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Evaluation of long-term dermal exposure to soil contaminated with spent engine oil in male Wistar rats: An experimental approach

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Abstract

Continuous occupational exposure to spent engine oil (SEO) poses physiological risks to humans, especially to automobile mechanics. This study investigated the physiological effects of SEO-contaminated soil in a male Wistar rat model. Animals were dermally exposed to soil contaminated with SEO for 120 consecutive days and compared with rats exposed to uncontaminated soil (negative control). Heavy metal (lead (Pb), nickel (Ni), zinc, and cadmium (Cd)) accumulations, hematology, biochemical (aspartate aminotransferase (AST), alanine aminotransferase, alkaline phosphatase (ALP), urea, and creatinine) parameters, sperm morphology, and histopathology (liver, kidney, lungs, brain, skin, and testis) were evaluated as end points. Results revealed that the heavy metals in SEO-contaminated soil are far greater than the World Health Organisation permissible limits, with significant ($p < 0.05$) increases of Pb and Ni present in the brain, and Pb and Cd in the serum compared with the uncontaminated soil for the negative control. Only significant ($p < 0.05$) values were observed in the lymphocytes, activities of AST and ALP, and sperm abnormalities of the exposed rats compared with those used for the negative control. Histopathological changes were not evident in the brain but lesions were observed in the liver, kidney, lungs, skin, and testis of the exposed rats. Results herein suggest that the constituents of SEO may elicit harmful physiological changes to humans who are directly exposed to them.

Keywords

Spent engine oil, chronic dermal exposure, contaminated soil, heavy metals, physiological alterations

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Introduction

Spent engine oil (SEO), also called crankcase oil or waste engine oil, isused oil drained from automobile engines, generators, and other machines and are one of the major environmental pollutants in Nigeria owing to the high demand for lubricant oil (Ahamefule et al., 2014; Okonokhua et al., 2007). SEO is produced from industries (machineries, fabricated metal products, and chemical products), transportation (automobiles, buses, airplanes, and trucks), and others (shock absorbers and refrigeration units) (Bamiro and Osibanjo, 2004). As the number of registered cars in Nigeria increases, there is the likelihood of increased SEO due to engine leaks and engine servicing (Anoliefo and Edegbai, 2000; Osubor and Anoliefo, 2003). Reports from Bamiro and Osibanjo (2004) indicated that 6 million registered vehicles

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produced an estimated 200 million liters of SEO per annum. As a result, there is the indiscriminate release and disposal of SEO onto farmlands, into drainage systems, and onto soil by automobiles in Nigeria resulting in challenges of managing its disposal.

SEO, usually a brown to black liquid, contains a mixture of chemicals ranging from aromatic/aliphatic hydrocarbons, PAHs as well as heavy metals such as V, Al, Pb, Cr, Ni, Cu, Cd, and Si (Arise et al., 2012; ATSDR, 1997). The metal concentrations found in SEO are far higher than those in new motor oils; therefore, SEO potentially constitutes health risks to living organisms. Many of these complex chemicals and heavy metals are nondegradable; they accumulate over a long period of time and become dangerous to human health and the environment (atmosphere, soil, and water) when spilled or burned (Vazquez-Duhalt, 1989). They may penetrate the soil into ground water and contaminate other sources of drinking water. They alter the physiology of organisms by generating reactive oxygen species (Abdullah et al., 2018; Arise et al., 2012; Omoruwa et al., 2015; Singru et al., 2017; Sunmonu and Oloyede, 2007).

In Nigeria, the indiscriminate disposal of SEO has led to direct and/or indirect exposures of humans, creating health risks, via dermal, oral, or inhalatory routes of exposure. Studies have shown the toxicity of used engine oil exposure to Wistar rats and mice orally (Babalola and Oni, 2018; Patrick-Iwuanyanwu et al., 2010; Wasiu et al., 2015), dermally to rabbits and rats (Sibomana et al., 2019; Sibomana and Mattie, 2020), and to aquatic organisms such as *Clarias gariepinus* (Ayoola and Akaeze, 2012).

Occupational workers such as automobile mechanics and other autoworkers are primarily exposed dermally to SEO that accumulates in automobile parts, equipment, open grounds, and work benches, among other places. With increasing consumption of engine oil because of increased utilization of machines, equipment, and vehicles, the chances of long-term SEO dermal toxicity exposure to mechanics and other autoworkers have continued to increase. Up until now, no study has investigated the effect of long-term exposure to SEO, modeling the occupational exposure route of auto mechanics who are continuously in contact with SEO contaminated soils.

Therefore, this study simulated, in a controlled laboratory, the scenario that often occurs among automobile mechanics who are repeatedly exposed dermally to SEO from vehicles and heavy-duty machines. Hypothetically, chronic dermal exposure to SEO-contaminated soil samples (SEOCS) is expected to alter physiological parameters in male Wistar rats. Specific hypotheses for this study include (1) chronic dermal exposure to SEO will bioaccumulate components in the viscera of Wistar rats, (2) chronic dermal exposure to SEO will alter biochemical and hematological parameters and histopathology of viscera in Wistar rats, and (3) chronic dermal exposure will induce sperm abnormalities in the Wistar rats. Hence, the study assessed the toxicity effects of long-term dermal exposure of SEO on male Wistar rats. Assessed toxicological end points were heavy metal bioaccumulation, hematological and biochemical parameters, sperm morphology, and histopathology.

Materials and methods

SEO and soil samples

SEO was collected from the automobile section of the Works and Maintenance Department of the University of Ibadan, Oyo State, Nigeria. Two different categories of soil samples were collected and used: uncontaminated soil sample, which was obtained from the botanical garden of the University of Ibadan, Oyo State, Nigeria, and the contaminated soil sample obtained from an auto-mechanic shop at the Agbowo Express Junction, also in Ibadan, Oyo State, Nigeria.

Experimental animals

Twenty-five healthy adult male Wistar rats (100–150 g) obtained from the Animal Facility of the Department of Physiology, University of Ibadan were acclimatized for 14 days in polypropylene cages and maintained at an average room temperature of 25°C with relative humidity of 60% with a 12-h light/12-h dark cycle. The animals were fed a standard rat diet, and potable water was provided *ad libitum*. The use and ethics of animals were according to the standard guidelines of ILAR (2011). The animal procedures were approved by the Research Ethics Committee of the University of Ibadan, Ibadan, Nigeria (UI-ACUREC/App/0195).

Experimental design

The rats were randomly divided into five groups of five rats each. They were continuously exposed to 2 kg soil samples mixed with SEO and used as their beddings for 120 consecutive days. These beddings were renewed every 48 h to maintain constant dermal exposure.

Group 1 (G1): Rats housed in a cage with uncontaminated soil collected from the Botanical Garden of the University of Ibadan. This group served as the negative control.

Group 2 (G2): Rats housed in a cage with contaminated soil collected from an auto-mechanic workshop in Ibadan. This group served as the positive control.

Group 3 (G3): Rats housed in a cage with uncontaminated soil mixed with 200 mL SEO.

Group 4 (G4): Rats housed in a cage with uncontaminated soil mixed with 300 mL SEO.

Group 5 (G5): Rats housed in a cage with uncontaminated soil mixed with 400 mL SEO.

Blood collection

At the end of 120 days of dermal exposure, the rats were euthanized by cervical dislocation. Five milliliters of blood was obtained by cardiac puncture with 3 mL transferred into ethylenediaminetetraacetic acid (EDTA) bottles for hematological analysis and 2 mL into plain bottles, which was centrifuged using Beckman centrifuge (JR-3.2) at 2555 g and stored at −20°C for further biochemical analyses.

