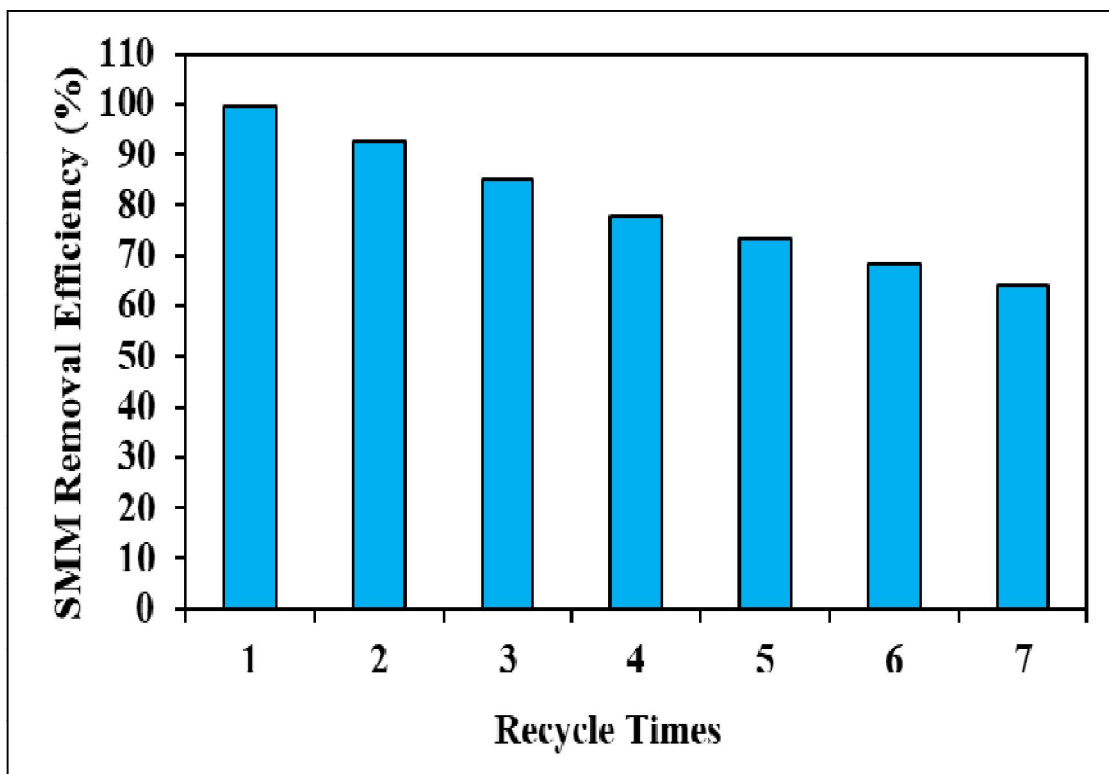


Figure 12: Effect of recycle times for SMM removal in PI ww during PDP, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 4/3wt CuO/ZrO₂ NCs mass ratio, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively.

3.9. Acute Toxicity Assays

3.9.1. Effect of Increasing SMM Concentrations on the Microtox Acute Toxicity Removal Efficiencies in PI ww at Increasing Photocatalytic Degradation Time and Temperature

In Microtox with *Aliivibrio fischeri* (*Vibrio fischeri*) acute toxicity test, the initial EC₉₀ values at pH=7.0 was found as 825 mg/l at 25°C (Table 2: SET 1). After 60, 120 and 180 min photocatalytic degradation time, the EC₉₀ values decreased to EC₅₇=414 mg/l to EC₂₂=236 mg/l and to EC₁₂=165 mg/l in SMM=20 mg/l at 30°C (Table 2: SET 3). The Microtox acute toxicity removals were 41.75%, 80.64% and 91.75% after 60, 120 and 180 min, respectively, in SMM=20 mg/l and at 30°C (Table 2: SET 3).

Table 2: Effect of increasing SMM concentrations on Microtox acute toxicity in PI ww after PDP, at 30°C and at 60°C, respectively.

No	Parameters	Microtox Acute Toxicity Values, * EC (mg/l)							
		25°C							
		0. min	60. min	120. min	180. min				
	*EC ₉₀	*EC	*EC	*EC					
1	Raw ww, control	825	EC ₇₀ =510	EC ₆₀ =650	EC ₄₉ =638				
		30°C				60°C			
		0.	60.	120.	180.	0.	60.	120.	180.

