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chemical and biological remediation of lindane residue in aqueous media

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For the first time in the history of the world, every human being is now subjected to contact with dangerous chemicals, from the moment of conception until death. The systemic pesticides have been so thoroughly distributed throughout the animate and inanimate world that they occur virtually every where. Residues of these chemicals have entered and lodged in the bodies of fish, birds and they have been found in water and in man himself (Carson 1962).

Water pre requisite for life and key reasons of humanity, is abundant on earth. However, 97% is salt water. The remaining 2.5% that is the fresh water, 70% is frozen in the polar ice caps. Only less than 1% of the world's fresh water resources are readily available for human use (WHO 2002).

Egypt, today is facing and suffering from two huge problems. The first one is the Renaissance Dam in Ethiopia, it can cause a shortage in water supply and reduction Egypt's share of the Nile water by 14.5 billion cubic meters, which can irrigate 3 million Fadden and the second problem is the environmental pollution in general, and particularly agrochemical pollutants in surface water through the intensive used of pesticides and fertilizers.

On the other hand, the pollution of surface water and wastewater has increased sharply and it constitutes a major problem due to an extensive use of these substances (Evgenidou *et al.,* 2007). The monitoring of pesticides in surface water showed the presence of organochlorines, organophosphorus and carbamates insecticides in water resources in Kafr El-sheikh Governorate (Hamed 1997; Abd El-Razik, 2006; Massoud *et al*., 2007a; Massoud *et al.,* 2011; Ismail *et al.,* 2015).

Nanotechnology is an emerging technology, which can lead to a new revolution in every field of science (Rico *et al.,* 2011). This technology is used with association to optics, electronics, and biomedical and materials sciences. Research in this field has gained momentum in the recent years by providing innovative solutions in different scientific disciplines.

Zinc oxide crystallizes in two main forms, hexagonal wurtzite (Fierro 2006) and cubic zincblende. ZnO semiconductor has several unique properties such as good transparency, high electron mobility, wide band gap, and strong room temperature luminescence. The wide band gap and large excitonic binding energy have made zinc oxide important both for scientific and industrial applications (Wang *et al.,* 2004).

Titanium dioxide (TiO_2) is considered very close to an ideal semiconductor for photocatalysis because of its high stability, low cost and safety toward both humans and the environment. TiO_2 belongs to the family of transition metal oxides. There are four commonly known polymorphs of TiO_2 found in nature: anatase (tetragonal), brookite (orthorhombic), rutile (tetragonal), and TiO_2 (B) (monoclinic) (Crap *et al.* 2004).

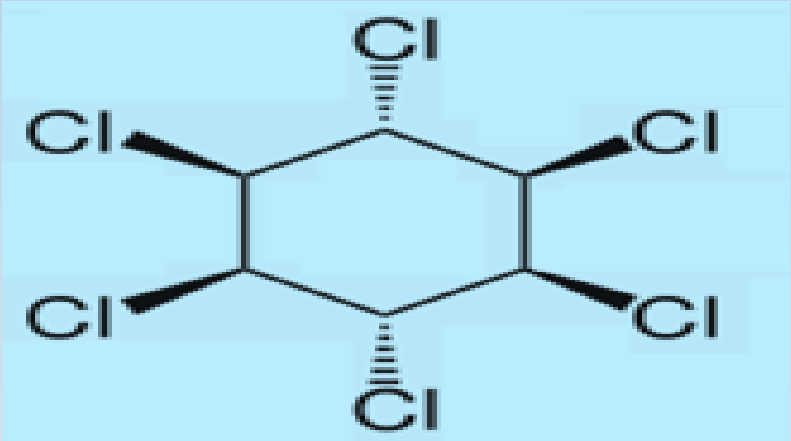
In this study the efficiency of advanced oxidation processes with different nano materials and bioremediation with *Xanthomonas campestris pv. Translucens* and *Aspergillus fumigatus* were evaluated to achieve the total degradation of lindane. The enzymes activity and histological changes in liver and kidney of rats was measured to confirm the complete detoxification of lindane-contaminated water.

MATERIALS AND METHODS

Tested insecticides

Chemical class: Organochlorine (Lindane with purity of 99.5%)

Empirical formula: $C_6H_6CL_6$.



Photochemical remediation

The scope of the experiments included the following treatments: Nano titanium dioxide combined with hydrogen peroxide(TiO_2 (nano)/H_2O_2/UV), nano photo zinc oxide combined with hydrogen peroxide (ZnO (nano)/H_2O_2/UV), photo Fenton reagent (Fe^{2+}/H_2O_2/UV), photo titanium dioxide combined with hydrogen peroxide (TiO_2 /H_2O_2/UV) and photo zinc oxide combined with hydrogen peroxide (ZnO/H_2O_2/UV).

For photo Fenon and Fenton like reaction UV Lamp as employed for the irradiation of lindane in aquatic solution. Ferric chloride and ferrous sulphate were used as source of iron catalyst**.** The solution was prepared by addition of desired amount of lindane (5 mg L^{-1}) distilled water and carefully agitated.

Synthesis of nonmaterials

1- Synthesis of ZnO nanoparticles (NPs)

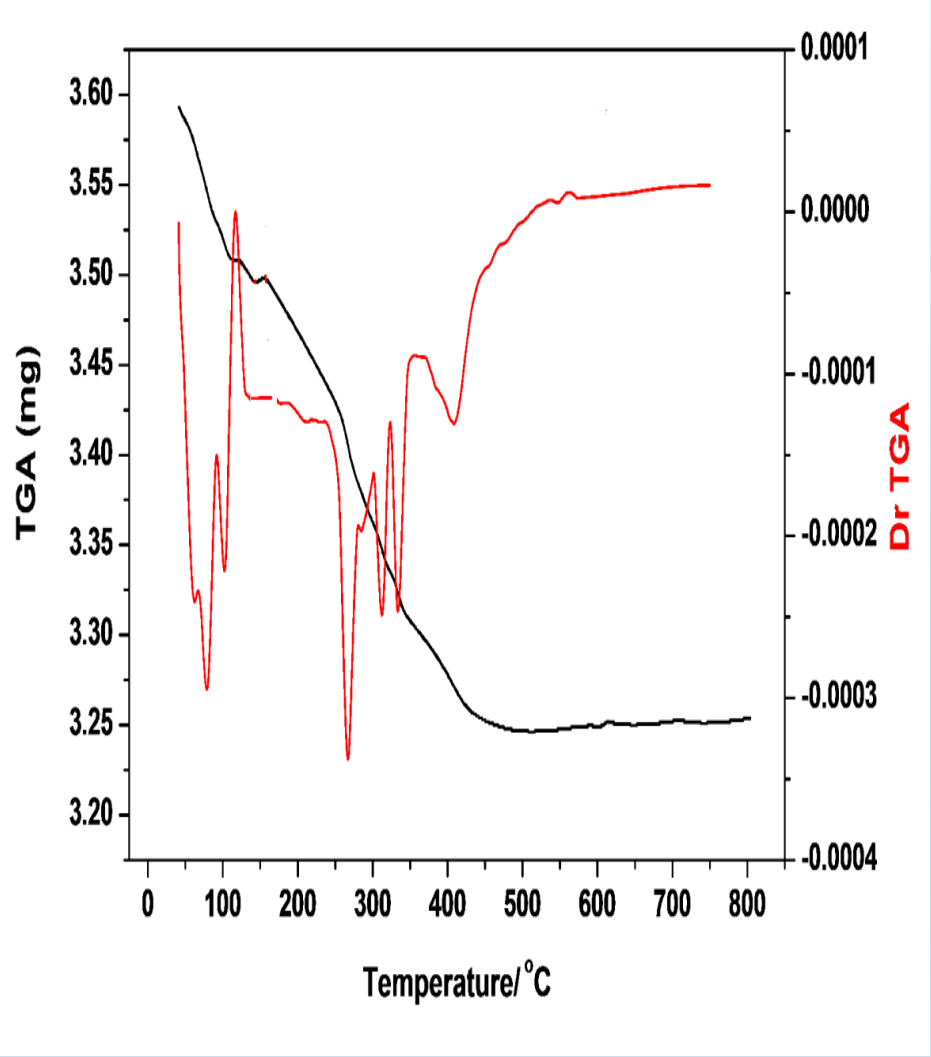
Zinc acetate dihydrate ($ZnAc_2.2H_2O$) and capping agent (triethylamine, TEA) were mixed at a molar ratio of 5:3 in ethanol with typically 3.2926 gm of zinc acetate dihydrate and 1.255 ml of triethylamine. The mixed solution was stirred for 60 min at 50-60º C in a condensation system until it turned white cloudy. The resulting cloudy solution indicates the growth of ZnO nanoparticles (El-Kemary *et al.,* 2010)..

