



# Standardized experimental model for cement dust exposure; tissue heavy metal bioaccumulation and pulmonary pathological changes in rats

M.W. Owonikoko<sup>a</sup>, B.O. Emikpe<sup>b</sup>, S.B. Olaleye<sup>a,\*</sup>

<sup>a</sup> Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

<sup>b</sup> Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

## ARTICLE INFO

Edited by DR. A.M Tsatsakis

### Keywords:

Exposure chamber  
Heavy metals  
Cement dust  
Histopathology  
Bioaccumulation  
Pulmonary toxicity

## ABSTRACT

A controlled experimental model of exposure to aerosols particularly for cement dust was recently invented in a study from the laboratory that found high serum levels of heavy metals, decrease gastrointestinal motility, and altered hematological variables in cement dust exposed rats. However, reproducibility was not considered. This work aims at standardizing the model and investigating preliminary toxicological indicators. Thirty male rats used in this study were divided into 3 groups ( $n = 10$ ). Group 1; control, while groups 2 and 3 were exposed to cement dust for 14 days and 28 days respectively. We assessed clinical signs of toxicity, tissue heavy metal concentration, histopathological, and body weight (BW) changes. We observed poor movement coordination, abnormal posture, cephalic fur loss. Evidence of ischemia and fibrotic pneumoconiosis were grossly observed in the lungs of the exposed groups. There was a significant increase in tissue level of heavy metals with pulmonary and gastric heavy metal content showing a trendy relationship during the period of the exposure as the value of Lead, Chromium, Cadmium, Iron, Calcium, and Nickel increased by nearly similar percentages in both tissues. Organs weights increased; the 14-day exposed ( $198 \pm 31$ ;  $168 \pm 22$ ) and 28-day exposed ( $198 \pm 22$ ;  $187 \pm 26$ ) groups had significantly reduced body weight at the first and second weeks of exposure compared to the control group ( $265 \pm 26$ ;  $357 \pm 40$ ) respectively. Exposure to cement dust induced low bone density in the exposed rats ( $p < 0.05$ ). Histopathological alterations include necrosis, inflammatory cellular infiltration, and alveolar hyperplasia suggestive of the proliferative response of pulmonary tissue to the dust. The operation of the standardized apparatus mimics a typical occupational exposure and the findings show that cement dust induces systemic toxicity via respiratory perturbation and body/organ weight discordance mediated by heavy metal bioaccumulation.

## 1. Introduction

Cement industries constitute a notable source of environmental toxicants [1,2] encountered during the manufacturing, distribution, and utilization of cement product. Occupational and environmental exposure to cement dust has been known to precede a number of systemic injuries with particular reference to the respiratory, gastrointestinal, and integumentary systems characterized by fibrosis, emphysema, cough, cancer, inflammation, and liver diseases among workers and host community residents of cement factories [3,4]. Cement product which has wide application in the construction industry is a homogenous mixture of hazardous heavy metals such as Cobalt (Co), Iron (Fe), lead (Pb), cadmium (Cd), Chromium (Cr), Nickel (Ni), Manganese (Mn), and arsenic (As) at different relative proportions [5–7] which have been

considered to be toxic to the body system. Deleterious health effects of cement production at host communities such as Kashmir valley and Krew in India [8,9]; Ewekoro in Nigeria [10] and Oromia, Addis Ababa in Ethiopia [11] have been severally reported. These reports have attracted the attention of researchers to cement dust studies.

Increased level of consciousness on the adverse health effects of cement dust culminates in scientific research since over two decades ago; an intervention which has been overtly impeded by the dearth of a known model of experimental exposure. Data hitherto analyzed stemmed basically from questionnaires [12–17,9], examination of health/medical records [18,19], interviews [16,20,21] and case report [22].

Toxicosis of cement dust is still poorly understood because, hitherto, empirical investigations have been achieved only by the deployment of crude experimental procedures of merely placing experimental animals

\* Corresponding author.