		min	min	min	min	min	min	min	min
		*EC ₉₀	*EC	*EC	*EC	*EC ₉₀	*EC	*EC	*EC
2	Raw ww, control	825	EC ₇₀ = 580	EC ₅₀ = 580	EC ₃₉ = 548	825	EC ₅₅ = 550	EC ₄₀ = 590	EC ₂₉ = 688
3	SMM=5 mg/l	825	EC ₆₃ = 421	EC ₂₈ = 241	EC ₁₈ = 167	825	EC ₅₈ = 418	EC ₂₃ = 265	EC ₁₃ = 149
	SMM=10 mg/l	825	EC ₆₂ = 420	EC ₂₇ = 238	EC ₁₇ = 166	825	EC ₅₇ = 413	EC ₂₂ = 231	EC ₁₂ = 160
	SMM=20 mg/l	825	EC ₅₈ =413	EC ₂₃ = 235	EC ₁₃ = 164	825	EC ₅₃ = 549	EC ₁₈ = 539	EC ₈ = 499
	SMM=40 mg/l	825	EC ₆₈ = 407	EC ₃₃ = 229	EC ₂₃ = 161	825	EC ₆₃ = 402	EC ₂₈ = 217	EC ₁₈ = 147

* EC values were calculated based on COD_{dis} (mg/l).

The EC₉₀ values decreased to EC₅₃, to EC₁₈ and to EC₈ after 60, 120 and 180 min, respectively, in SMM=20 mg/l, at 60°C (**Table 2: SET 3**). The EC₅₁=549 mg/l, EC₁₁=539 mg/l and EC₇=499 mg/l values were measured in SMM=20 mg/l, at 60°C. The toxicity removals were 47.30%, 86.29% and 97.31% after 60, 120 and 180 min, respectively, in SMM=20 mg/l, at 60°C (**Table 2: SET 3**). 97.31% maximum Microtox acute toxicity removal was found in SMM=20 mg/l after 180 min and at 60°C (**Table 2: SET 3**).

The EC₉₀ values decreased to EC₆₃=421 mg/l to EC₂₈=241 mg/l and to EC₁₈=167 mg/l after 60, 120 and 180 min, respectively, in SMM=5 mg/l at 30°C (**Table 2: SET 3**). The EC₉₀ values decreased to EC₆₂=420 mg/l to EC₂₇=238 mg/l and to EC₁₁=166 mg/l after 60, 120 and 180 min, respectively, in SMM=10 mg/l at 30°C. The EC₉₀ values decreased to EC₆₈=407 mg/l to EC₃₃=229 mg/l and to EC₂₃=161 mg/l after 60, 120 and 180 min, respectively, in SMM=40 mg/l at 30°C. The Microtox acute toxicity removals were 86.19%, 86.17% and 80.64% in 5, 10 and 40 mg/l SMM, respectively, after 180 min, at 30°C. It was obtained an inhibition effect of SMM=40 mg/l to *Vibrio fischeri* after 180 min and at 30°C (**Table 2: SET 3**).

The EC₉₀ values decreased to EC₅₈=418 mg/l to EC₂₃=265 mg/l and to EC₁₃=149 mg/l after 60, 120 and 180 min, respectively, in SMM=5 mg/l at 60°C (**Table 2: SET 3**). The EC₉₀ values decreased to EC₅₇=413 mg/l to EC₂₂=231 mg/l and to EC₁₂=160 mg/l after 60, 120 and 180 min, respectively, in SMM=10 mg/l at 60°C. The EC₉₀ values decreased to EC₆₃=402 mg/l to EC₂₈=217 mg/l and to EC₁₈=147 mg/l after 60, 120 and 180 min, respectively, in SMM=40 mg/l at 60°C. The Microtox acute toxicity removals were 91.75%, 91.72% and 86.21% in 5, 10 and 40 mg/l SMM, respectively, after 180 min, at 60°C. It was observed an inhibition effect of SMM=40 mg/l to Microtox with *Vibrio fischeri* after 180 min, and at 60°C (**Table 2: SET 3**).

3.9.2. Effect of Increasing SMM Concentrations on the *Daphnia magna* Acute Toxicity Removal Efficiencies in PI ww at Increasing Photocatalytic Degradation Time and Temperature

The initial EC₅₀ values were observed as 850 mg/l at 25°C (**Table 3: SET 1**). After 60, 120 and 180 min, the EC₅₀ values decreased to EC₃₂=349 mg/l to EC₁₈=239 mg/l and to EC₁₃=89 mg/l in SMM=20 mg/l, at 30°C (**Table 3: SET 3**). The toxicity removals were

43.85%, 73.76% and 83.54% after 60, 120 and 180 min, respectively, in SMM=20 mg/l at 30°C (Table 3: SET 3).

Table 3: Effect of increasing SMM concentrations on *Daphnia magna* acute toxicity in PI ww after PDP, at 30°C and at 60°C.