2-Synthesis of TiO_2 nanoparticles (NPs)

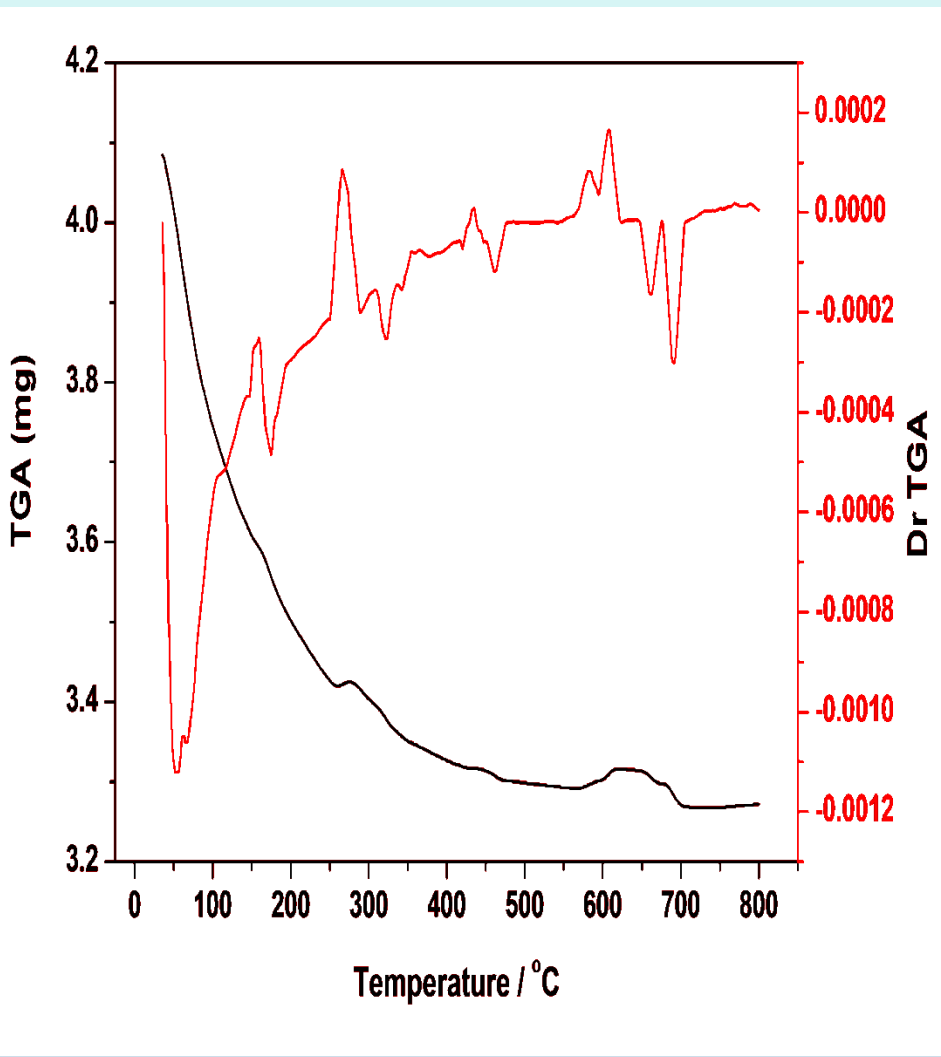
Nanostructured TiO_2 catalysts have been prepared by hydrolysis of titanium isopropoxide or titanium tetrachloride (Addamo *et al.*, 2004). The nanostructured TiO_2 sample was prepared by hydrolysis of titanium tetrachloride $TiCl_4$. In a typical synthesis: 4 ml $TiCl_4$ solution was dropped into 400 ml of a mixture of ethanol and distilled water (4:1). The mixture was refluxed at 80 ºC with stirring; a white suspension of TiO_2 nanoparticles was formed in about 120 min of reflux.

3- photo Fenon and Fenton like reaction

photo Fenon and Fenton like reaction UV Lamp as employed for the irradiation of lindane in aquatic solution. Ferric chloride and ferrous sulphate were used as source of iron catalyst. The solution was prepared by addition of desired amount of lindane (5 mg L^{-1}) distilled water and carefully agitated. Then, freshly prepared ferric chloride or ferrous sulphate at concentration level of 50 mg L^{-1} as Fe^{3+} was added followed by the addition of H_2O_2 at concentration level of 20mg/L. After that the solution was completed with water up to 1000 mL. The initial pH of the solution was adjusted to be 2.8 by using hydrochloric acid 1 Molar for all experiments [Derbalah et al. 2004].



TGA-DrTGA of the synthesized ZnO isolated at a heating rate of 10 °C/min under nitrogen atmosphere.



TGA-DrTGA of the synthesized TiO_2 powders measured at a heating rate of 10 °C/min under nitrogen atmosphere.

Scanning Electron Microscope (SEM)

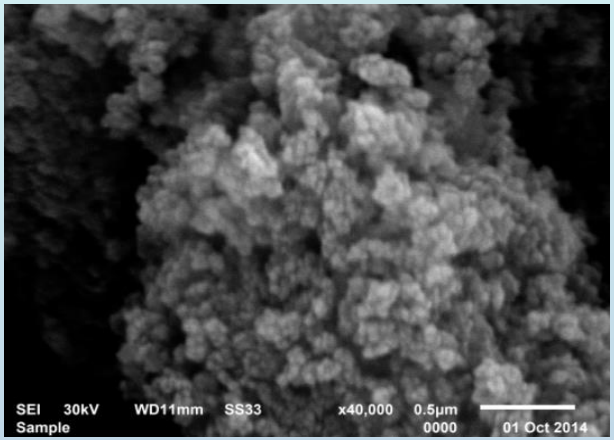


Fig. (22):SEM image of the synthesized ZnO (s). spherically shape (size :30-49 nm)

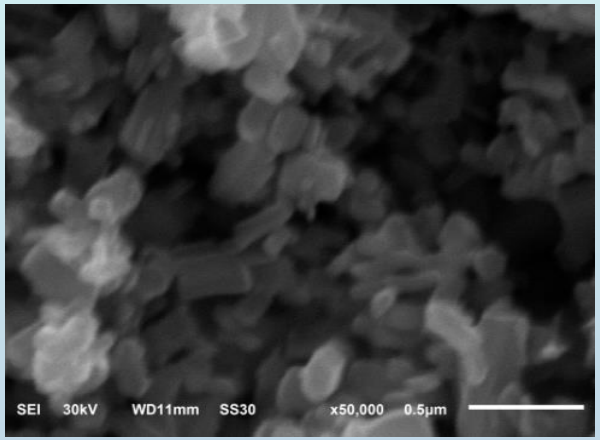


Fig. (23): SEM image of commercial ZnO.(size d:34 to 119 nm L:169 to 399 nm hexagonal prisms

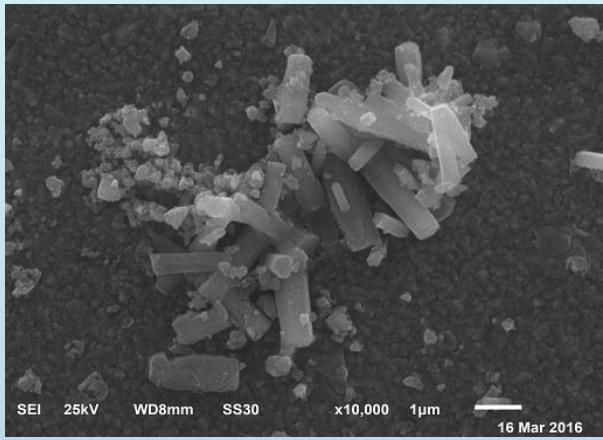


Fig. (24):SEM image of synthesized ZnO thin film on FTO substrate.(sized:100-450 nm , l: 0.4 3µm)hexagonal nanorods

