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Biosafety assessment of *Pleurotus ostreatus* and *P. pulmonarius* cultivated on Bonnylight crude oil contaminated soils

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Abstract

This study investigated the biosafety assessment of Pleurotus ostreatus and P. pulmonarius harvested from soil contaminated with Bonnylight crude oil. Twenty seven albino rats of both sexes were allocated into three groups with three animals each viz: animals fed without P. ostreatus and P. pulmonarius (CON), animals fed with P. ostreatus harvested from soil contaminated with crude oil (POCO) and animals fed with P. pulmonarius harvested from soil contaminated with crude oil (PPCO). The animals were sacrificed after 21 days and their haematological and histopathological parameters were determined. Weights of animals fed with P. ostreatus and P. pulmonarius were higher than the control. The PCV, haemoglobin content, RBC of animals fed with POCO were higher than that of PPCO and control while WBC of animals fed with PPCO was higher than POCO and control. The heart of the rats fed with POCO extract showed little signs of histological deformation. However, there were major signs of histological abnormalities in the heart, kidney, liver and intestine of animals fed with PPCO. This study has shown that large consumption of P. ostreatus grown on crude oil contaminated soil should be done with caution to avoid slight haemorrhage and necrosis of the heart while P. pulmonarius grown on such soils should not be consumed.

Keywords: Pleurotus ostreatus, P. pulmonarius, biosafety assessment, crude oil, contaminated soils.

Introduction

Petroleum pollution is a common problem in oil producing regions of the world[1]. Pollution of environments with these hydrocarbons is hazardous to humans, plants and animals[2]. Physicochemical methods of remediation of polluted sites are becoming obsolete due to the alternative use of bioremediation techniques. Bioremediation proves to be advantageous as it is less expensive and promotes the enzymatic products or microbial growth necessary to transform the noxious pollutants to nontoxic end products[3]. Fungi have been found to be better degraders of petroleum than bacteria[4]. Fungi display several advantages such as penetration of the hyphae and secretion of oxidative enzymes in the polluted sites[5]

White rot fungi (WRF) have been used to degrade an extremely diverse range of persistent or toxic environmental pollutants[6, 7]. This is because they produce a lot of ligninolytic enzymes which are capable of degrading different xenobiotic compounds[6]. *Pleurotus ostreatus* and *P. pulmonarius* which have many industrial applications as potential degrader of various xenobiotics and industrial pollutants[8] are prospective sources of valuable food protein[9]. They are consumed mainly because of their delicious taste and high nutritive value as they are rich in protein, carbohydrate, minerals, vitamins and low fat contents[10].

Several authors have worked on the ability of some *Pleurotus* species to ameliorate crude oil contaminated soils. However, there is no information on the assessment of the toxicological effects of consuming *P. ostreatus and P. pulmonarius* cultivated on crude oil contaminated soils to the best of our knowledge. Therefore, the aim of this present investigation was to ascertain the safety of consuming *Pleurotus ostreatus and P. pulmonarius* cultivated on soil contaminated with Bonnylight crude oil.

Material and Methods

Collection of materials: Spawns of *P. ostreatus* and *P. pulmonarius* were obtained from the Department of Microbiology, University of Ibadan, Nigeria while Bonny light crude oil was collected from Port Harcourt. Soil samples were obtained from FUTA research farm while rice bran was purchased at Oba market, Akure. Also, eight to ten weeks old albino rats comprising of both sexes were used. The animals were purchased from the Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State.

Cultivation of *P. ostreatus* and *P. pulmonarius* on crude oil contaminated soil: One hundred grammes of moistened sterilized soil were weighed into 9 x 9 x 4 cm (350 cm^3) jam bottles and then mixed thoroughly with 30% bonny light crude oil. Each bottle was then inoculated with two agar plugs of a vigorously growing mycelium of *P. ostreatus* and *P. pulmonarius* using a 7 mm sterile cork borer. The bottles were incubated at room temperature for 3 months.