E-mail addresses: [owonikoko.mathew@yahoo.com](mailto:owonikoko.mathew@yahoo.com) (M.W. Owonikoko), [sb.olaleye@yahoo.com](mailto:sb.olaleye@yahoo.com) (S.B. Olaleye).

<https://doi.org/10.1016/j.toxrep.2021.06.001>

Received 21 October 2020; Received in revised form 31 May 2021; Accepted 1 June 2021

Available online 3 June 2021

2214-7500/© 2021 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

in proxy distance to cement factories [10,23]. Meanwhile, this method of exposure gives wide room for controversies as a number of other confounding environmental factors could be responsible for the reported pathologies considering the appreciable physical distance between the location of the experimental animals and the actual cement factory. The need for experimental research on the pathophysiological mechanisms involved in the reported effects of cement dust is required for validation and further investigation of its toxicosis. Therefore, an experimental/laboratory method of exposure characterized by simplicity and reproducibility is required. Recently, our laboratory developed a model for regulated exposure which has been deployed in a preliminary study to access the effect of cement dust on some hematological variables and indices with interesting outcomes. Emanating data suggest that an increase in serum concentration of some heavy metals including the alteration of hematological variables accompany the exposure to cement dust [24]. Although the results from this study mimic earlier reports in the literature on human subjects, however, the efficacy and reproducibility of the model cannot be guaranteed. The initial generation and distribution of dust by the chamber is characterized by significant entropy, a condition that may be substantially different from the instantaneous distribution. These provisions make the effusion rate of the chamber dust generation unquantifiable. Also, some important parameters of the chamber such as the diameter were not taken into cognizance which further precipitates the challenge of reproducibility. Hence, the rationale for this study is to standardize the chamber and since inhalation is one of the three major routes of exposure to respirable particulate matters from the external environment, we set to investigate the attendant effect of cement dust exposure on the pulmonary cytoarchitecture and the probable accompanying heavy metal accumulation tendency particularly in the visceral tissues.

## 2. Materials and methodology

### 2.1. Animals

Thirty (30), 3-month-old male Wistar rats weighing between 150–180 g were purchased from the animal house of the college of Medicine, University of Ibadan, and were kept in plastic cages with wood shavings. They were housed under standard conditions of temperature ( $23 \pm 2$  °C), humidity ( $55 \pm 15$  %), and natural 12 h light and dark cycle in the Animal house of Department of Physiology, University of Ibadan, Ibadan. They were allowed access to water and standard

laboratory chow *ad libitum*.

Following two weeks of acclimatization, they were exposed to cement dust with the aid of an exposure chamber fabricated according to the specifications below (Fig. 1A and B). This study was conducted in accordance with the current Animal Care Regulations and standards approved by the Institute for Laboratory Animal Research [25] and the experimental protocol was approved by the Animal Care and Use Research Ethics Committee, University of Ibadan, Ibadan, Nigeria having been assigned the approval number UI-ACUREC/18/0129.

### 2.2. Standardized design and operation of the Exposure Apparatus

The dust exposure was carried out with the use of a fabricated non-mobile apparatus designed to simulate a cement factory environment for the exposure of experimental animals to particulate matters. Unlike the previous report [24], the method was standardized by modifying it into a perfect square of 60 cm in height, breadth, and width, made of transparent plexiglass. An internal subchamber of height 20 cm, breadth 26 cm, and diameter of 32.8 cm is specifically designed to house the dust. Made of plexiglass, the subchamber is designed to contain cement dust with its walls adequately perforated to ensure constant effusion of cement dust-laden air into the portion of the chamber housing the rats. There is no available entrance to the subchamber from the internal portion of the apparatus. This is with the intention to prevent the explorative animals from mechanical injury, to prevent the interruption of the dispensing circuitry of the chamber to ensure sustained; maintaining constant effusion rate, and to prevent direct ingestion of the dust. The apparatus which is particularly designed to avail the experimental animals a wide range of movement consists of a manipulative chimney of 30 cm by 10 cm dimension located on the apparatus lid (dorsally) and diagonally to the internal subchamber, perpendicularly to the internal subchamber and opened during operational periods but closed thereafter.