Collection of organs

The liver, lungs, kidney, skin, and testis were excised and rinsed in normal saline. A portion (0.3 g) of each organ was stored in 10% formalin for histopathological analysis while the remaining portions were kept at −4°C for heavy metal analysis.

Heavy metal analysis in soil and tissue samples

The soil samples and tissue (liver, kidney, lungs, brain, and serum) samples from the exposed rats were analyzed for the presence of heavy metals (lead (Pb), nickel (Ni), zinc (Zn), and cadmium (Cd)). The digestion of the tissue samples was carried out according to the method described by Babalola et al. (2009). Heavy metal concentrations were determined in replicates using the Buck 200 Atomic Absorption Spectrophotometer (AOAC, 975.23 model), USA.

Biochemical analysis

Blood collected into plain bottles were analyzed using Randox kits, United Kingdom, for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and levels of urea (BUN) and creatinine (CREA).

Hematological parameters

Blood samples collected into EDTA bottles were also analyzed for hematological profiles, which included packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell (RBC) count, white blood cell (WBC) count, and their differentials.

Sperm morphology assay

Sperm morphology assay was carried out according to Wyrobek et al. (1983). The cauda epididymis of each rat was surgically excised, rinsed in normal saline, and excess fats were removed. The tissues were further transferred and teased in a mixture of normal saline and 1% eosin Y aqueous stain (9:1 v/v), and left for 45 min. Smears were prepared from the suspension on clean labeled glass slides and allowed to air dry for 24 h. Six slides were prepared per animal, out of which four slides were randomly selected for scoring under a light microscope at 1000 × magnification. A total of 1000 sperm cells per animal were examined and assessed for abnormalities according to Wyrobek and Bruce (1975).

Histopathological examination

The harvested organs (lungs, kidney, liver, brain, and skin) were collected into containers and fixed with 10% formalin, while the testes were fixed in Bouin's fluid for routine histological analysis. The organs were washed and processed through an ascending series of ethanol, cleared in methyl salicylate, and infiltrated with wax at 57°C, then embedded in paraffin. Sections of 5 µm were cut and stained with aqueous hematoxylin and alcoholic-eosin, then examined using a light microscope at magnification of 400×.

Statistical analysis

All data were analyzed as mean ± SE using one-way analysis of variance followed by Dunnett *post hoc* test. Means were considered statistically different at $p < 0.05$. GraphPad Prism 5.01 for Windows, GraphPad Software (San Diego California, USA) was used in performing all analyses.

Table 1. Heavy metal concentrations from contaminated soil samples.

Sample	Pb (mg/kg)	Cd (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
US	17.84	5.88	13.57	29.69
CSMW	123.87	16.29	36.79	326.58
SEOCS	129.65	19.23	44.25	345.76
WHO	85.00	8.00	35.00	50.00

US: uncontaminated soil sample; CSMW: contaminated soil from the mechanic's workshop; SEOCS: spent engine oil contaminated soil sample; WHO: World Health Organisation.

Results

Heavy metal concentrations in contaminated soil samples

Table 1 presents the heavy metal concentrations (mg/kg) of Pb, Ni, Zn, and Cd in the soil samples. Uncontaminated soil sample had the least concentrations of these metals which were below the WHO maximum permissible limit. The spent engine oil contaminated soil sample (SEOCS) and contaminated soil from the mechanic's workshop (CSMW) groups contained higher concentrations of these metals with the SEOCS having the highest concentrations of the heavy metals. Pb concentrations in the CSMW and SEOCS groups were 1.46- and 1.53-fold higher than the WHO permissible limit. Ni concentrations from the CSMW and SEOCS samples were 1.05- and 1.26-fold higher than the WHO permissible limit. Likewise, the concentrations of Zn for the CSMW and SEOCS groups were 6.53- and 6.92-fold higher than the WHO value. Cd concentrations for the CSMW and SEOCS groups had 2.03- and 2.40-fold increase than the WHO maximum permissible value of heavy metals in soil.

Heavy metal concentrations in tissues of rats exposed to contaminated soil samples

Table 2 presents the heavy metal concentrations in the liver, kidney, lungs, brain, and serum of rats dermally exposed to contaminated soil samples. The heavy metal concentrations measured in the tissues were not significantly ($p > 0.05$) altered compared with the concentrations in the tissues under negative control except for Pb and Ni in the brain of rats exposed to CSMW, and 400 mL of SEO, and Pb and Cd in the serum of rats exposed at greater volumes of SEO, which were significantly ($p < 0.05$) greater than that of the negative control.

Biochemical parameters in rats exposed to contaminated soil samples

Table 2 also presents the different enzyme parameters in the rat sera across the different exposure groups. There were no significant ($p > 0.05$) increases in the values of ALT, BUN, and CREA in the sera of rats exposed to the different soil samples contaminated with SEO. The AST activity in the group exposed to 200 mL and 400 mL of SEOCS was significantly ($p < 0.05$) greater than the negative control group. Likewise, the ALP activity in the rats exposed to 400 mL of SEO contaminated soil was significantly ($p < 0.05$) greater than that of the negative control.

Hematological parameters in rats exposed to SEO contaminated soil

Hematological parameters of the rats exposed to SEOCS samples are presented in Table 3. No significant ($p > 0.05$) changes were observed in the values of PCV, Hb, RBC count, WBC count, platelets, and other WBC differentials except for the percentage lymphocytes that were significantly ($p < 0.05$) increased in the G2, G4, and G5 groups compared with the negative control.

Sperm abnormalities in rats induced by contaminated soil samples

The frequency of abnormal spermatozoa in rats exposed to the contaminated soil samples is shown in Figure 1. Rats exposed to CSMW (G2) had the highest number of aberrations (9.4 ± 2.9), while the control group (G1) had the least (4.8 ± 1.76). Subsequently, there was a concentration-dependent increase in sperm abnormalities in the SEO contaminated soil groups (Figure 1). Figure 2 depicts the various abnormalities in rats exposed to the different soil samples contaminated with SEO.

Histological evaluations of rat organs

Figures 3 to 8 represent the photomicrographs of the histopathological lesions of the liver, kidney, lungs, testis, skin, and brain of rats exposed to contaminated soil samples. The photomicrographs of the liver of rats exposed to different concentrations of contaminated soil showed normal cords of hepatocytes with sinusoids for the negative control group, while the photomicrographs for the experimental groups showed periportal hepatocellular necrosis, vasculitis,

Table 2. Heavy metal analysis in tissues and biochemical parameters in rats exposed to contaminated soil samples for 120 days.[a]