No	Parameters	<i>Daphnia magna</i> Acute Toxicity Values, * EC (mg/l)							
		25°C							
		0. min		60. min		120. min		180. min	
*EC ₅₀		*EC		*EC		*EC			
1	Raw ww, control	850		EC ₄₅ =625		EC ₄₀ =370		EC ₂₉ =153	
		30°C				60°C			
		0. min	60. min	120. min	180. min	0. min	60. min	120. min	180. min
		*EC ₅₀	*EC	*EC	*EC	*EC ₅₀	*EC	*EC	*EC
2	Raw ww, control	850	EC ₃₉ =468	EC ₃₄ =228	EC ₂₃ =111	850	EC ₃₄ =373	EC ₂₉ =210	EC ₁₈ =71
3	SMM=5 mg/l	850	EC ₃₃ =449	EC ₂₃ =144	EC ₁₈ =259	850	EC ₃₃ =129	EC ₁₈ =424	EC ₁₃ =339
	SMM=10 mg/l	850	EC ₃₇ =449	EC ₂₂ =174	EC ₁₇ =99	850	EC ₃₂ =424	EC ₁₇ =139	EC ₇ =89
	SMM=20 mg/l	850	EC ₃₃ =349	EC ₁₈ =239	EC ₁₃ =89	850	EC ₂₈ =149	EC ₁₃ =59	EC ₈ =374
	SMM=40 mg/l	850	EC ₄₃ =299	EC ₂₈ =169	EC ₂₃ =51	850	EC ₃₈ =249	EC ₂₃ =109	EC ₁₈ =10

* EC values were calculated based on COD_{dis} (mg/l).

The EC₅₀ values decreased to EC₂₈ to EC₁₃ and to EC₈ after 60, 120 and 180 min, respectively, in SMM=20 mg/l at 60°C (Table 3: SET 3). The EC₂₈=149 mg/l, EC₁₃=59 mg/l and EC₈=374 mg/l values were measured in SMM=20 mg/l at 60°C. The toxicity removals were 53.83%, 83.51% and 93.27% after 60, 120 and 180 min, respectively, in SMM=20 mg/l at 60°C (Table 3: SET 3). 93.27% maximum *Daphnia magna* acute toxicity removal was obtained in SMM=20 mg/l after 180 min and at 60°C, respectively (Table 3: SET 3).

The EC₅₀ values decreased to EC₃₈=449 mg/l to EC₂₃=144 mg/l and to EC₁₈=259 mg/l after 60, 120 and 180 min, respectively, in SMM=5 mg/l at 30°C (Table 3: SET 3). The EC₅₀ values decreased to EC₃₇=449 mg/l to EC₂₂=174 mg/l and to EC₁₇=99 mg/l after 60, 120 and 180 min, respectively, in SMM=10 mg/l and at 30°C. The EC₅₀ values decreased to EC₄₃=299 mg/l to EC₂₈=169 mg/l and to EC₂₃=51 mg/l after 60, 120 and 180 min, respectively, in SMM=40 mg/l and at 30°C. The *Daphnia magna* acute toxicity removals were 73.11%, 73.45% and 64.10% in 5, 10 and 40 mg/l SMM, respectively, after 180 min and at 30°C. It was observed an inhibition effect of SMM=40 mg/l to *Daphnia magna* after 180 min and at 30°C (Table 3: SET 3).

The EC₅₀ values decreased to EC₃₃=129 mg/l to EC₁₈=424 mg/l and to EC₁₃=339 mg/l after 60, 120 and 180 min, respectively, in SMM=5 mg/l and at 60°C (Table 3: SET 3). The EC₅₀ values decreased to EC₃₂=424 mg/l to EC₁₇=139 mg/l and to EC₇=89 mg/l after 60, 120 and 180 min, respectively, in SMM=10 mg/l and at 60°C. The EC₅₀ values decreased to EC₃₈=249 mg/l to EC₂₃=109 mg/l and to EC₁₈=10 mg/l after 60, 120 and 180 min, respectively, in SMM=40 mg/l and at 60°C. The *Daphnia magna* acute toxicity removals were 84.95%,

93.54% and 74.22% in 5, 10 and 40 mg/l SMM, respectively, after 180 min and at 60°C. It was observed an inhibition effect of SMM=40 mg/l to *Daphnia magna* after 180 min and at 60°C (**Table 3: SET 3**).

Increasing the SMM concentrations from 5 to 40 mg/l did not have a positive effect on the decrease of EC₅₀ values as shown in **Table 3 at SET 3**. SMM concentrations >20 mg/l decreased the acute toxicity removals by hindering the PDP. Similarly, a significant contribution of increasing SMM concentration to acute toxicity removal at 60°C after 180 min was not observed. Low toxicity removals found at high SMM concentrations could be attributed to their detrimental effect on the *Daphnia magna* (**Table 3: SET 3**).