Toxicological studies on *P. ostreatus* and *P. pulmonarius* using albino rats: Albino rats were housed in separate cages, kept in a clean environment and provided with food pellets (standard diet Feed Master©grower mash) and water *ad libitum.* Twenty seven albino rats of both sexes with average weight of 93.5g were allocated into three groups (CON, POCO, PPCO) of three animals each. The animals in CON group were given food pellets without *P. ostreatus* and *P. pulmonarius*. Those in POCO and PPCO groups were fed with food pellets fortified with 1.0, 1.5 and 2.0 g of mushrooms (*P. ostreatus* and *P.pulmonarius*) extracts respectively harvested from soils contaminated with 30% bonnylight crude oil. The experiment was carried out for 21 days and changes in body weight were monitored. The albino rats were anaesthetized with chloroform and sacrificed at the end of the experiment.

Hematological and histological analyses: Blood from sacrificed animals was collected from cervical area with the aid of syringes and needles collection into labelled EDTA bottles. The haematological analysis (determination of Erythrocyte Sedimentation Rate, packed cell volume, haemoglobin level, red blood cell and total and differential white blood cell counts) of the different groups was done according to the method of Momoh et al[11]. Histopathological tests were carried out on the various organs namely heart, intestine, kidney and liver) of the laboratory animals according to the method of Silva et al[12].

Statistical analysis: All data were expressed as mean ± SEM. Statistical analysis was performed using ANOVA. Inter-group comparison was achieved by Duncan Multiple Range Test using SPSS window 15. Differences were judged to be statistically significant when p was less than 0.05.

Results and Discussion

Growth rate of albino rats fed with and without *Pleurotus* species harvested from soil contaminated with crude oil: Generally, the weights of animals fed with *P. ostreatus* (POCO) and *P. pulmonarius* (PPCO) were higher than weight of those that were fed without *P. ostreatus* and *P. pulmonarius* the control (CON). There was increase in the weight of the animals fed with POCO and PPCO extracts as the weight of *Pleurotus* consumed was increasing from day 1 to 21as shown in table 1.

Haematological analyses of albino rats fed with and without *Pleurotus* species harvested from soil contaminated with crude oil: Table 2 shows the packed cell volume (PCV), red blood cell (RBC) and haemoglobin (Hb) of albino rats fed with *P. ostreatus* (POCO) was higher than that of animals fed with *P. pulmonarius* (PPCO) and without mushroom (CON). In addition, the PCV of PPCO was less than CON. The white blood cell count of PPCO was higher than that of POCO and CON. Also, the erythrocyte sedimentation rates (ESR) of albino rats fed with 1.0 and 1.5g of *P. pulmonarius* ($PPCO_1$ and $PPCO_2$) were higher than those fed with 1.0 and 1.5g of *P. ostreatus* ($POCO_1$ and $POCO_2$) respectively. The ESR of POCO and PPCO was higher than CON.

White blood cell differential of albino rats fed with or without *Pleurotus* species harvested from soil contaminated with crude oil: Lymphocytes of PPCO were higher than POCO and CON. Neutrophils were higher in both POCO and PPCO than CON. Monocytes were lower in $PPCO_1$ than $PPCO_1$and in $PPCO_3$ than $POCO_3$. Monocyte count was almost equal in CON and $PPCO_3$. Also, eosinophils and basophils were lowest in PPCO. Basophil count was noted to be between 0 and 0.35% in PPCO as shown in table 3.