Heavy metal concentrations						Serum biochemical parameters				
Tissues	Groups	Pb (μg/L)	Ni (μg/L)	Zn (μg/L)	Cd (μg/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	BUN (mg/dL)	CREA (mg/dL)
Liver	G1	0.43 ± 0.10	0.65 ± 0.07	45.61 ± 4.05	0.06 ± 0.01	40.75 ± 0.95	29.00 ± 1.08	94.25 ± 6.36	16.78 ± 0.50	0.65 ± 0.05
	G2	0.52 ± 0.04	0.67 ± 0.01	46.98 ± 5.71	0.07 ± 0.00	40.75 ± 1.49	29.25 ± 1.65	99.25 ± 10.06	17.33 ± 0.12	0.65 ± 0.03
	G3	0.41 ± 0.02	0.72 ± 0.03	44.77 ± 1.51	0.06 ± 0.01	45.50 ± 0.50[b]	33.00 ± 1.00	75.00 ± 3.00	16.95 ± 0.35	0.65 ± 0.05
	G4	0.86 ± 0.27	1.13 ± 0.44	46.73 ± 8.60	0.08 ± 0.01	39.25 ± 1.32	28.25 ± 1.44	87.75 ± 7.35	17.10 ± 0.20	0.65 ± 0.03
	G5	0.88 ± 0.54	1.15 ± 0.58	35.17 ± 6.00	0.08 ± 0.03	44.50 ± 1.44[b]	32.25 ± 1.11	108.00 ± 4.64[b]	17.20 ± 0.24	0.70 ± 0.00
Kidney	G1	0.50 ± 0.15	0.68 ± 0.06	36.98 ± 1.31	0.06 ± 0.02					
	G2	0.95 ± 0.02	1.27 ± 0.05	48.38 ± 5.00	0.08 ± 0.01					
	G3	0.83 ± 0.48	1.17 ± 0.44	40.65 ± 2.77	0.07 ± 0.03					
	G4	0.92 ± 0.40	1.15 ± 0.54	35.40 ± 6.03	0.08 ± 0.01					
	G5	1.30 ± 0.17	1.37 ± 0.28	33.23 ± 3.98	0.08 ± 0.02					
Lungs	G1	0.86 ± 0.46	1.17 ± 0.50	32.87 ± 4.48	0.08 ± 0.03					
	G2	0.99 ± 0.10	1.35 ± 0.02	35.32 ± 6.25	0.08 ± 0.01					
	G3	0.93 ± 0.40	1.10 ± 0.61	35.06 ± 5.61	0.07 ± 0.03					
	G4	0.92 ± 0.31	1.25 ± 0.50	40.59 ± 12.15	0.09 ± 0.02					
	G5	0.46 ± 0.03	0.51 ± 0.03	40.85 ± 0.27	0.05 ± 0.01					
Brain	G1	0.49 ± 0.02	1.25 ± 0.22	32.30 ± 3.45	0.07 ± 0.01					
	G2	1.31 ± 0.04[b]	1.65 ± 0.21[b]	33.14 ± 4.81	0.10 ± 0.01					
	G3	0.65 ± 0.23	0.57 ± 0.21	43.57 ± 2.75	0.07 ± 0.01					
	G4	1.08 ± 0.16	0.93 ± 0.41	34.81 ± 6.44	0.07 ± 0.02					
	G5	1.12 ± 0.10[b]	1.53 ± 0.00[b]	33.47 ± 4.71	0.08 ± 0.01					
Serum	G1	0.24 ± 0.01	0.37 ± 0.02	62.90 ± 0.04	0.04 ± 0.00					
	G2	0.30 ± 0.03	0.44 ± 0.02	63.31 ± 0.03	0.05 ± 0.00[c]					
	G3	0.24 ± 0.02	0.45 ± 0.03	62.19 ± 1.95	0.05 ± 0.00[c]					
	G4	0.20 ± 0.01	0.43 ± 0.02	61.78 ± 0.02	0.05 ± 0.00[c]					
	G5	0.32 ± 0.03[b]	0.41 ± 0.02	63.79 ± 0.03	0.05 ± 0.00[c]					

G1: rats exposed to uncontaminated soil sample; G2: rats exposed to CSMW soil sample; G3: rats exposed to 200 mL SEO contaminated soil sample; G4: rats exposed to 300 mL SEO contaminated soil sample; G5: rats exposed to 400 mL SEO contaminated soil sample. SEO: spent engine oil; CSMW: contaminated soil from the mechanic's workshop; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; BUN: urea; CREA: creatinine; Pb: lead; Ni: nickel; Zn: zinc; Cd: cadmium.

[a]Data are expressed as mean ± SEM ($n = 5$).

[b]$p < 0.05$: significantly different from the negative control (G1).

[c]$p < 0.01$: significantly different from the negative control (G1).

Table 3. Hematological parameters of rats exposed to contaminated soil samples for 120 days.[a]

Groups	PCV (%)	Hb (g/dL)	RBC (cell/L)	WBC (10^3 μ/L)	Platelets (10^3 μ/L)	LYM (%)	NEU (%)	EO (%)	MO (%)
G1	30.00 ± 4.14	10.98 ± 1.20	5.58 ± 0.72	5.73 ± 1.24	100.80 ± 4.99	58.75 ± 1.93	37.50 ± 2.10	2.00 ± 0.41	1.75 ± 0.48
G2	37.75 ± 1.97	10.77 ± 1.86	6.19 ± 0.33	6.01 ± 1.77	159.00 ± 27.44	66.50 ± 1.85[b]	29.25 ± 1.44	1.75 ± 0.48	2.50 ± 0.289
G3	37.00 ± 0.00	12.10 ± 0.10	6.21 ± 0.02	7.73 ± 2.93	126.00 ± 14.00	57.00 ± 3.00	39.50 ± 3.50	1.50 ± 1.50	2.00 ± 1.00
G4	40.00 ± 1.47	13.05 ± 0.44	6.62 ± 0.44	6.99 ± 1.55	134.00 ± 25.89	67.25 ± 2.75[b]	29.00 ± 2.86	2.00 ± 0.41	1.75 ± 0.25
G5	36.50 ± 2.72	11.95 ± 0.90	5.91 ± 0.54	6.34 ± 2.49	141.00 ± 46.87	64.75 ± 2.87[b]	32.50 ± 3.07	0.75 ± 0.25	2.00 ± 0.41

G1: rats exposed to uncontaminated soil sample; G2: rats exposed to CSMW soil sample; G3: rats exposed to 200 mL SEOCS; G4: rats exposed to 300 mL SEOCS; G5: rats exposed to 400 mL SEOCS. CSMW: contaminated soil from the mechanic's workshop; SEOCS: SEO contaminated soil sample; PCV: packed cell volume, Hb: Hemoglobin, RBC: red blood cell, WBC: white blood cell, LYM: lymphocyte, NEU: Neutrophils, EO: Eosinophil, MO: Monocyte.

[a]Values are expressed as mean ± SEM ($n = 5$).

[b]$p < 0.05$: significantly different from the negative control.

atrophy, and centrilobular hepatocellular degeneration (Figure 3). The kidney of the rats in the negative control group showed no lesions while those of the rats in the experimental groups showed lesions such as degeneration of tubular epithelium, ectasia, and congestion of interstitial capillaries (Figure 4). It was also observed that the lungs of the rats in the negative control group showed no lesions while those of the rats in the experimental groups showed capillary and pulmonary congestion, and interstitial pneumonia (Figure 5). The testis of the negative control group also showed no lesions. However, those in the experimental groups showed tubular degeneration and spermatogenic arrest (Figure 6). The skin of the negative control group also showed no lesions while those in the experimental groups showed hydropic degeneration of keratinocytes and increased pigmentation (Figure 7). Also, the brain of the rats in the experimental groups exposed to different contaminated soil samples did not exhibit any lesions (Figure 8).

Discussion

Our study showed high concentrations of Pb, Ni, Zn, and Cd in the SEO contaminated soil, which are significantly above the WHO (1996) permissible heavy metal content in soil. Increased levels of these heavy metals in the SEO are most likely a result of the increased activities of motor engine's wear and tear and exhausts of vehicles. Nevertheless, the toxicity of these heavy metals is likely to increase when absorbed in bio-systems (Garba et al., 2013). Studies have shown that these heavy metals are not advantageous to the body, and at higher levels can induce physiological dysfunction. For instance, Pb and Cd toxicities are associated with alterations in the kidneys and liver tissues (Andejelkovic et al., 2019), hemopoietic system (Alwaleedi, 2016), and the reproductive system (Zhao et al., 2017).