3.9.3. Direct Effects of SMM Concentrations on the Acute Toxicity of Microtox and *Daphnia magna* without PI ww after PDP

The acute toxicity test was performed in the samples containing 5, 10, 20 and 40 mg/l SMM concentrations, at 25°C. In order to detect the direct responses of Microtox and *Daphnia magna* to the increasing SMM concentrations the toxicity test were performed without PI ww after PDP, at 25°C. The initial EC values and the EC₅₀ values were measured in the samples containing increasing SMM concentrations after 180 min. **Table 4** showed the responses of Microtox and *Daphnia magna* to increasing SMM concentrations.

Table 4: The responses of Microtox and *Daphnia magna* acute toxicity tests in addition of increasing SMM concentrations without PI ww during PDP after 180 min, at 25°C.

SMM Conc. (mg/l)	Microtox Acute Toxicity Test			<i>Daphnia magna</i> Acute Toxicity Test		
	Initial EC ₅₀ Value (mg/l)	Inhibitions after 180 min	EC Values (mg/l)	Initial EC ₅₀ Value (mg/l)	Inhibitions after 180 min	EC Values (mg/l)
5	EC ₁₁ = 23	-	-	EC ₁₁ = 38	-	-
10	EC ₁₅ = 78	3	EC ₂ = 3	EC ₂₀ = 98	5	EC ₃ = 4
20	EC ₂₁ = 148	6	EC ₄ = 6	EC ₃₁ = 198	7	EC ₇ = 10
40	EC ₂₆ = 218	8	EC ₆ = 9	EC ₄₁ = 298	10	EC ₉ = 14

The acute toxicity originating only from 5, 10, 20 and 40 mg/l SMM were found to be low (**Table 4**). 5 mg/l SMM did not exhibit toxicity to *Aliivibrio fischeri* and *Daphnia magna* before and after 180 min. The toxicity attributed to the 10, 20 and 40 mg/l SMM were found to be low in the samples without PI ww after PDP for the test organisms mentioned above. The acute toxicity originated from the SMM decreased significantly to EC₂, EC₄ and EC₆ after 180 min. Therefore, it can be concluded that the toxicity originating from the SMM is not significant and the real acute toxicity throughout PDP was attributed to the PI ww, to their metabolites and to the photocatalytic degradation by-products (**Table 4**).

4. CONCLUSIONS

The maximum 99.6% SMM removal was obtained during PDP in PI ww, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively.

The maximum 99.2% SMM removal was found with PDP in PI ww, at 5 mg/l SMM, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively.

The maximum 99.5% SMM removal was measured to 800 mg/l CuO/ZrO₂ NCs with PDP in PI ww, at 5 mg/l SMM, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively.

The maximum 99.4% SMM removal was measured at 4/3wt CuO/ZrO₂ NCs mass ratios, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively.

The maximum 99.5% SMM recovery was measured in PI ww during PDP, after 1. recycle time, at 5 mg/l SMM, at 800 mg/l CuO/ZrO₂ NCs, at 4/3wt CuO/ZrO₂ NCs mass ratio, at 350 W UV-vis, at 147 mW/cm², after 120 min, at pH=12.0 and at 25°C, respectively.

97.31% maximum Microtox acute toxicity removal was found in SMM=20 mg/l after 180 min and at 60°C. It was observed an inhibition effect of SMM=40 mg/l to Microtox with *Vibrio fischeri* after 180 min, and at 60°C. 93.27% maximum *Daphnia magna* acute toxicity removal was obtained in SMM=20 mg/l after 180 min and at 60°C, respectively. It was obtained an inhibition effect of SMM=40 mg/l to *Daphnia magna* after 180 min and at 60°C. Increasing the SMM concentrations from 5 to 40 mg/l did not have a positive effect on the decrease of EC₅₀ values. SMM concentrations > 20 mg/l decreased the acute toxicity removals by hindering the PDP. Similarly, a significant contribution of increasing SMM concentration to acute toxicity removal at 60°C after 180 min was not observed. Low toxicity removals found at high SMM concentrations could be attributed to their detrimental effect on *Aliivibrio fischeri* and *Daphnia magna*.

As a result, the CuO/ZrO₂ NCs during PDP in PI ww was stable in harsh environments such as acidic, alkaline, saline, and then was still effective process. When the amount of contaminant was increased, the a novel CuO/ZrO₂ NCs during PDP performance was still considerable. The synthesis and optimization of CuO/ZrO₂ heterostructure photocatalyst provides insights into the effects of preparation conditions on the material's characteristics and performance, as well as the application of the effectively designed photocatalyst in the removal of antibiotics, which can potentially be deployed for purifying wastewater, especially PI ww. Finally, the combination of a simple, easy operation preparation process, excellent performance and cost effective, makes this CuO/ZrO₂ NCs a promising option during PDP in PI ww treatment.

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