Table -1
Weight of albino rats fed with grower mash and *Pleurotus* species harvested from soil contaminated with crude oil

Ays of feeding albino rats	CON	$POCO_1$	$PPCO_1$	$POCO_2$	$PPCO_2$	$POCO_3$	$POCO_3$
1	86.00±0.06	85.00±0.00	92.00±0.02	89.00±0.50	94.00±0.13	90.00±0.15	96.00±0.11
4	87.00±0.05	87.00±0.05	93.00±0.05	90.00±0.30	95.00±0.03	92.00±0.05	97.00±0.08
6	88.00±0.10	89.00±0.05	94.00±0.01	92.00±0.15	97.00±0.11	93.00±0.05	98.00±0.04
9	89.00±0.05	90.00±0.06	96.00±0.04	95.00±029	98.00±0.25	95.00±0.07	98.00±0.09
12	90.00±0.11	92.00±0.05	96.00±0.01	95.00±0.27	98.00±0.13	97.00±0.02	98.00±0.01
15	92.00±0.03	93.00±0.03	95.00±0.00	97.00±0.13	98.00±0.11	98.00±0.05	97.00±0.08
18	93.00±0.02	94.00±0.00	94.00±0.08	98.00±0.15	97.00±0.12	98.00±0.08	96.00±0.06
21	95.00±0.05	96.00±0.02	91.00±0.04	100.00±0.11	94.00±0.05	101.00±0.05	96.00±0.06

Key: CON= Weight of albino rats fed without *P. ostreatus* and *P. Pulmonarius,* $POCO_1$= Weight of albino rats fed with 1.0g of *P. ostreatus* harvested from soil contaminated with crude oil, $PPCO_1$= Weight of albino rats fed with 1.0g of *P. pulmonarius* harvested from soil contaminated with crude oil, $POCO_2$= Weight of albino rats fed with 1.5g of *P. ostreatus* harvested from soil contaminated with crude oil, $PPCO_2$= Weight of albino rats fed with 1.5g of *P. pulmonarius* harvested from soil contaminated with crude oil, $POCO_{3=}$Weight of albino rats fed with 2.0g of *P. ostreatus* harvested from soil contaminated with crude oil, $PPCO_{3=}$Weight of albino rats fed with 2.0g of *P. pulmonarius* harvested from soil contaminated with crude oil.

Table-2
Haematological parameters of albino rats fed with grower mash and *Pleurotus* species harvested from soil contaminated with crude oil

Treatment	ESR%	PCV%	Hb%	WBC(x10^3)/mm^3	RBC%
CON	1.0 ± 2.55	34.05 ± 6.52	11.50 ± 7.25	5.50 ±1.02	6.86 ± 2.51
$POCO_1$	1.0 ± 1.68	38.07 ± 9.71	13.50 ± 8.22	5.70 ± 3.34	7.66 ± 1.33
$PPCO_1$	2.0 ± 5.20	31.67 ± 14.43	11.10 ± 7.00	6.13 ± 0.49	6.35 ± 2.63
$POCO_2$	1.4 ± 2.34	38.67 ± 2.52	13.10 ± 8.00	5.57 ± 1.15	7.25 ± 2.44
$PPCO_2$	1.7 ± 3.27	32.33 ± 7.64	11.00 ± 6.25	7.29 ± 1.29	6.31 ± 4.21
$POCO_3$	1.5 ± 2.33	38.00± 6.56	13.00 ± 7.51	4.63 ± 2.36	7.38 ± 3.11
$PPCO_3$	1.4 ± 2.44	27.67 ± 6.50	10.00 ± 5.11	7.93 ±1.02	6.30 ± 4.41

Key: CON= Albino rats fed without *P. ostreatus* and *P. Pulmonarius,* $POCO_1$= Albino rats fed with 1.0g of *P. ostreatus* harvested from soil contaminated with crude oil, $PPCO_1$= Albino rats fed with 1.0g of *P. pulmonarius* harvested from soil contaminated with crude oil, $POCO_2$= Albino rats fed with 1.5g of *P. ostreatus* harvested from soil contaminated with crude oil, $PPCO_2$= Albino rats fed with 1.5g of *P. pulmonarius* harvested from soil contaminated with crude oil, $POCO_{3=}$ Albino rats fed with 2.0g of *P. ostreatus* harvested from soil contaminated with crude oil, $PPCO_{3=}$ Albino rats fed with 2.0g of *P. pulmonarius* harvested from soil contaminated with crude oil, ESR= Erythrocyte Sedimentation Rate; PCV= Packed cell Volume; Hb= Haemoglobin; RBC= Red blood cell, WBC= White blood cell