Exposure to heavy metals and other organic compounds present in SEO mainly occurs through inhalation, ingestion, and dermal routes, of which ATSDR ranks Pb and Cd as second and seventh, respectively, on the list of priority dangerous substances (ATSDR, 2019). Our study showed significant levels of Pb and Ni in the brain, and Pb and Cd in the serum of rats dermally exposed to SEO contaminated soil. The study also observed that the brain may be a possible target organ of Pb accumulation owing to its relative sensitivity to microenvironmental changes (Liu et al., 2010). Sanders et al. (2009) showed that calcium ions

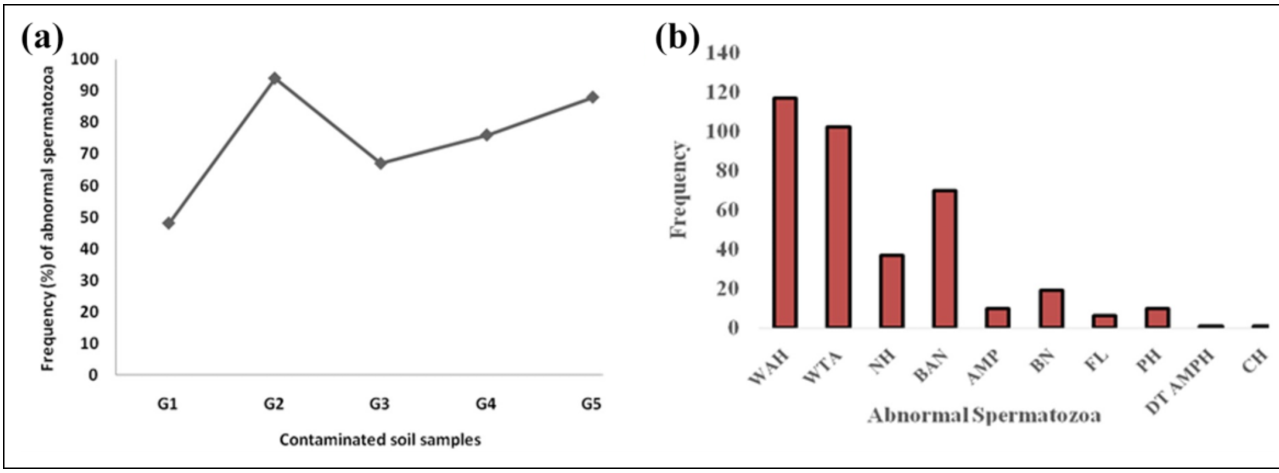

Figure 1. (a) Frequency of sperm aberrations in rats exposed to contaminated soil samples for 120 days. G1: rats exposed to uncontaminated soil sample; G2: rats exposed to CSMW sample; G3: rats exposed to 200 mL SEOCS; G4: rats exposed to 300 mL SEOCS; G5: rats exposed to 400 mL SEOCS. (b) Frequency of abnormal spermatozoa in rats induced by the contaminated soil samples. CSMW: contaminated soil from the mechanic's workshop; SEOCS: SEO contaminated soil sample; WAH: wrong-angled hook; WTA: wrong tail attachment; NH: no hook; BAN: banana; AMPH: amorphous head; BN: bent neck; FL: folded; PH: pin head; DT AMPH: double tails with amorphous head, and CH: curvy hook.

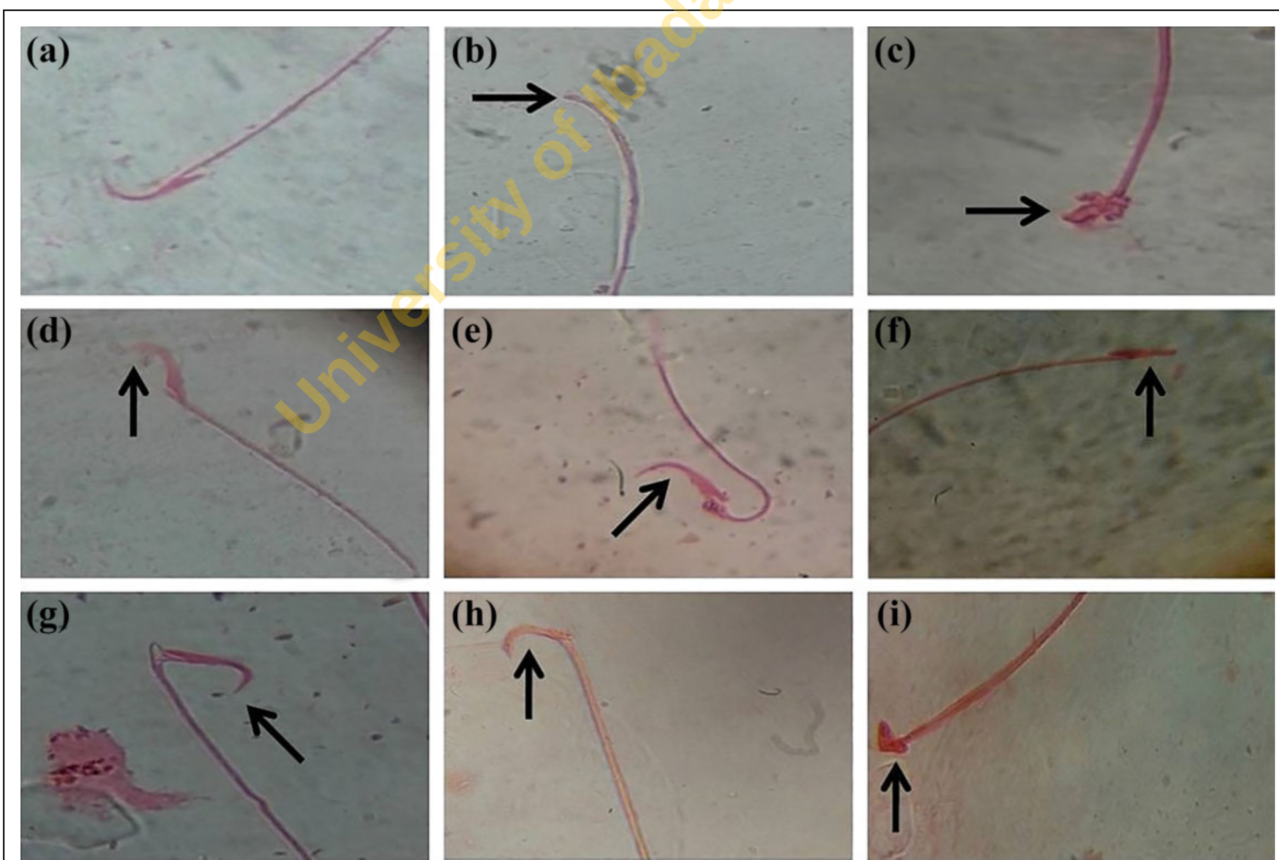

Figure 2. Photomicrographs showing examples of abnormal spermatozoa in rats exposed to different contaminated soil samples for 120 days. Normal sperm cell (a), pin head (b), amorphous head (c), wrong angled hook (d), banana head (e), no hook (f), wrong tail attachment (g), curvy hook (h), and double tail with amorphous head (i). Magnification: ×1000.