The subchamber houses two miniature metallic quadri-bladed aerators situated at an anterodorsal right angle to each other. The aerators are heavy duty type of model CNS-3–20/620 (serial number S40141392) running in main alternating current of 220 V, power of 27 W, current of 0.25A, and frequency of 50/60 Hz. They are capable of moving with a speed of 2400/3400 rpm. When connected to a power source, sufficient torque equips the aerators as they synergistically generate, propel and deliver the dust in inhalable form from the subchamber to the internal portion of the apparatus housing the

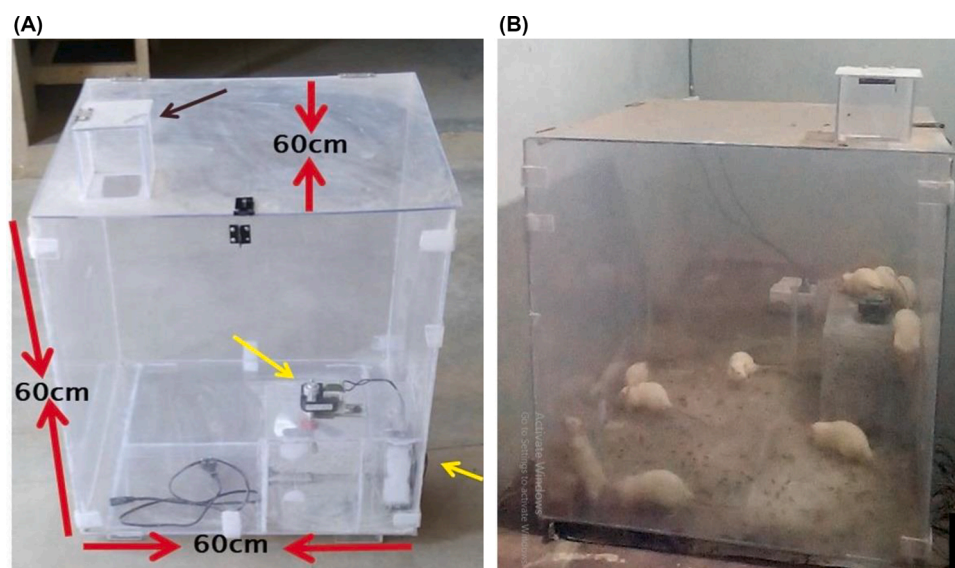


Fig. 1. A: The Description of the apparatus. B: The apparatus in operation (Advancement and modifications stated in Table 2).

experimental animals. The specificity of the aerators enables the generation and dispensation of about 0.2 g/h of the dust.

### 2.3. Cement material and exposure

A full intact and freshly supplied bag of Nigeria Portland cement was purchased from an accredited depot in Ibadan, Oyo state, Nigeria. The exposure began daily by introducing 100 g of cement dust into the subchamber. Old and remnant dust were evacuated prior to exposure every other day. This routine practice was maintained on daily basis. Exposure was 5 h daily for periods of 14 days and 28 days using the exposure chamber. The control rats received sham exposure to normal atmospheric oxygen. They were all sacrificed thereafter.

### 2.4. Experimental rats grouping

There were three (3) groups in this study each with 10 animals. The first group (Group 1) of rats were the control group. The second group (Group 2) received cement dust exposure for 14 days (14-day group) while the last group (Group 3) were exposed to cement dust for 28 days (28-day group). Exposure was carried out 5 h daily. The control Group animals were allowed to thrive in completely dust-free environment. The experimental animals were all allowed free access to standard laboratory chow and water.

### 2.5. Body and organ weights

The weekly weight changes of the animals in the groups each were determined using Acculab® USA, Model-vic-303 electronic analytical weighing balance and recorded while weekly percentage change in weight throughout the study was calculated as:

$$[(B - A)/A \times 100] \quad (1)$$

Where “A” represents the “initial weight”, “B” is the “final weight”.