Table-3
White blood cell differential of the experimental animals

Treatments	L%	N%	M%	Eos%	Bas%
CON	65.07 ± 3.06	15.00 ± 8.96	7.00 ± 0.52	2.55 ± 1.24	1.00 ± 0.80
$POCO_1$	65.33 ± 10.60	25.33 ± 9.71	6.00 ± 0.00	2.50 ± 1.00	1.00 ± 0.80
$PPCO_1$	68.33 ± 12.50	25.67 ± 12.50	5.00 ± 0.00	1.40 ± 0.91	0.35 ± 0.53
$POCO_2$	66.33 ± 6.50	27.33 ± 7.02	5.00 ± 0.00	2.00 ± 0.85	0.35 ± 0.53
$PPCO_2$	70.33 ± 11.72	25.33 ± 11.93	5.00 ± 0.54	1.40 ± 0.91	0.00 ± 0.00
$POCO_3$	64.66 ± 11.15	26.00 ±10.58	7.33 ± 0.58	2.50 ± 1.00	0.35 ± 0.53
$PPCO_3$	70.67 ± 3.06	26.33 ± 8.96	4.00 ± 0.50	0.35 ± 0.53	0.00 ± 0.00

Key: CON= Albino rats fed without *P. ostreatus* and *P. Pulmonarius,* $POCO_1$= Albino rats fed with 1.0g of *P. ostreatus* harvested from soil contaminated with crude oil, $PPCO_1$= Albino rats fed with 1.0g of *P. pulmonarius* harvested from soil contaminated with crude oil, $POCO_2$= Albino rats fed with 1.5g of *P. ostreatus* harvested from soil contaminated with crude oil, $PPCO_2$= Albino rats fed with 1.5g of *P. pulmonarius* harvested from soil contaminated with crude oil, $POCO_{3=}$ Albino rats fed with 2.0g of *P. ostreatus* harvested from soil contaminated with crude oil, $PPCO_{3=}$ Albino rats fed with 2.0g of *P. pulmonarius* harvested from soil contaminated with crude oil, L= Lymphocytes; N=Neutrophils; M=Monocytes; Eos= Eosinophils; Bas= Basophils.

Histopathological analyses of experimental animals: The photomicrographs of heart muscles of CON albino rats showed no histological deformation and have similar structures with POCO although there was slight haemorrhage and necrosis in animals fed with POCO. However, PPCO albino rat showed dilation of heart strata, necrosis and extreme haemorrhage as shown in figure 1.

Also, the kidney sections of CON and POCO albino rats showed normal nephrotic cells. However, PPCO showed cell infiltrations and presence of cast cells. This resulted to haemorrhage, reduction and detachment of the glomerula of the kidney as shown in figure 2.

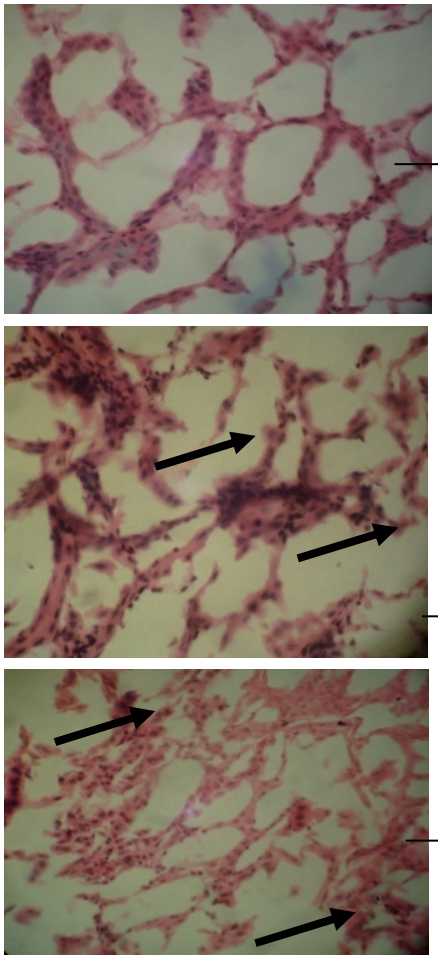
The photomicrographs of the liver of CON and POCO albino rats showed normal hepatic cells with its sinusoids in place but the liver of PPCO showed cell vacuolation, packed sinusoids and spaceous hepatocytic cells as shown in figure 3.