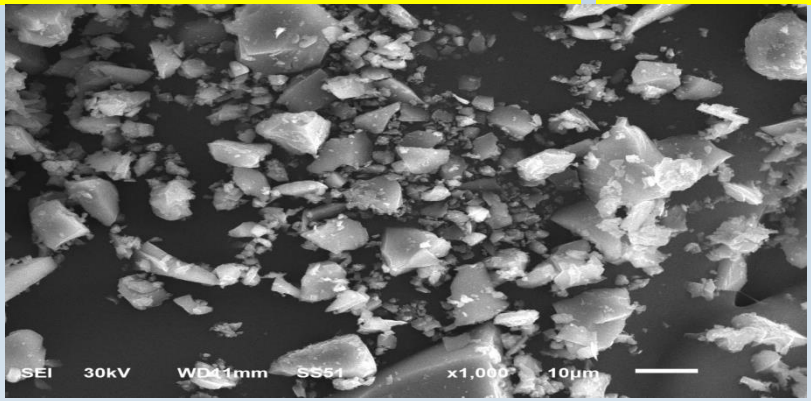


Fig. (25): SEM images of the synthesized TiO_2 (s). (spherically shape) (size d:30 to 40 nm)

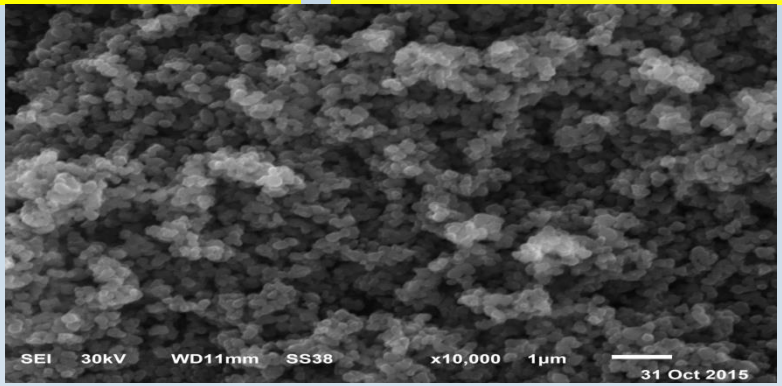


Fig. (26): SEM images of the commercial TiO_2. (spherically shape) (size: 126-206 nm)

Transmission Electron Microscope (TEM)

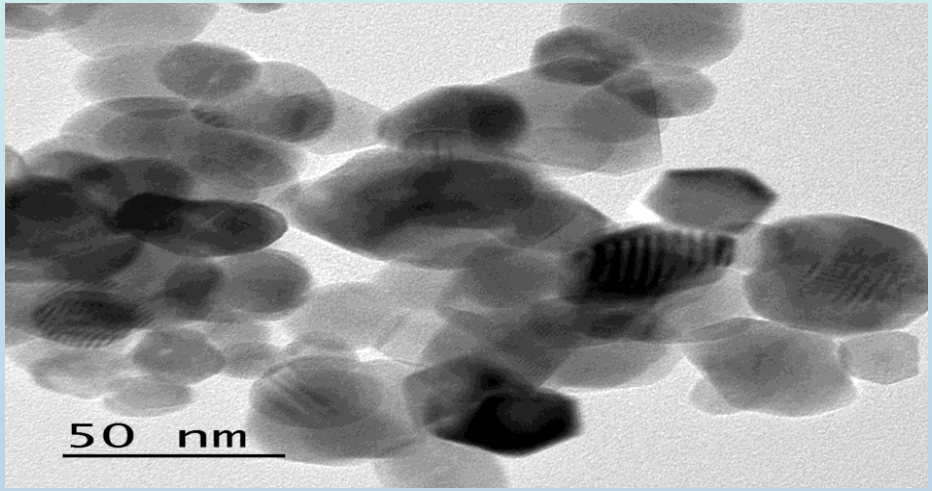


Fig. (27): TEM images of ZnO (s). (size:12.48-26.12 nm)

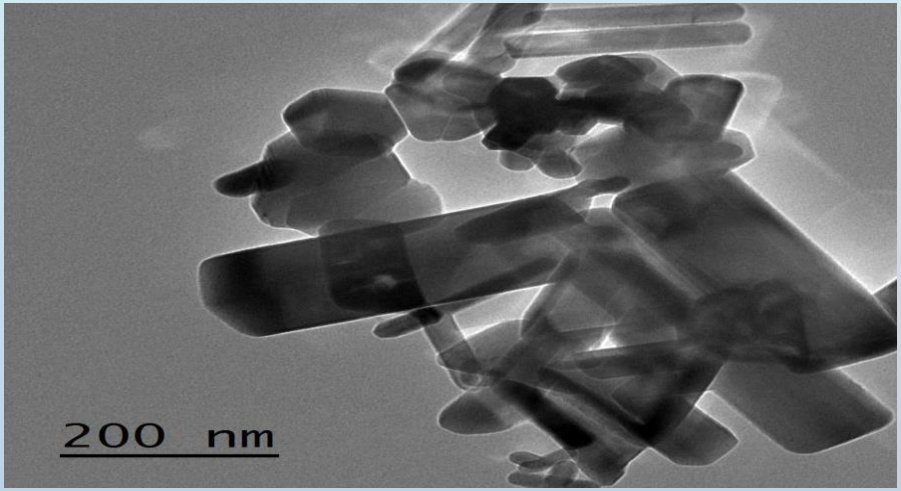


Fig. (28): TEM images of ZnO (c) nanowire. (size d:30-83.87, l: 70.9-406.5 nm)

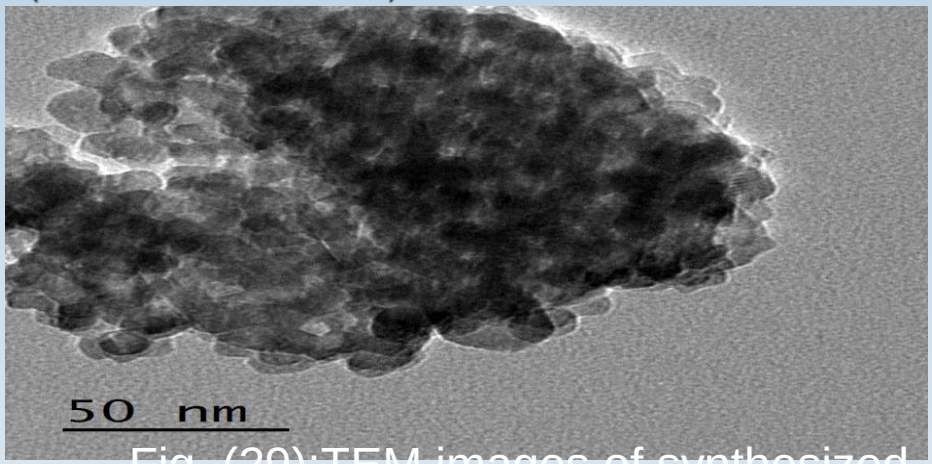


Fig. (29):TEM images of synthesized TiO_2 .