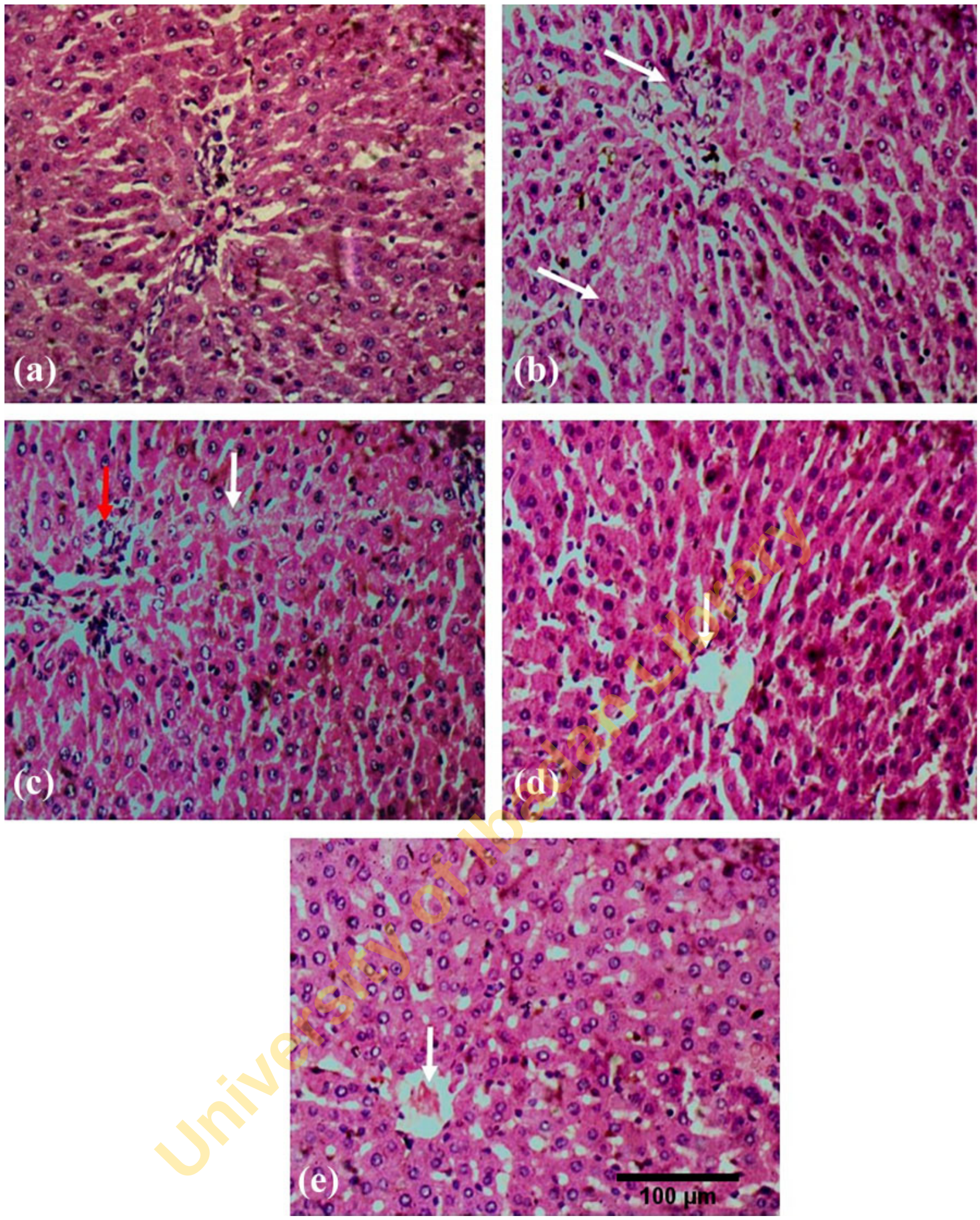

Figure 3. Histopathological lesions of the liver in rats exposed to different contaminated soil samples for 120 days. Liver: (a) no lesion in the control group, (b) moderate periportal hepatocellular necrosis in group exposed to CSMW sample, (c) moderate periportal hepatocellular necrosis and vasculitis in group exposed to 200 mL SEO soil sample, (d) centrilobular hepatocellular atrophy in group exposed to 300 mL SEO soil sample, and (e) centrilobular hepatocellular degeneration in group exposed to 400 mL SEO soil sample. Magnification: ×400. US: uncontaminated soil; CSMW: contaminated soil from the mechanic's workshop; SEO: spent engine oil.

can be substituted with Pb ions once Pb penetrates the blood–brain barrier, interfering with calcium regulatory action and several intracellular activities. Similar results of Pb accumulation in the brain have been reported (Ahmed et al., 2013; Ghareeb et al., 2010; Li et al., 2015). Our study is also in consonance with that of Topal et al. (2015), which shows a significant decrease in acetylcholine esterase and demyelination, and necrotic changes in brain tissues of rainbow trout exposed to Ni concentrations. It has been confirmed that sulfhydryl groups, which are essentially important for the functioning of several enzymes, are mainly targeted by Ni and Pb to induce toxicity (Senatori et al., 2009).