Moreover, the intestine of CON and POCO albino rats showed normal intestinal villi without necrosis and washing off of intestinal walls. The intestine of PPCO albino rats showed haemorrhage and spaceous intestinal villi and washed intestinal wall as shown in figure 4.

Discussion: Ogbonnia *et al.*[13] reported that experimental screening is important to ascertain the safety and efficacy of natural products. Food pellets fortified with *P. ostreatus* and *P. pulmonarius* led to increase in the weights of albino rats. The increase in weights of albino rats fed with *P. ostreatus* and *P. pulmonarius* indicated growth[14]. The increase in PCV, RBC and Hb of rats fed with *P. ostreatus* harvested from soil contaminated with crude oil showed that there was no occurrence of anaemia. This implies that the animals were healthy. However, the decrease in these parameters in rats fed with *P. pulmonarius* from the same kind of soil was an indication that there is tendency for hypochromic anaemia to occur in those animals[15]. Also, the increase in WBC rats fed with *P. pulmonarius* showed that there was an immune disorder or certain infection thus leading to multiplication of WBC to fight the infection[16]. Generally, neutrophils, basophils and eosinophils of albino rats fed *Pleurotus pulmonarius* from soil contaminated with 30% bonnylight crude

oil were higher than those fed with *P.ostreatus* from the same kind of soil. These parameters were lower in animals fed without any of the above mushrooms. Neutrophils and eosinophils have been found to respond chemotactically to bacterial products and complement fragments[17].