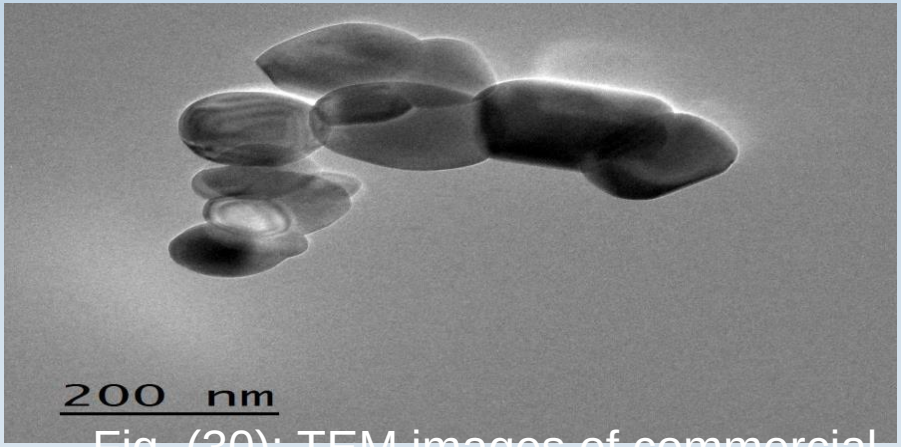
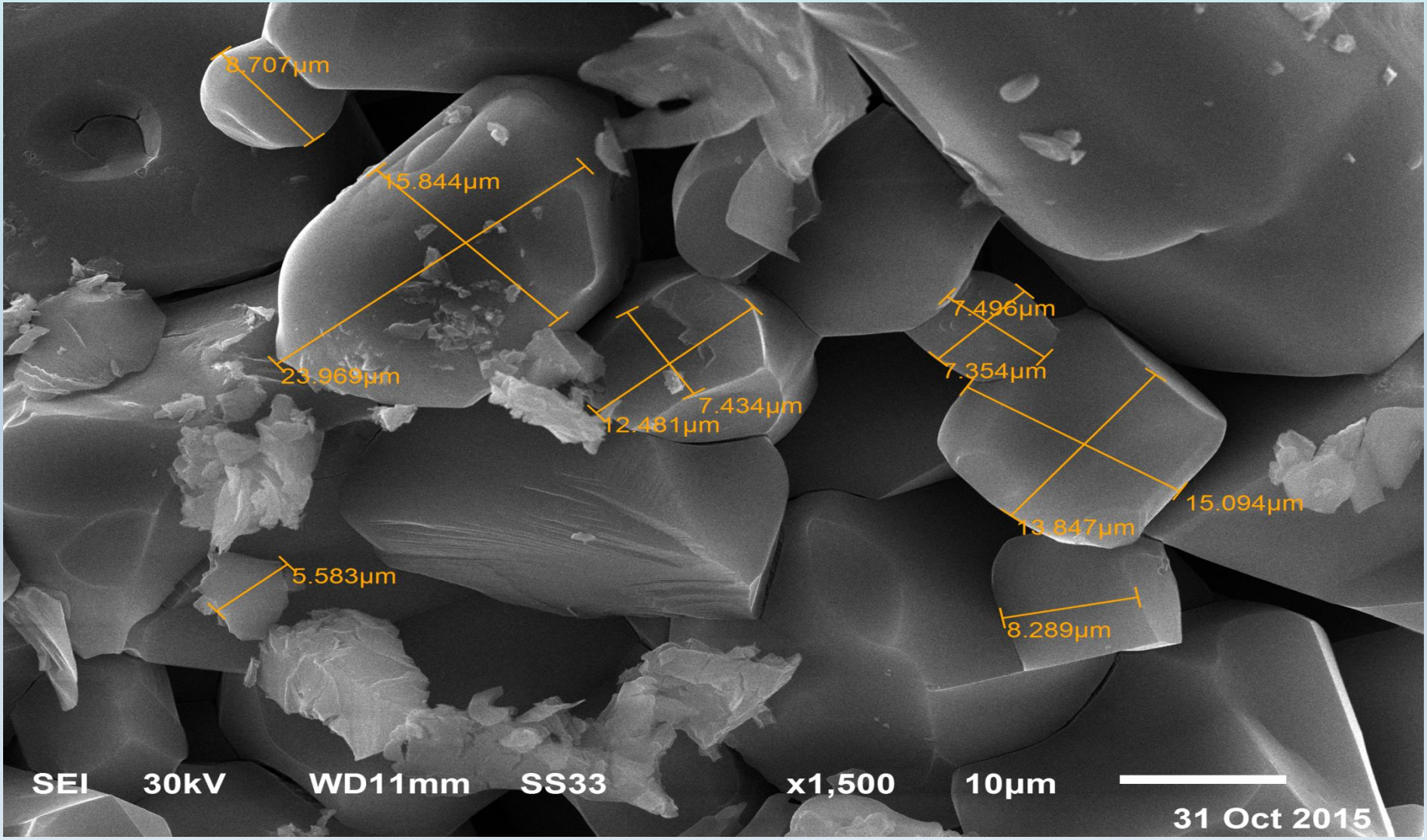


Fig. (30): TEM images of commercial TiO_2 (antase). (size d: 33.3-100nm, l: 100-250nm)

Scanning Electron Microscope (SEM) from Iron



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Bioremediation Technique

The bioremediation test was carried out at pesticides laboratory, Dep. of Pesticides chemistry and Toxicity, Fac. of Agric. Kafrelsheikh Univ. The selected microbial isolates (A1) *Aspergillius fumigatus and* (A2) *Xanthomonas campestris pv. Translucens* were cultured onto MSL spiked with the tested insecticides (lindane), separately for 7 days and then the growing colonies were washed with three ml sterilized MSL liquid medium. The cell suspension of 10^8cfu/ml (cfu:colony forming unit) was used to inoculate 100 ml MSL liquid medium containing 5ppm of the tested insecticide.

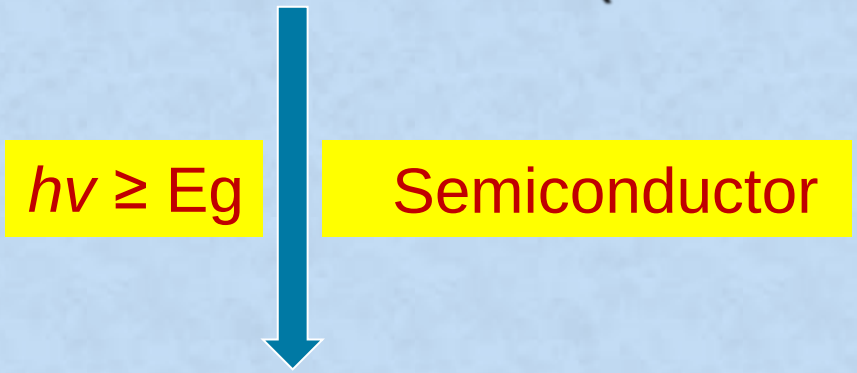
Toxicity test

To confirm the complete detoxification of lindane in treated water, toxicity test was conducted on rats. Lindane contaminated-waters after treatment with $ZnO/H_2O_2/UV$, and *Xanthomonas campestris pv. Translucens* were orally administrated to the tested rats. This test was carried out to measure the effect of the possible remaining lindane (parent or metabolites) in the water samples after remediation on rats with respect to histological changes in liver and kidney of treated rats relative to control.

Adult rats (*Sprague Dawley*) with 100-120 gm of weight, obtained from Faculty of Veterinary Medicine, Kafr-El-Shiekh University were used. Rats were housed in polypropylene cages under standard conditions with free access to drinking water and food. The animals were randomly divided into five groups each comprising of three animals and the treated samples that possibly contain lindane were given to rats as oral administration. Control group rats were fed with normal diet and given oral dose containing no lindane. After 28 days, the rats were scarified under anesthesia and the kidney and liver organs were removed and prepared for histopathological examination according to the method described by Bancroft and Stevens (1996).

Results and Discussion

Organic pollutant + AOPs ($^{\bullet}OH$, $O_2^{\bullet-}$)