During sacrifice, visceral organs including the stomach, lungs, heart, spleen and brain were collected. The relative organ weight was calculated by using the formula;

$$(X/Y) \times 100 \text{ g} \quad (2)$$

Where “x” represents the “Absolute Organ Weight”; the raw weight of the organ as obtained from the weighing balance while “y” is the “Terminal Body Weight (TBW)”; the instantaneous weight of the animal at the point of sacrifice.

Mean Femoral Weight (MFW) is the sum of the weight of the femur divided by “n” per group while Relative Femoral Weight (RFW) was calculated from the equation below

$$(FW/TBW) \text{ g} \quad (3)$$

Mean Relative Femoral Weight (MRFW) is the sum of the RFW divided by “n” per group

The same as above was applicable to the femur after collecting and the attached muscles carefully trimmed off.

### 2.6. Clinical observations

Each animal in the different groups were carefully examined on daily basis before and after experimental exposure for possible clinical signs of cement dust-induced toxicity in the respiratory and behavioural patterns, skin, fur, eyes and other mucous areas while morbidity/mortality case was equally noted. At the end of the experiment, the animals were fasted overnight but were allowed access to water. All visceral organs including the stomach, spleen, lungs and brain were excised, carefully examined before weighing and thereafter digested for heavy metal analysis except the lungs tissue that was divided into two and part of it was fixed in 10 % formalin for histopathology.

### 2.7. Digestion of tissue and heavy metal analysis

Heavy metal level in the lungs, brain, stomach and spleen of the exposed animals were investigated according to [26]. Nitric acid (1 mL) followed by perchloric acid (1 mL) was added to 100 mg of the tissues each in a clean sample bottle. The mixtures were then digested over a sand bath until the solution becomes clear and yellow in colour. In the instance of the outcome of brown-coloured digest, the above process was repeated. The digests were aliquoted after being made up to known volume of ionized water and read using Atomic Absorption spectrophotometer model (Buck Scientific AAS Model 210/211 VGP, Connecticut, USA) at various wavelengths according to the standard working parameters stated in Table 1 below. Results of accumulated heavy metals were recorded in mg/L and presented as mean  $\pm$  SEM. Radiation source were the hollow cathode lamp of Lead (Pb), Chromium (Cr), Cadmium (Cd), Nickel (Ni), Iron (Fe), Manganese (Mn), Cobalt (Co) and Calcium (Ca) while the fuel was air acetylene.

### 2.8. Macroscopy and histomorphological investigation

Following the sacrifice of the animals, the lungs were excised and carefully examined for any macroscopic pathology before fixing in 10 % formalin for histological examinations. They were thereafter embedded in paraffin wax; sectioned at 5  $\mu$ m and were stained with haematoxylin and eosin before viewing under light microscope (PEC MEDICAL USA; X400 Mag) for any pneumopathological alterations according to [27]. The histological and pathological evaluations were carried out by a blinded pathologist.

### 2.9. Statistical analysis

Statistical analyses were done using Graphpad prism 5.0® and data presented as mean  $\pm$  SEM for n = 5 per group while One-way ANOVA and Dunnett post-hoc test were used for mean comparison between the different groups with p < 0.05 considered significant as stated at each case.

## 3. Results

The chamber as modified and standardized is pictorially represented above in Fig. 1A and B. The major discrepancies and the standardizing factors are analysed in Table 2 below.

The width of the chamber and the subchamber had been slightly adjusted to suit that of a perfect square. Although the height of the chamber remains the same but that of the subchamber was slightly reduced. The diameter and the dust effusion rate were determined. This is expected not only to increase the internal space of the major part of the chamber but also to modify the aerosolized dust. Overall, the modifications ensured effective and calculable dust effusion rate.