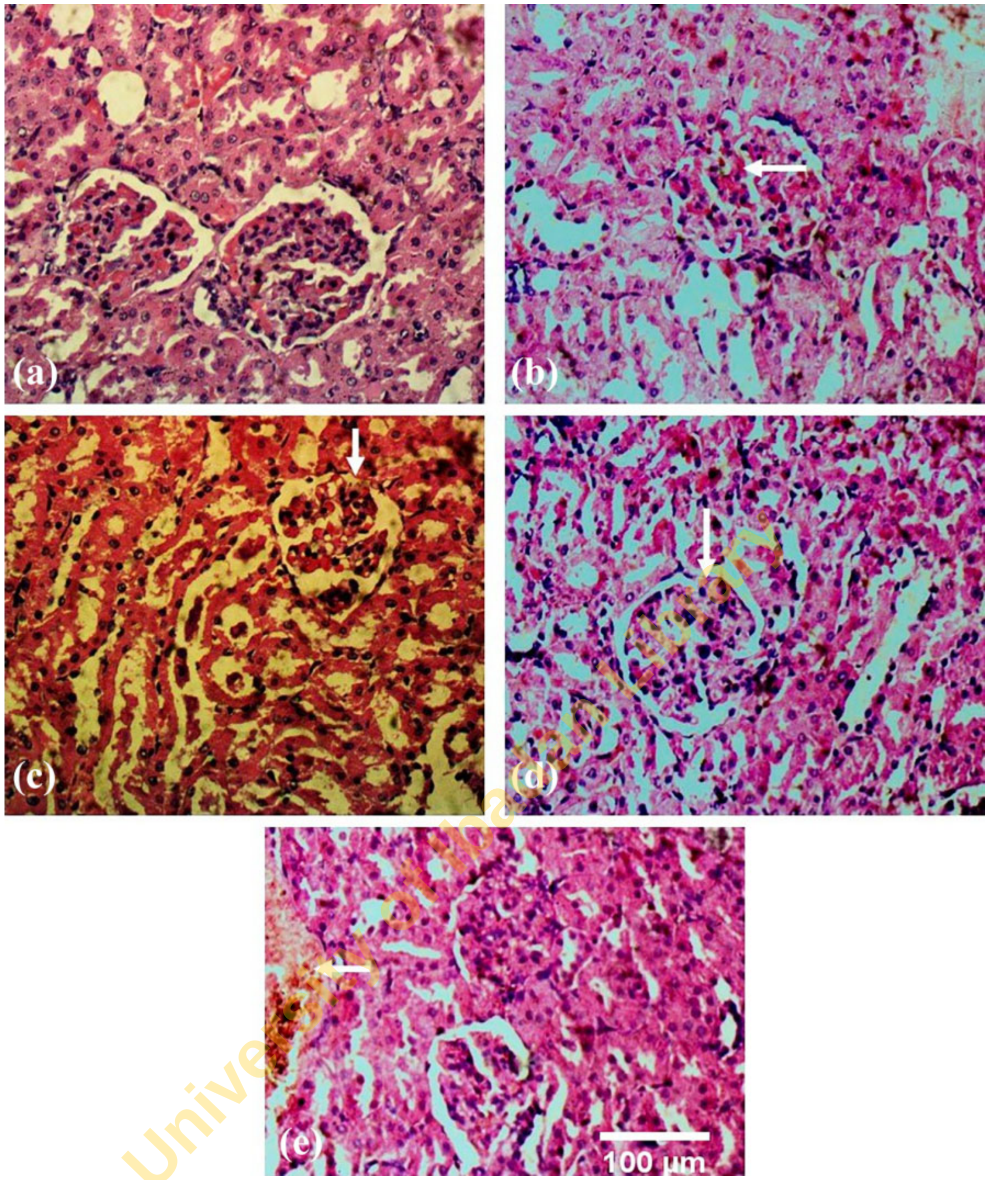

Figure 4. Histopathological lesions of the kidney in rats exposed to different contaminated soil samples for 120 days: (a) no observable lesion in the control group, (b) moderate degeneration of tubular epithelium and ectasia in group exposed to CSMW group, (c) moderate tubular epithelial degeneration in group exposed to 200 mL SEO soil sample, (d) moderate foci of tubular coagulation necrosis (arrows) in group exposed to 300 mL SEO soil sample, and (e) congested interstitial capillaries in group exposed to 400 mL SEO soil sample. Magnification: ×400. CSMW: contaminated soil from the mechanic's workshop; SEO: spent engine oil.

The study further demonstrated that only Pb and Cd were significantly increased in the serum across the volumes of SEO compared with the uncontaminated soil. We hypothesize that Cd and Pb have a slower clearance from the blood. The toxicokinetics of Cd and Pb in organs and tissues is highly dependent on the characteristics and forms of metals, route, dose and exposure duration, ligand binding, and sensitivity of species (Andejelkovic et al., 2019). Cd is a heavy metal and a major concern to public health. It is found in Ni-Cd batteries, cigarette smoke, and television screens (Debby, 2018). Cd interferes with hormones, thus acting as an endocrine disruptor (De Angelis et al., 2017). Previous studies have reported Cd as a developmental and reproductive toxicant (Dix-Cooper and Kosatsky, 2018), as

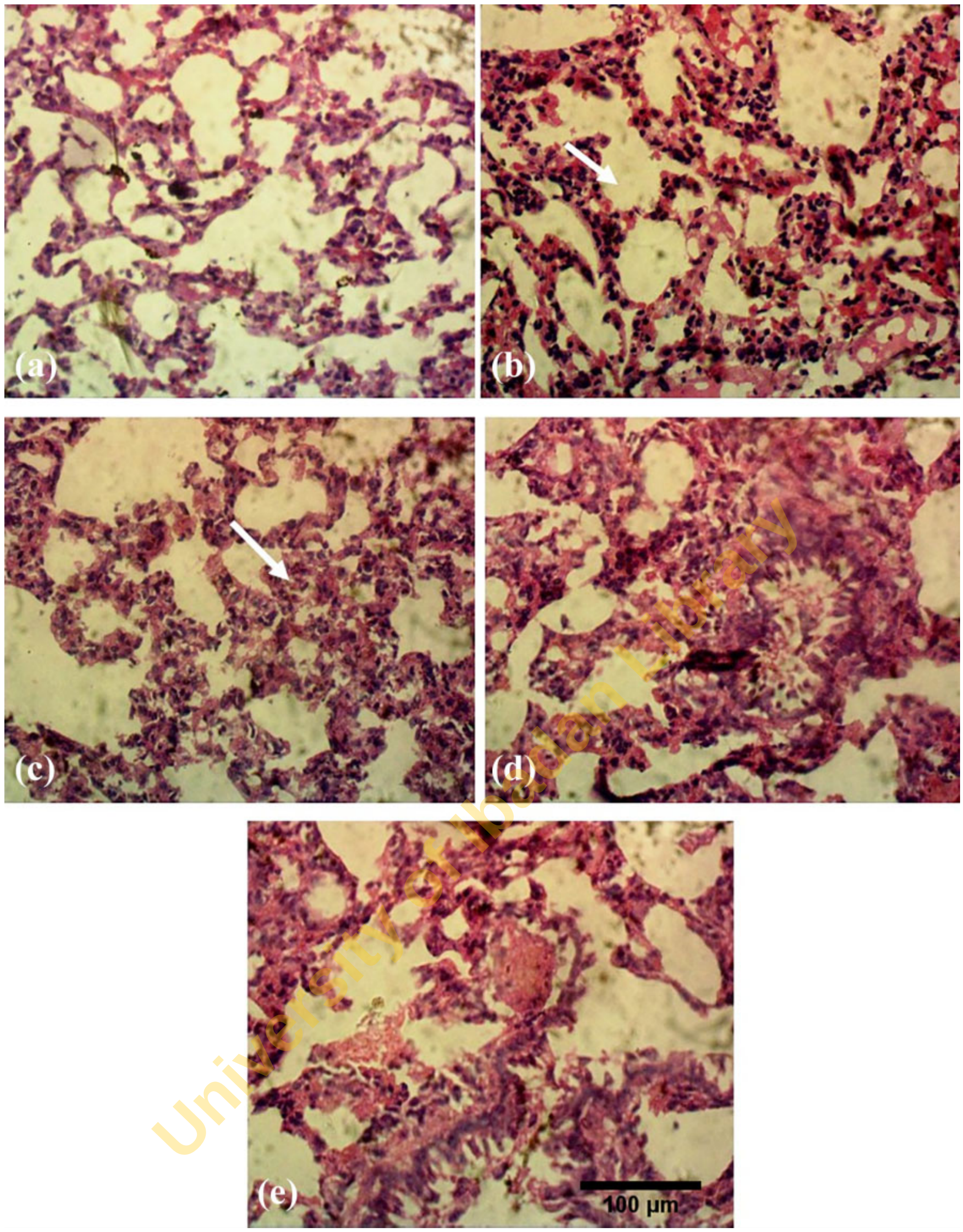

Figure 5. Histopathological lesions of the lungs in rats exposed to different contaminated soil samples for 120 days: (a) no observable lesion in the control group, (b) capillary congestion in group exposed to CSMW, (c) diffuse mild pulmonary congestion in group exposed to 200 mL SEO soil sample, (d) mild interstitial pneumonia in group exposed to 300 mL SEO soil sample; mild interstitial pneumonia in group exposed to 400 mL SEO soil sample. Magnification: ×400. CSMW: contaminated soil from the mechanic's workshop; SEO: spent engine oil.

well as nephro- and hepato-toxicant (Andejelkovic et al., 2019).

Hemopoietic is one of the most sensitive systems where Cd and Pb critically target the blood (ATSDR, 2012). RBCs or proteins distribute these heavy metals in the system after absorption (TimChalk et al., 2006). Cd is mostly bound to high molecular-weight proteins in the RBCs while it is bound to hemoglobin minimally. For Pb, it is the opposite. Pb enters the blood and is mostly bound to the hemoglobin and minimally to the RBCs (Abadin et al., 2007). Not only were Cd and Pb significantly increased in the serum of rats exposed to contaminated soil, the constituent of SEO induced lymphocytopenia at the same higher doses.