CO_2+H_2O+ Inorganic ions

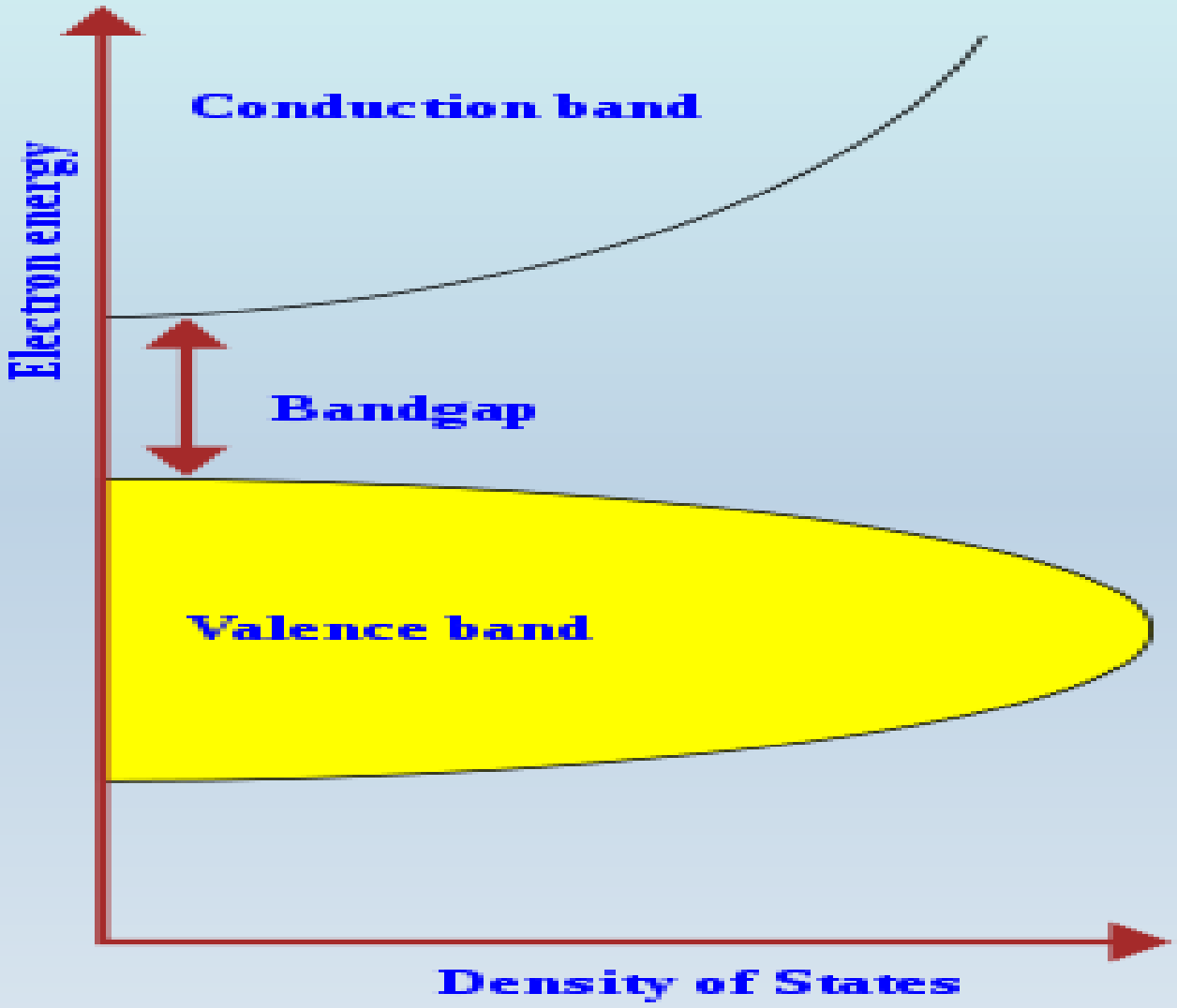
Total mineralization

UV-Visible Spectra of ZnO

The band gap energy (E) was calculated as per the literature report using the following equation (**Hoffmann *et al.*, 1995**):

$$\textbf{Band gap energy (E)} = hc / \lambda$$

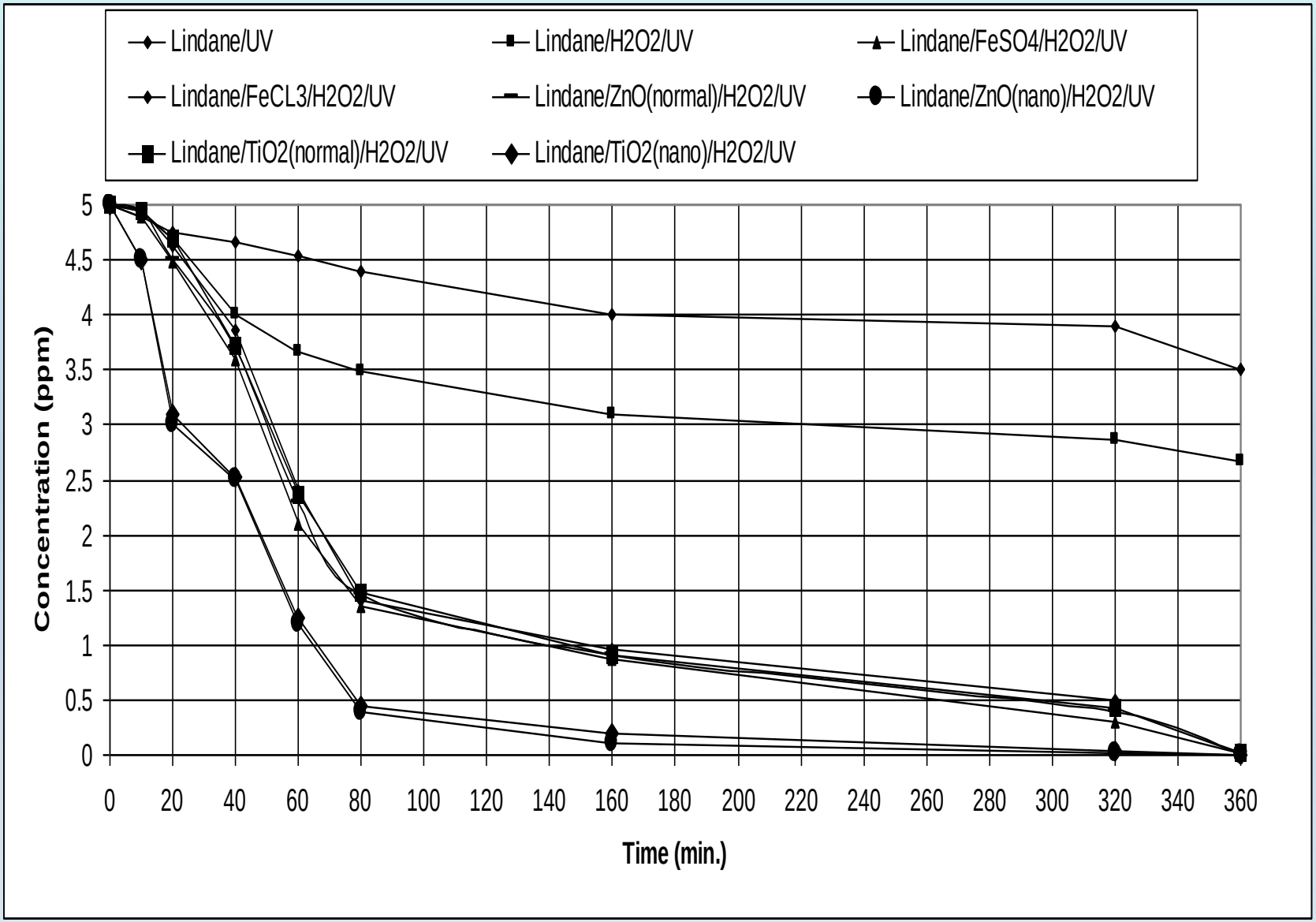
Where h is the Plank's constant (6.625×10^{-34} Js), c is the speed of light (3.0×10^{8} m/s) and (λ) is the wavelength. According to this equation, the band gap of the synthesized ZnO(s) is 3.36 eV while the band gap of the commercial ZnO is 3.29 eV. The wide band gap energy of semiconductor nanoparticles is the more reactive in a photocatlytic degradation of the organic pollutants **(Dhal *et al.*, 2015)**.