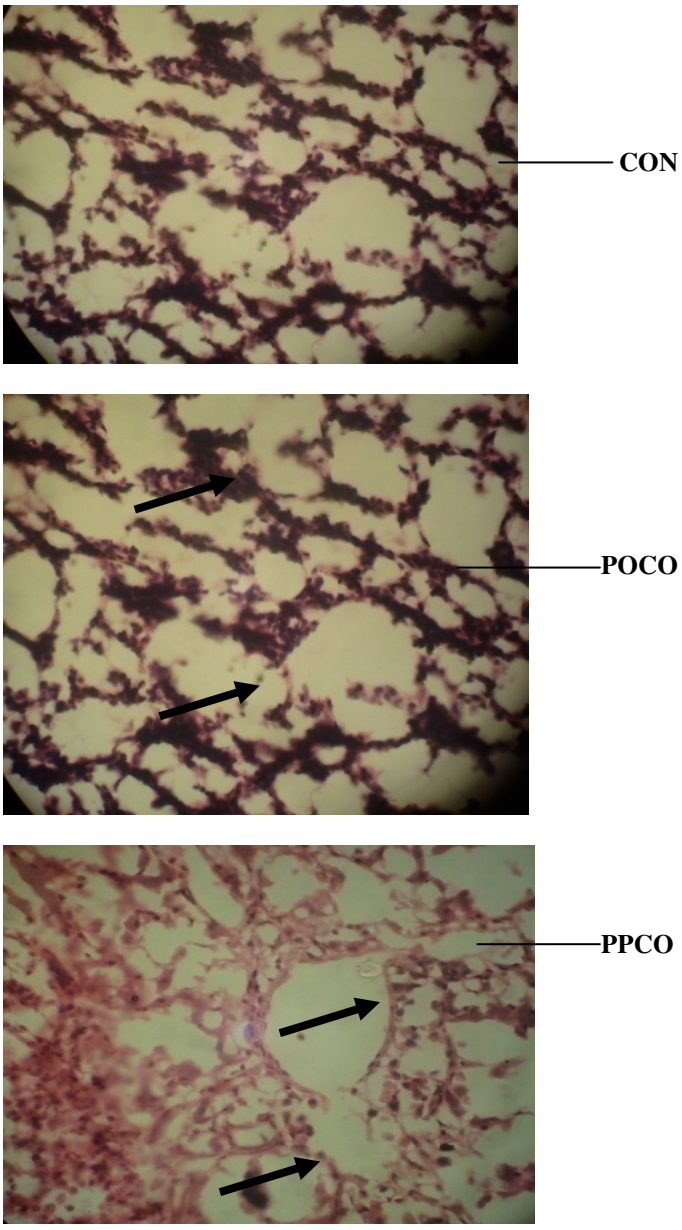
The albino rats fed with *P. ostreatus* harvested from soil contaminated with 30% crude oil were as healthy as those fed with normal grower mash diet without mushrooms. Both rats showed no histopathological deformation of the heart, kidney, liver and intestine. It has been reported that toxic effects of natural products on host cells must be considered as a substance that may exhibit an apparent biological activity by virtue of toxic effect on cells[18]. The histopathological results of rats fed with *P. pulmonarius* showed that damages were done to the tissues of liver, kidney, heart and intestine. These were evident by the clinical signs of toxicity such as cell vacuolation, packed sinusoids, spaceous hepatocytic cells of the liver as well as haemorrhage, infiltration of cells, reduction and detachment of the glomerula of the kidney[19].



(H+E × 100)

Figure-1
Photomicrographs of heart muscles of albino rats at different feeding treatments