### 3.1. Clinical signs

One rat died after two weeks of exposure out of the 28-day exposed animals (Group 3) while the period of exposure lasted. A number of clinical signs of toxicities such as mortality, laboured breathing,

**Table 1**  
Operational parameters of atomic absorption spectrophotometer.

S/NO	METAL	WAVELENGTH (nm)	SLIT WIDTH
1	Lead	283.3	0.7
2	Chromium	357.9	0.7
3	Cadmium	228.9	0.7
4	Cobalt	352.7	0.7
5	Manganese	279.5	0.7
6	Iron	248.3	0.7



**Table 2**  
Modifications entrenched to standardize the earlier model of cement dust exposure.

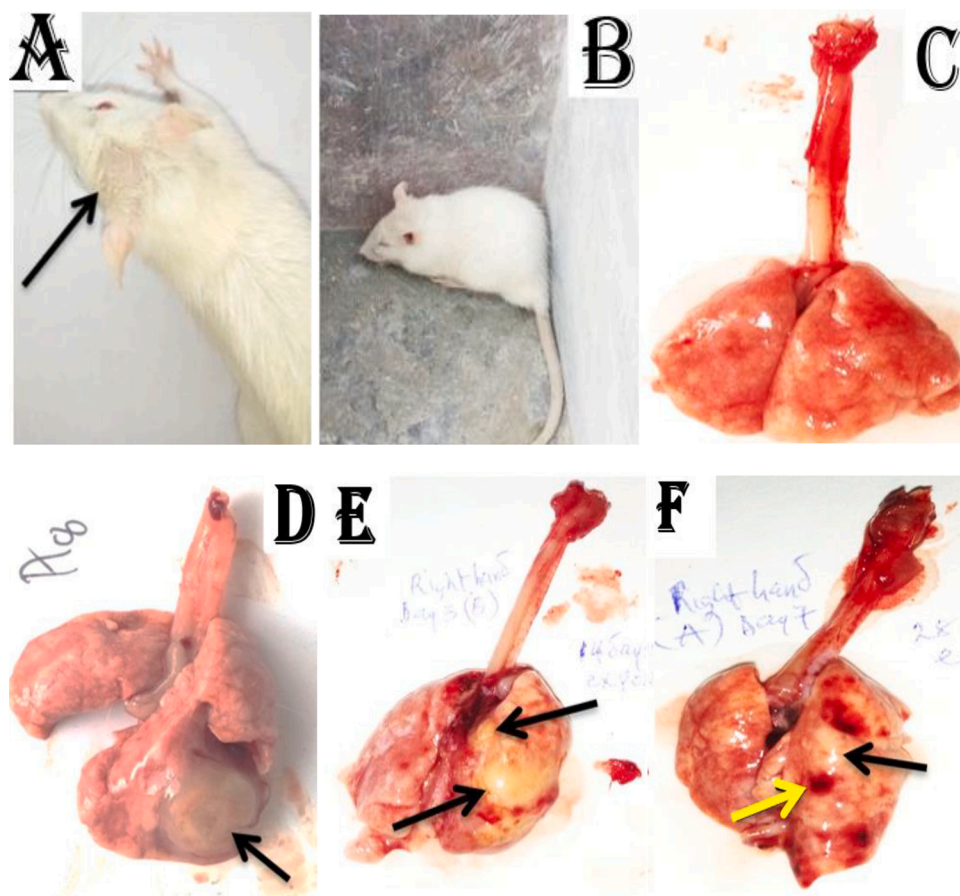
PARTS	SPECIFICATIONS	MODIFICATIONS	FUNCTIONAL ALLOWANCES
DUST GENERATOR	1 Plastic and 1 iron bladed industrial fan	2 iron bladed aerators	
CHAMBER WIDTH	59.9cm	60 cm	
CHAMBER HEIGHT	60 cm	60 cm	
CHAMBER DIAMETER	Unknown	84.9cm	
SUBCHAMBER WIDTH	26.1cm	26 cm	
SUBCHAMBER HEIGHT	19.6 cm	20 cm	
SUBCHAMBER DIAMETER	Not stated	32.8 cm	
CHIMNEY AREA	10.6cm × 9.9cm	30cm × 10cm	
FAN SPEED	2400–3000rpm	2400–3000rpm	
DUST EFFUSION RATE	Unknown	0.2 g/hr	