Conduction band
Electron energy
Bandgap
Valence band
Density of States

1- Degradation of lindane by advanced oxidation processes

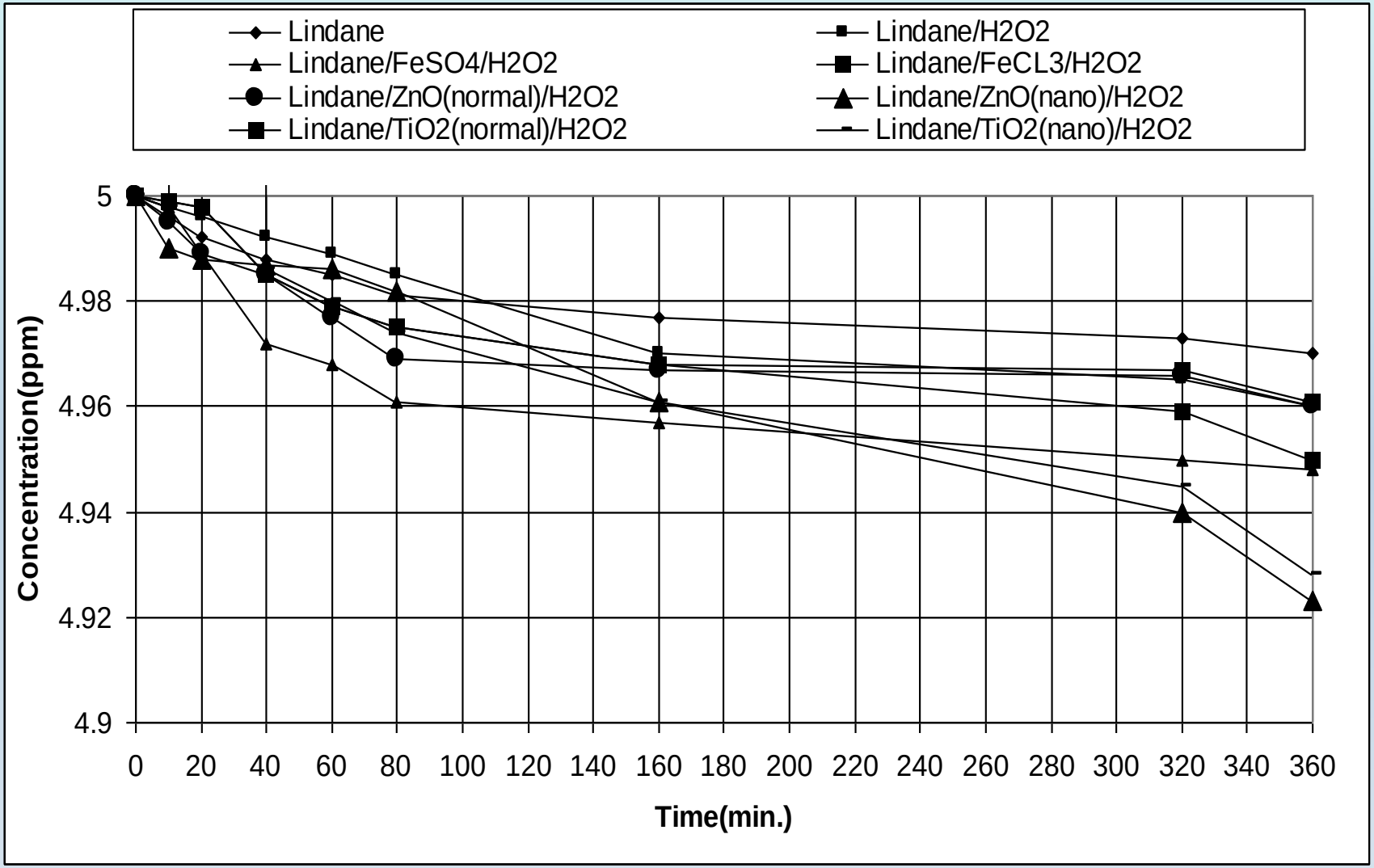
Degradation of lindane at initial concentration of 5ppm under UV, H_2O_2/UV, Fe^{3+}/H_2O_2/UV, Fe^{2+}/H_2O_2/UV, ZnO(nano)/H_2O_2/UV, ZnO/H_2O_2/UV, TiO_2(nano)/H_2O_2/UV and TiO_2/H_2O_2/UV systems by HPLC analysis.

Time (min)	UV	H_2O_2/ UV	$FeCl_3$/ H_2O_2/ Uv	$FeSO_4$/ H_2O_2/ UV	ZnO/ H_2O_2/ UV	ZnO(nano) H_2O_2/ UV	TiO_2/ H_2O_2/ UV	TiO_2(nano)/ H_2O_2/ UV
0	5	5	5	5	5	5	5	5
10	4.89	4.95	4.9	4.96	4.94	4.5	4.95	4.5
20	4.75	4.7	4.49	4.62	4.5	3	4.69	3.1
40	4.67	4.0	3.6	3.87	3.7	2.51	3.72	2.52
60	4.53	3.67	201	2.4	2.3	1.2	2.37	1.25
80	4.4	3.49	1.35	1.41	1.46	0.4	1.47	O.45
160	4.0	3.09	0.87	0.955	0.91	0.1	0.9	0.2
320	3.9	2.87	0.3	0.5	0.4	0.025	0.42	0.03
360	3.5	2.67	0.01	0.02	0.025	0.0	0.025	0


Lindane/UV
Lindane/H2O2/UV
Lindane/FeSO4/H2O2/UV
Lindane/FeCL3/H2O2/UV
Lindane/ZnO(normal)/H2O2/UV
Lindane/ZnO(nano)/H2O2/UV
Lindane/TiO2(normal)/H2O2/UV
Lindane/TiO2(nano)/H2O2/UV
Concentration (ppm)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
Time (min.)

Degradation of lindane at initial concentration of 5ppm under insecticide only, H_2O_2, Fe^{3+}/H_2O_2, Fe^{2+}/H_2O_2, ZnO(nano)/H_2O_2, ZnO/H_2O_2, TiO_2/H_2O_2 and TiO_2(nano)/H_2O_2 systems in dark conditions by HPLC analysis.

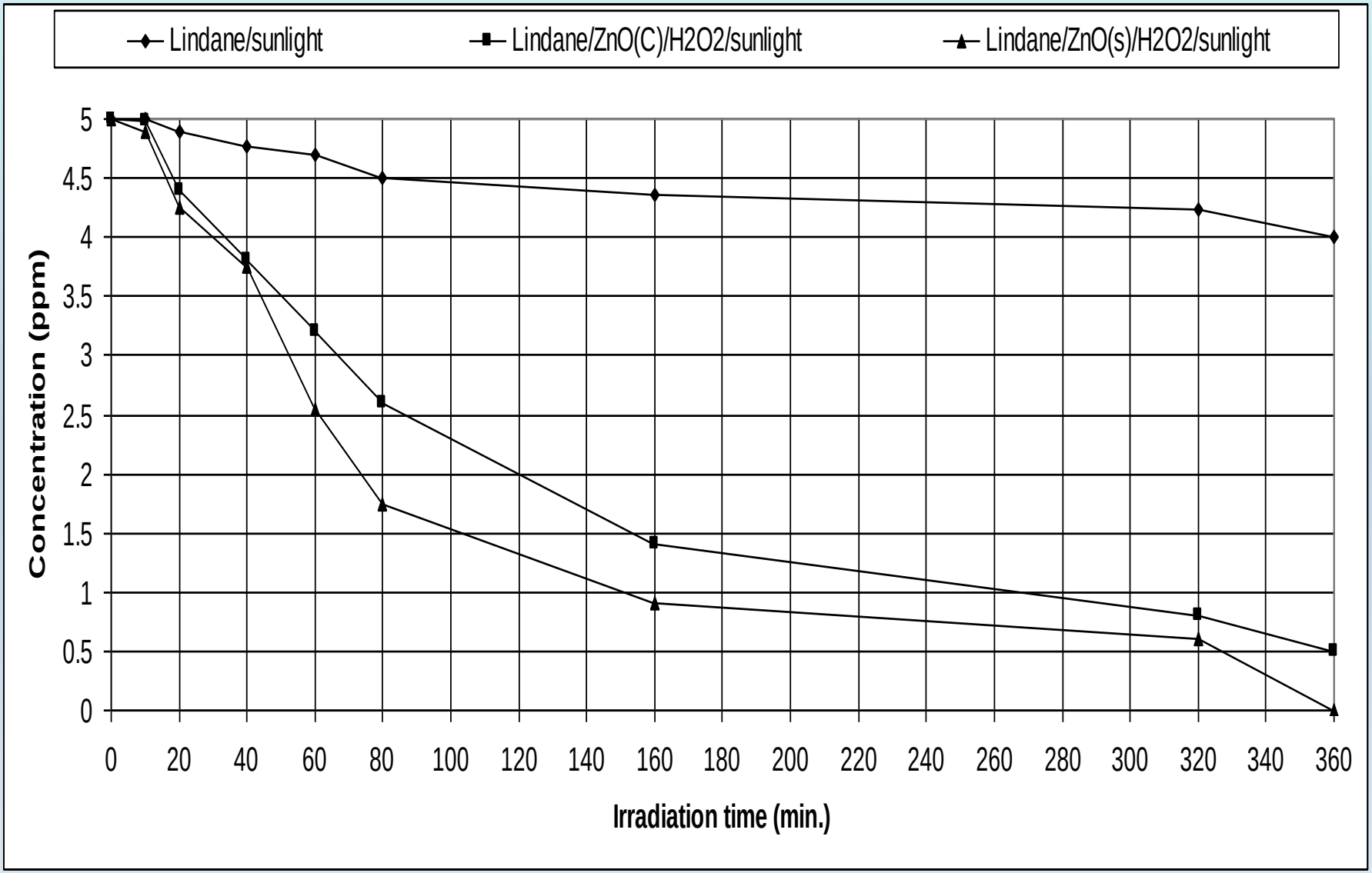
Time (min)	Lindane	H_2O_2	$FeCl_3/H_2O_2$	$FeSO_4/H_2O_2$	ZnO/ H_2O_2	ZnO(nano) H_2O_2	TiO_2/H_2O_2	TiO_2(nano)/ H_2O_2
0	5	5	5	5	5	5	5	5
10	4.996	4.998	4.998	4.999	4.995	4.99	4.999	4.998
20	4.992	4.996	4.989	4.998	4.989	4.988	4.998	4.992
40	4.988	4.992	4.972	4.985	4.985	4.987	4.985	4.986
60	4.985	4.989	4.968	4.979	4.977	4.986	4,979	4,98
80	4.981	4.985	4.961	4.975	4.969	4.982	4.975	4.974
160	4.977	4.97	4.957	4.968	4.967	4.961	4.968	4.961
320	4.973	4.965	4.95	4.959	4.966	4.94	4.967	4.945
360	4.970	4.96	4.948	4.95	4.96	4.923	4.961	4.928