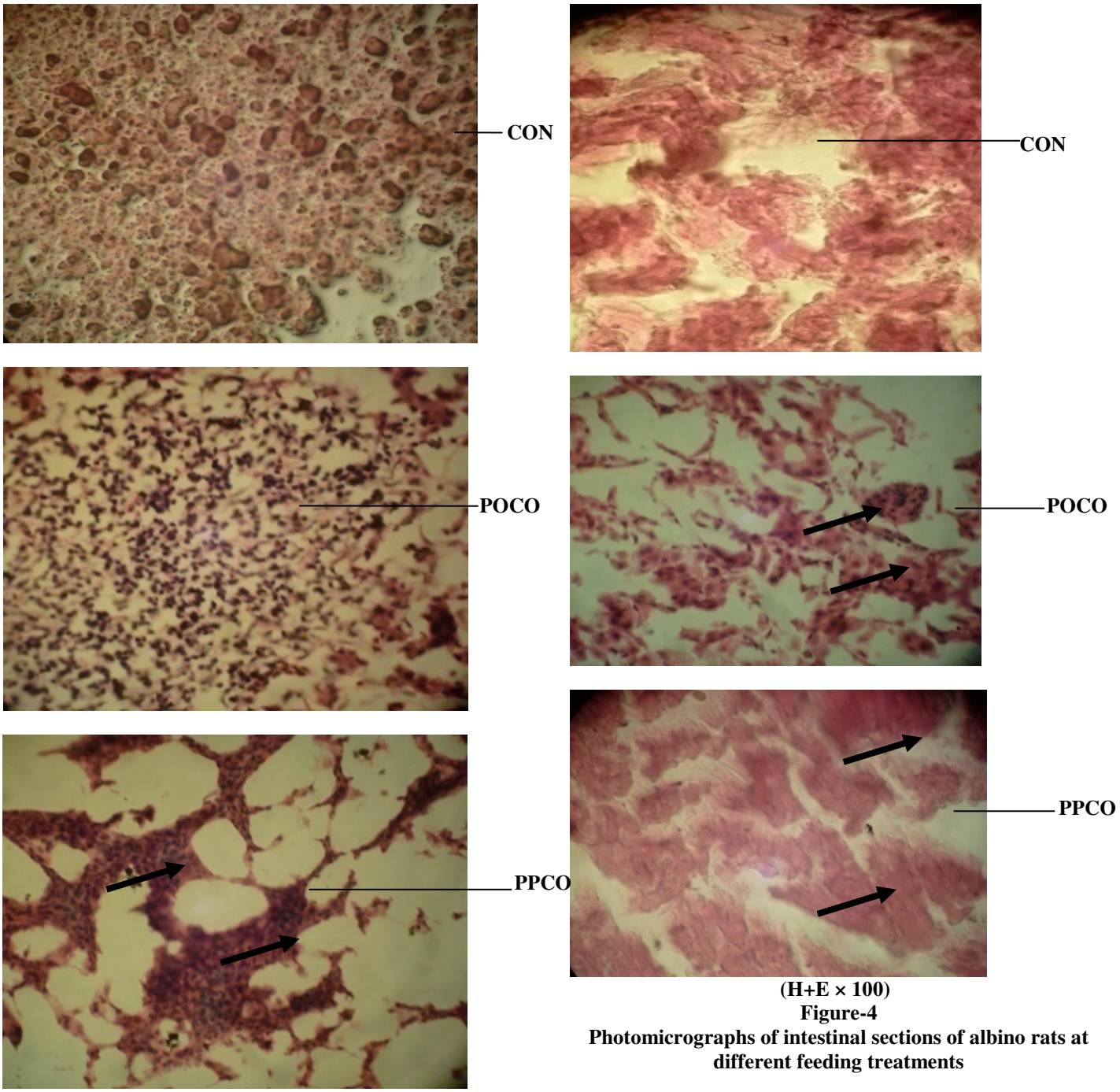
Key: CON: Heart muscle of rat fed without *P. ostreatus* and *P. Pulmonarius,* POCO: Heart muscle of rat fed with *P. ostreatus* harvested from soil contaminated with 30% crude oil, PPCO: Heart muscle of rat fed with *P. pulmonarius* harvested from soil contaminated with 30% crude oil.



(H+E × 100)

Figure-2
Photomicrographs of kidney sections of albino rats at different feeding treatments

Key: CON: Kidney section of rat fed without *P. ostreatus* and *P. Pulmonarius,* POCO: Kidney section of rat fed with *P. ostreatus* harvested from soil contaminated with 30% crude oil, PPCO: Kidney section of rat fed with *P. pulmonarius* harvested from soil contaminated with 30% crude oil



(H+E × 100)
Figure-3
Photomicrographs of liver sections of albino rats at different feeding treatments

Key: CON: Liver section of rat fed without *P. ostreatus* and *P. Pulmonarius,* POCO: Liver section of rat fed with *P. ostreatus* harvested from soil contaminated with 30% crude oil, PPCO: Liver section of rat fed with *P. pulmonarius* harvested from soil contaminated with 30% crude oil.

(H+E × 100)
Figure-4
Photomicrographs of intestinal sections of albino rats at different feeding treatments

Key: CON: Intestinal section of rat fed without *P. ostreatus* and *P. Pulmonarius,* POCO: Intestinal section of rat fed with *P. ostreatus* harvested from soil contaminated with 30% crude oil, PPCO: Intestinal section of rat fed with *P. pulmonarius* harvested from soil contaminated with 30% crude oil.

Conclusion

Pleurotus ostreatus cultivated on bonnylight crude oil may be consumed with caution because of slight necrosis and haemorrhage of the hearts of albino rats fed with POCO while

P. pulmonarius is not safe for consumption based on the major clinical signs of toxicity and damages done to the tissues.

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