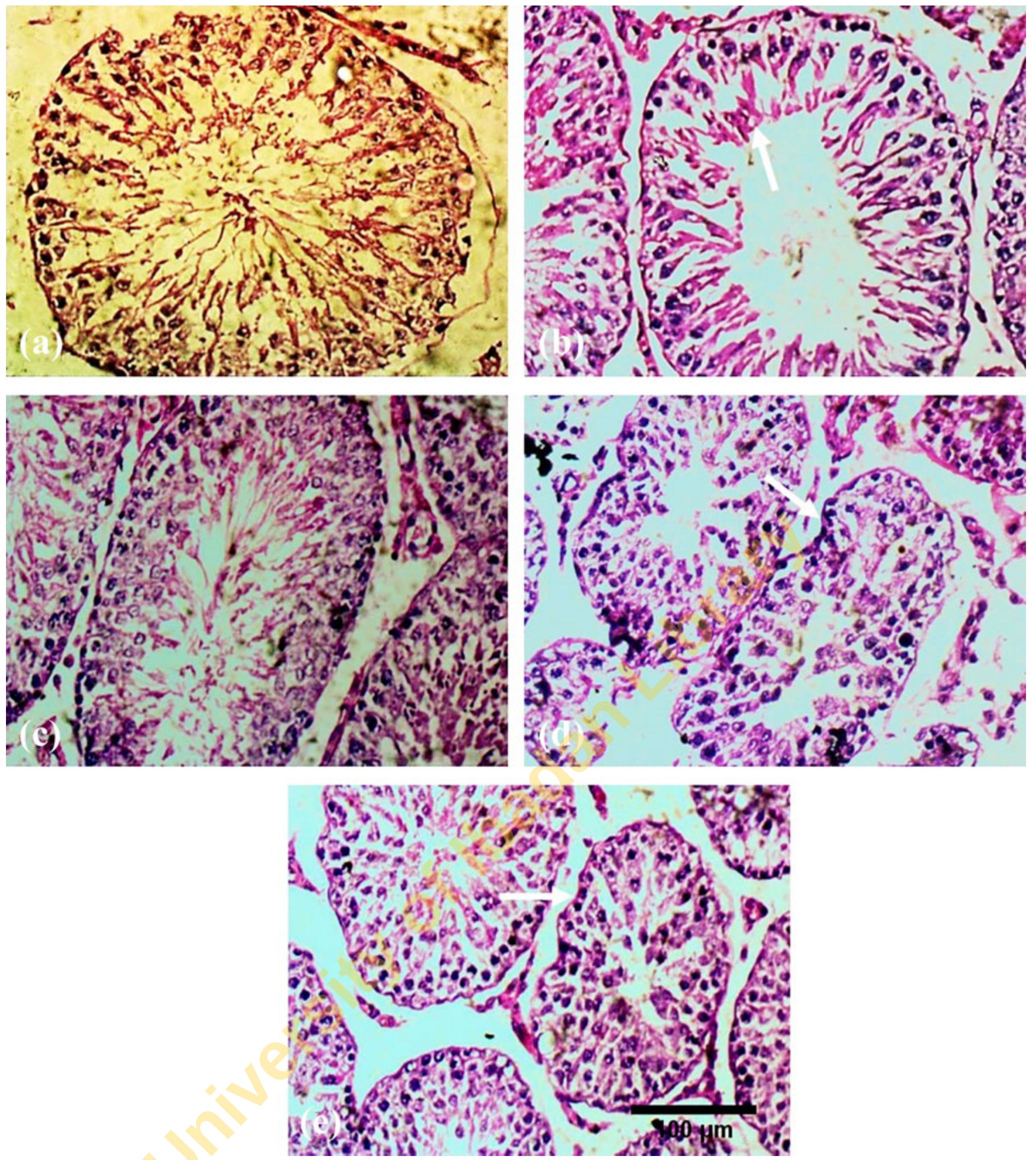

Figure 6. Histopathological lesions of the testes in rats exposed to different contaminated soil samples for 120 days: (a) normal tubules with elongated spermatids in the control group, (b) spermatogenic arrest in group exposed to CSMW, (c) normal tubules in group exposed to 200 mL SEO soil sample, and ((d) and (e)) tubular degeneration and spermatogenic arrest in groups exposed to 300 mL and 400 mL SEO soil samples, respectively. Magnification: ×400. CSMW: contaminated soil from the mechanic's workshop; SEO: spent engine oil.

This equally reveals that the significant increase in lymphocytes is an adaptive response of the peripheral blood cells to infection, physiological, and immunological changes due to stress (Kondo and Morimoto, 1996). Similar hematotoxic effects have been observed by Yildirim et al. (2018) and Mladenović et al. (2014). Their studies revealed a presence of lymphocytopenia in rats exposed to a single Cd treatment. Similar results have also been reported in BLAB/c mice treated with Cd for 14 days (Karmakar et al., 2000) and with $CdCl_2$ for 4 weeks via drinking water (El-Boshy et al., 2015). To assess the effects of the chemical constituents of SEO -contaminated soil on the hepatorenal functions in rats, the activities of serum ALT, AST, ALP, and levels of BUN and CREA were evaluated. Our results showed significant increases in the enzymatic activities of AST and ALP, which run parallel with an increase in SEO contaminated soil. AST is highly expressed in the mitochondria and cytoplasm of hepatocytes and is