Lindane
Lindane/H2O2
Lindane/FeSO4/H2O2
Lindane/FeCL3/H2O2
Lindane/ZnO(normal)/H2O2
Lindane/ZnO(nano)/H2O2
Lindane/TiO2(normal)/H2O2
Lindane/TiO2(nano)/H2O2
Concentration(ppm)
5
4.98
4.96
4.94
4.92
4.9
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
Time(min.)

Dark

Photochemical degradation of lindane at initial concentration 5 ppm under ZnO(c)/H_2O_2/sunlight, ZnO(s)/H_2O_2/sunlight and sunlight alone systems using HPLC analysis.

Time (min)	Lindane + sun	Lindane +ZnO (normal) + H_2O_2+ sun	Lindane +ZnO (nano) + H_2O_2+ sun
0	5	5	5
10	5	4.98	4.9
20	4.89	4.39	4.25
40	4.76	3,8	3.75
60	4.69	3.2	2.54
80	4.495	2.6	1.75
160	4.368	1.4	1.25
320	4.24	0.8	0.6
360	4	0.5	0.012


Lindane/sunlight
Lindane/ZnO(C)/H2O2/sunlight
Lindane/ZnO(s)/H2O2/sunlight
Concentration (ppm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
Irradiation time (min.)

Biodegradation of Lindane using microbe isolates

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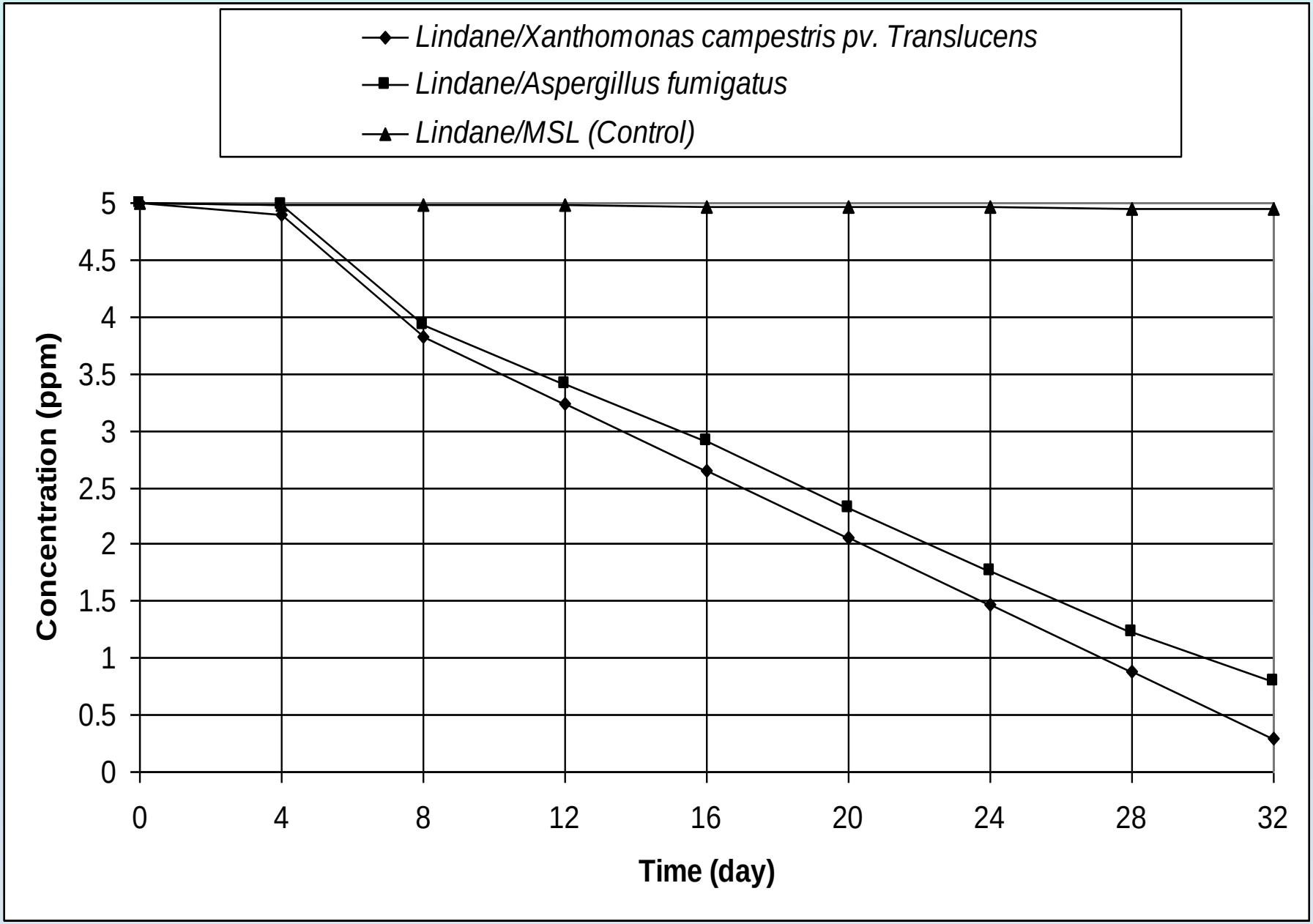
Biodegradation of lindane at concentration level 5ppm by *Xanthomonas campestris pv. Translucens* and *Aspergillus fumigatus* in aqueous media.

Time (min)	Lindane + Xanthomonas campestris pv.	Lindane + Aspergillus fumigatus	Lindane +MSL (Control)
0	5	5	5
10	4.89	4.98	4.99
20	3.82	3.93	4.985
40	3.23	3.4	4.981
60	2.65	2.9	4.971
80	2.06	2.31	4.963
160	1.47	1.76	4.958
320	0.88	231	4.953
360	0.30	0.8	4.94


Lindane/Xanthomonas campestris pv. Translucens
Lindane/Aspergillus fumigatus
Lindane/MSL (Control)
Concentration (ppm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
4
8
12
16
20
24
28
32
Time (day)