Enhance effective and sustained delivery of dust from the subchamber to the larger compartment housing the experimental animals

increased fur lability and cephalic fur loss. Other signs were frequent sneezing, abnormal posture and hypoactivity. There was evidence of poor nervous coordination resembling that of hemiballism and tremor. It was equally observed that the vigor and boisterous tendencies exhibited by the exposed animals at the beginning of the experiment gave way for docility, weakness and anorexia occasioned by restricted movement before the end of the experiment Fig. 2B. The exposed animals also show sign of drastic weight loss than the control. During sacrifice, there were grossly observable conditions of fibrotic pneumoconiosis at the caudal lobe alongside with being pus-gorged portions (black arrows in Fig. 2D, E and F) near the deep respiratory zone. There was also evidence of pulmonary ischemia with grossly observable pale red patches at the serosal surface of the anterior lobes (yellow arrow in Fig. 2F) in the 14- and 28-days exposed animals.

**3.2. Body and organ weight changes**

Table 4 shows that the 14-day exposed group and 28-day exposed group had significantly reduced body weight at the first and second weeks of exposure (Week 1 – week 4 in the table) compared to the control group. The rate of body weight gain in the test groups (14-day and 28-day exposed) was significantly reduced in the weeks of exposure when compared with the control. Also, the TBW and weight changes of the femur present an interesting statistic with the 14-day group showing marginal difference and 28-day showing a significant difference when compared with the control group. For instance, TBW of the 14-day exposed groups decreased by 10.67 % while the 28-day group decreased by 16.42 % when compared with the control. The MFW of the 14-day exposed group present 9.34 % while the 28-day exposed group present 31.78 %. MRFW shows the same trend with the 28- and 14-days exposed group showing 27.14 % and 8.57 % respectively. The foregoing



**Fig. 2.** A-F: Observed clinical signs of toxicity among cement dust exposed animals; A: Cephalic fur loss (black arrow) and abnormal posture following 28-day cement dust exposure. B: Docility and hypoactivity of exposed animals showing maintenance of stationary position. D, E and F: pulmonary emphysema and haemorrhages of caudal lobe of the lungs in the 14- and 28-days exposed animals. E and F: pus-gorged and pulmonary ischemia of the caudal lobes respectively (black arrows) and pale red patches on the anterior lobe of the lungs (yellow arrow); all compared with C: A normal lungs (from the control group) showing normal appearance and intact gross morphology. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



shows that the body weight of the 28-day exposed group is more affected than the 14-day group.

### 3.3. Histoarchitectural alterations

Fig. 3A-F, represent the photomicrographs of experimental animals from the control, 14-day and 28-day exposed groups respectively. The control animals (Fig. 3A and B) show normal lungs cytoarchitecture while the treated groups (3C-F) had an array of pathological manifestations including fibrinoid necrosis, evidence of emphysema and inflammatory response of the tissue marked by mononuclear cell infiltration. Alveolar type II pneumocyte hyperplasia was observed in addition to those alterations at the 28-day exposed group. Table 6 shows

the distribution and severity of the pulmonary histopathological changes observed in this study with hyperplasia of histiocytes, mononuclear cell infiltration in alveoli, medial hypertrophy of muscular arteries and fibrinoid necrosis being the most predominant pathologies while presence of eosinophilic substance, fibroblast proliferation, oedema and alveolar septal thickening were the least observed which were all completely absent in the control animals.