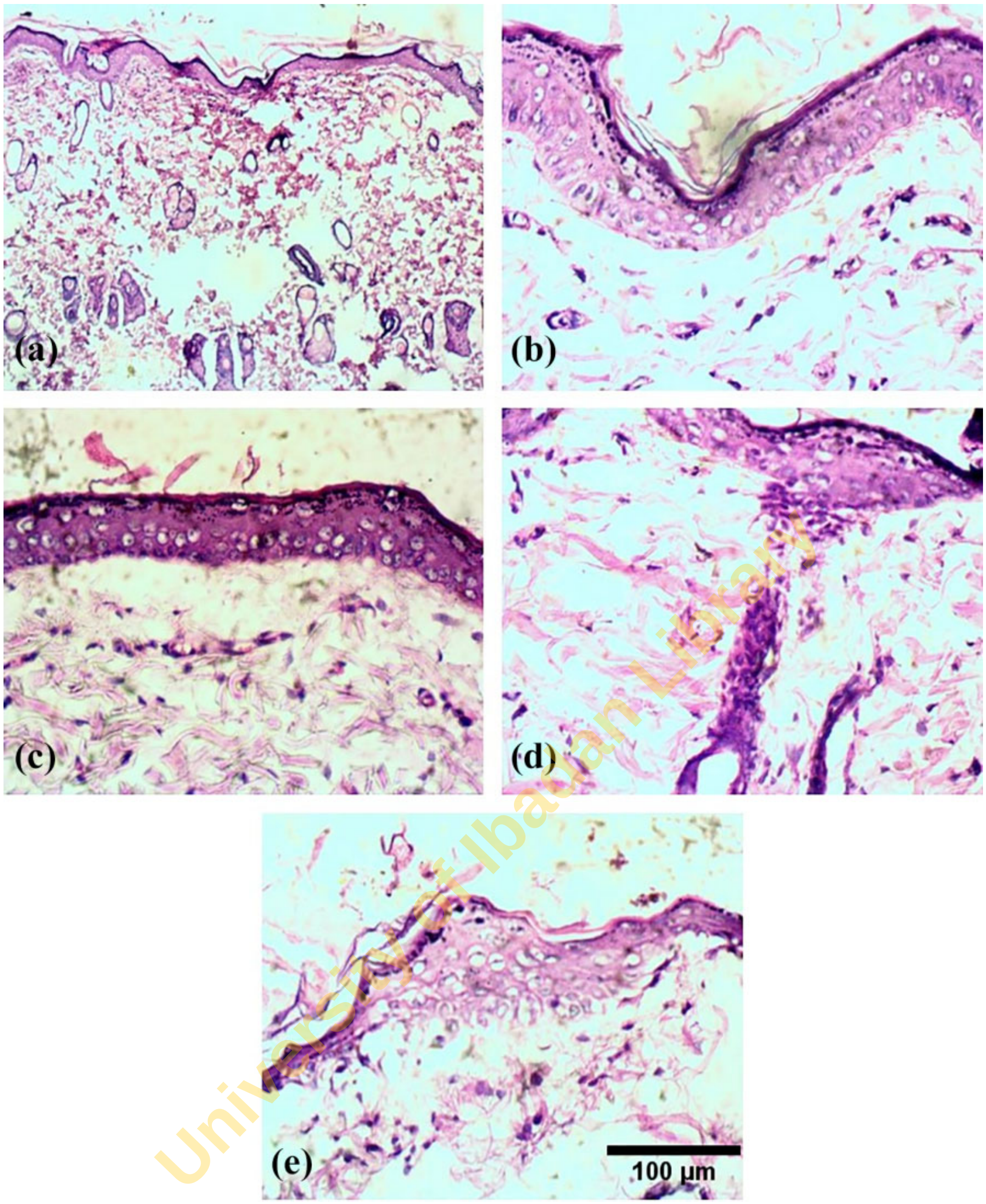

Figure 7. Histopathological lesions of the skin in rats exposed to different contaminated soil samples for 120 days. Skin (epidermis): (a) no observable lesion in the control group, (b) increased pigmentation in group exposed to CSMW group and ((c), (d), and (e)) hydropic degeneration of keratinocytes in groups exposed to 200 mL, 300 mL, and 400 mL SEO soil samples, respectively. Magnification: ×400. CSMW: contaminated soil from the mechanic's workshop; SEO: spent engine oil.

released into the blood stream as a result of cell membrane permeability or damaged hepatocytes owing to the presence of heavy metals and other inorganic constituents. While the integrity of the plasma membrane and endoplasmic reticulum is assessed using ALP (Akanji et al., 1993), our result tallies with Patrick-Iwuanyanwu et al. (2011) who reported significant increases in elevated activities of AST and ALP after exposure of petroleum products fed to rats.

One of the contributing factors that increase reproductive anomalies in the general population worldwide is the prevalence of xenobiotics and environmental toxicants. The seminiferous tubules in the testis are the primary sites where spermatogenesis occurs to produce highly differentiated spermatozoa.

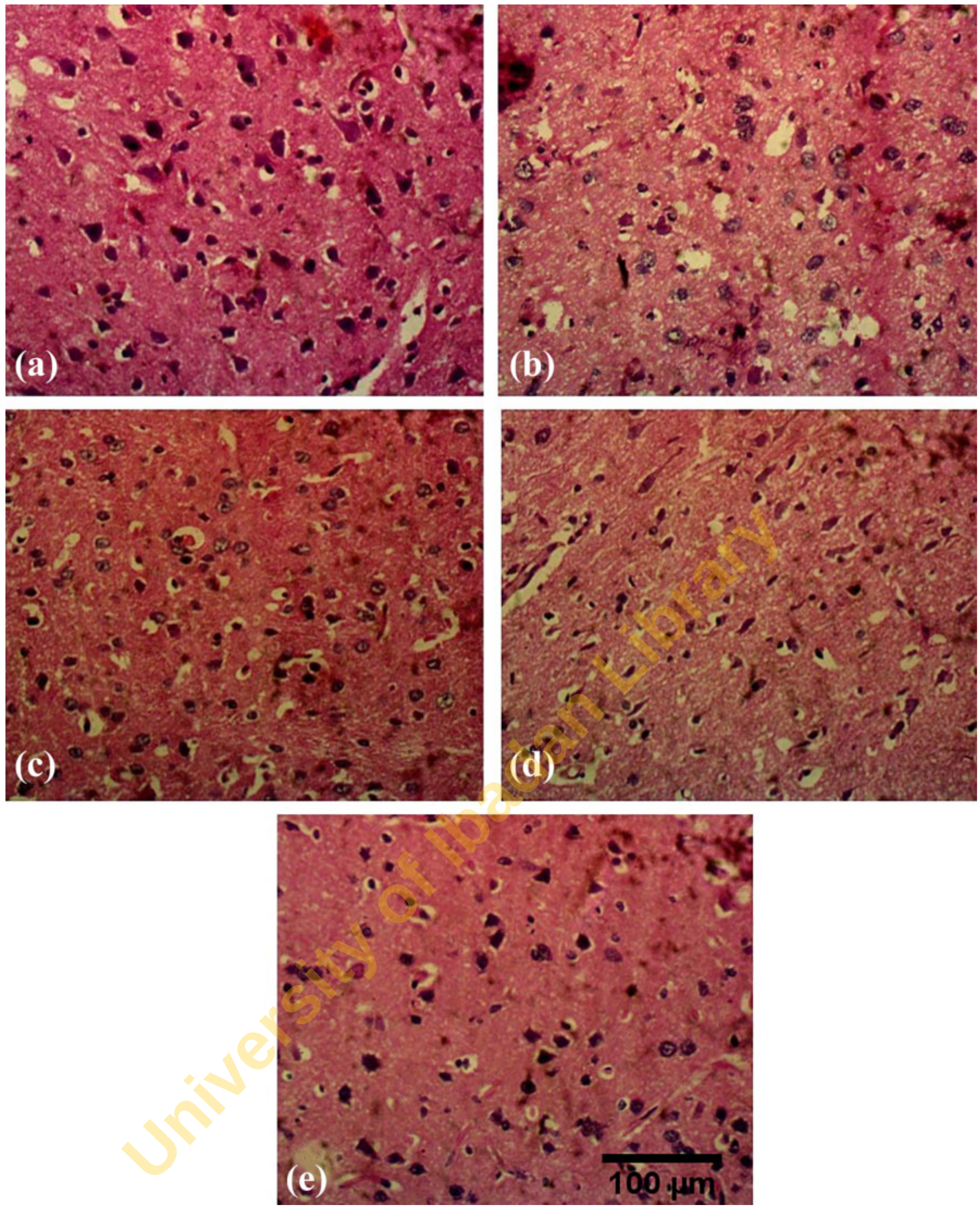

Figure 8. Histopathological lesions of the brain in rats exposed to different contaminated soil samples for 120 days. No lesions observed in (a) the control group, (b) group exposed to CSMW, (c) group exposed to 200 mL SEO soil sample, (d) group exposed to 300 mL SEO soil sample, and (e) group exposed to 400 mL SEO soil sample. Magnification: ×400. CSMW: contaminated soil from the mechanic's workshop; SEO: spent engine oil.

This study showed that there was a concentration-dependent increase in abnormal spermatozoa in rats in the groups exposed to SEO-contaminated soil compared with those in the negative control group. The study also showed that rats in the CSMW group had the highest frequency of abnormal spermatozoa. This is an indication that the organic and inorganic contents of the SEO may have the capacity of penetrating the testicular tissues to interfere with the spermatogenic process. Our result is similar to that of Wasiu et al. (2015) who observed increased sperm abnormalities induced by SEO.

Histopathology result from the treated rats revealed lesions such as hepatocellular necrosis and atrophy accompanied by significant increases of AST and ALP activities. In addition, tubular degeneration and congested interstitial capillaries in the kidney, capillary and pulmonary congestion in the lungs, hydropic degeneration of keratinocytes in the skin, and tubular degeneration in the testis were all observed. However, no lesions were observed in the brain of rats exposed to SEO contaminated soil.

In conclusion, our findings suggest that long-term dermal exposure to soil contaminated with SEO can accumulate in vital organs with Pb and Cd being the most significant heavy metals. The findings show that constituents of the SEO are able to induce sperm abnormalities as well as histopathology in exposed rats. The results obtained from this study require further investigations to identify the organic and inorganic elements present in the SEO and to fully highlight the mechanisms of toxicity induced directly or indirectly by the composition of SEO. The study, however, concludes that chronic dermal exposure to SEO is a potential risk to public health.

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