The histopathological changes in the kidney and Liver

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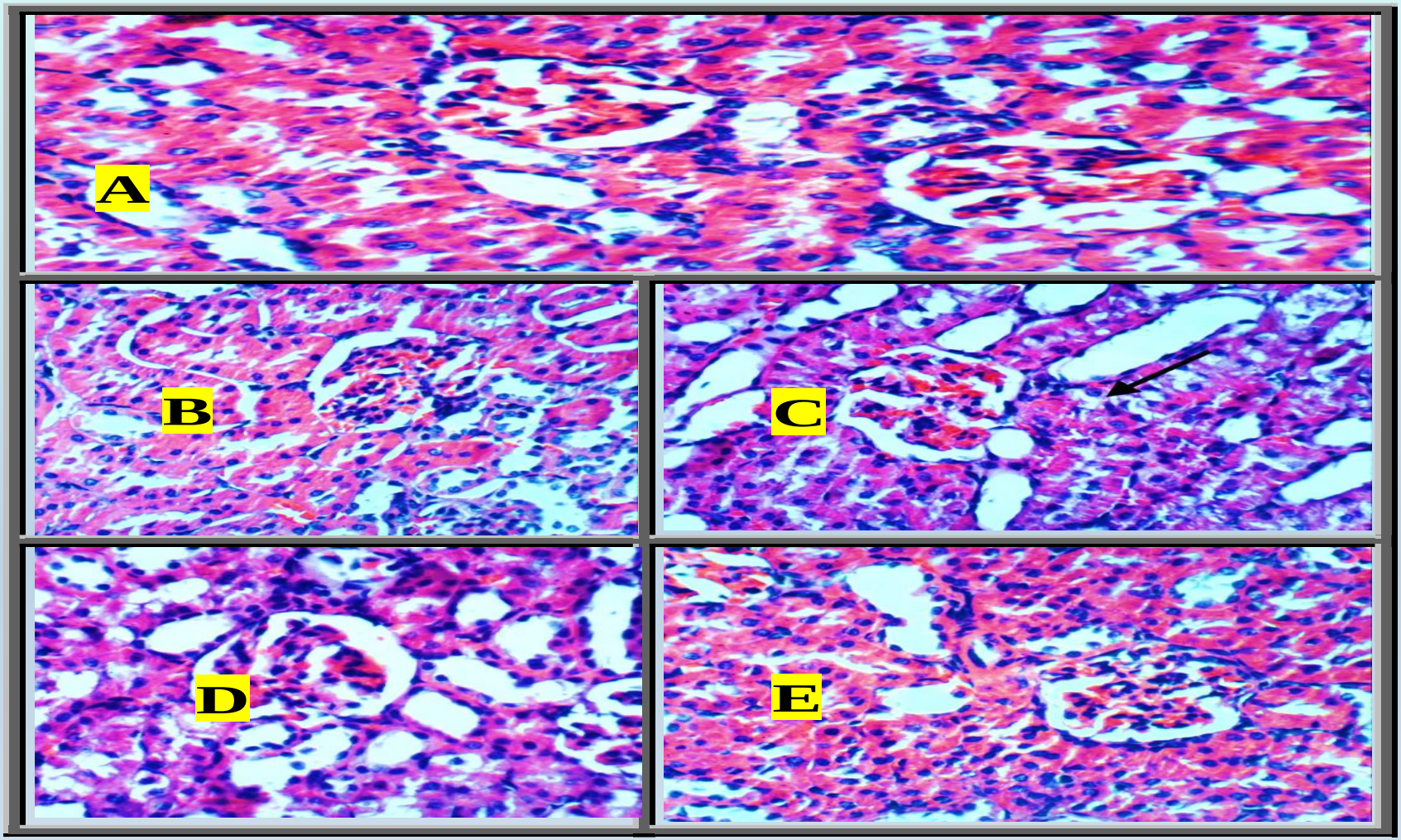
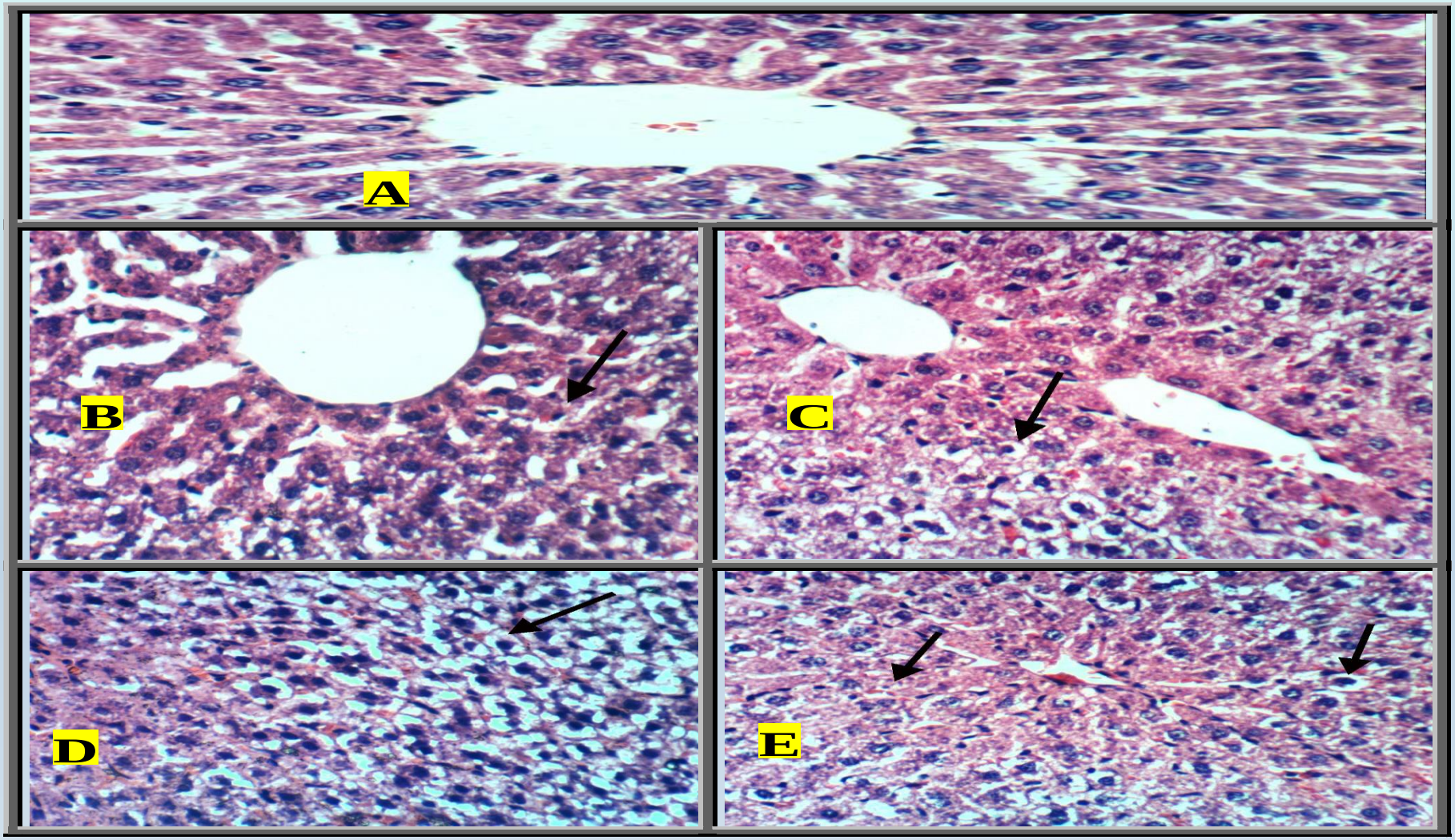


Sections in kidney of rats treated with remediated water from lindane- with ZnO (nano)/H_2O_2/UV (B), and *Xanthomonas campestris pv. Translucens*(C), as well as ZnO (nano)/H_2O_2 (D) and *Xanthomonas campestris pv. Translucens* (E) without lindane relative to control (A).



Sections in liver of rats treated with remediated water polluted from lindane with ZnO (nano)/H_2O_2/UV (B), and *Xanthomonas campestris pv. Translucens*(C), as well as ZnO (nano)/H_2O_2/UV and *Xanthomonas campestris pv. Translucens* (E) without lindane relative to control (A).

CONCLUSIONS

From previous results, it can be concluded that, the better understanding for photochemical degradation mechanism through advanced oxidation processes (AOPs) with Zinc oxide (nano particles) and titanium dioxide (nano particles).,the minerals are generated from hetero atoms such as S,N,Cl, containing organic pollutants and Eg is the band gap of semiconductors and electron-hole pairs are generated in valence and conduction bands of a semiconductor due to presence of free radical, attack the molecules of pollutants such as (Lindane).

The photo titanium dioxide and photo zinc oxide combined with hydrogen peroxide showed much promise in the complete degradation and detoxification of lindane in contaminated water, especially by using titanium and zinc oxide nanoparticles.

Lindane as long life resistance in soil and in water have a good stability chemical structure and resist against degradation. Therefore the use of advanced oxidation processes in presence of ZnO or TiO_2 in nanoparticles play an important role for the mechanism of the chemical degradation. The nanoparticles exhibit photocatalytic activity and the mechanism might be due to cascade electron transfer in the ternary phase (Dhal et. al., 2015)

The degradation of the tested insecticide may be attributed to the secretion of enzymes from either tested bacterial or fungal strains which are capable of degrading pesticides. *Xanthomonas campestris pv. Translucens* and *Aspergillus fumigatus* are promising as effective and safe bioremediation for lindane removal in water.


شكرا لكم

Thank you

Thank you