Fig. 4A-F represent the relative weights of the stomach, spleen, lungs, heart, brain and femur respectively while Table 3 shows femoral bone weight changes. The relative weight of the lungs (F-value = 16.25, p-Value = 0.0010), stomach (F-value = 5.307, p-Value = 0.0223), and spleen (F-value = 27.64, p-Value = 0.009) were significantly higher in 14-day and 28-day groups when compared to the control. However, the

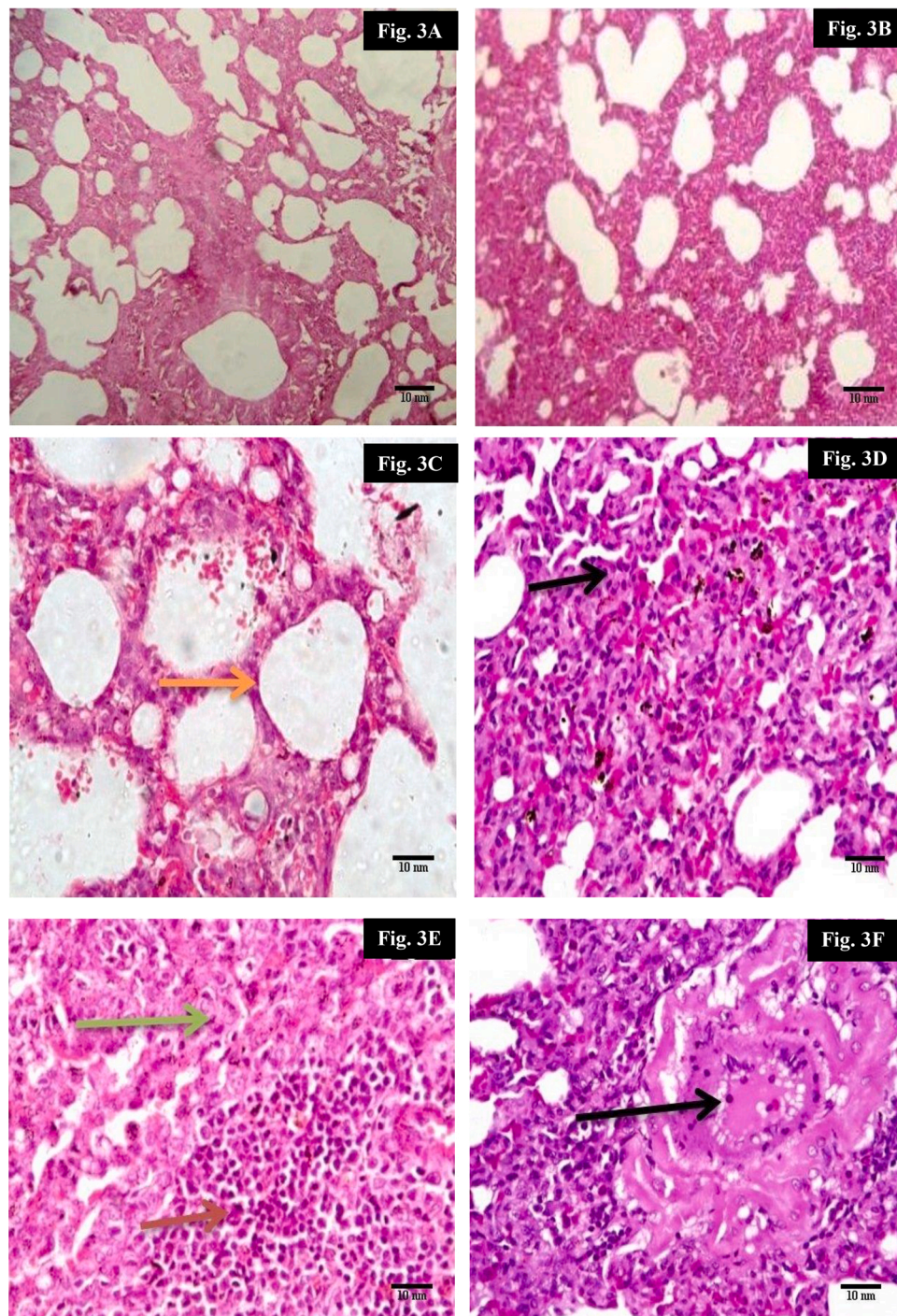
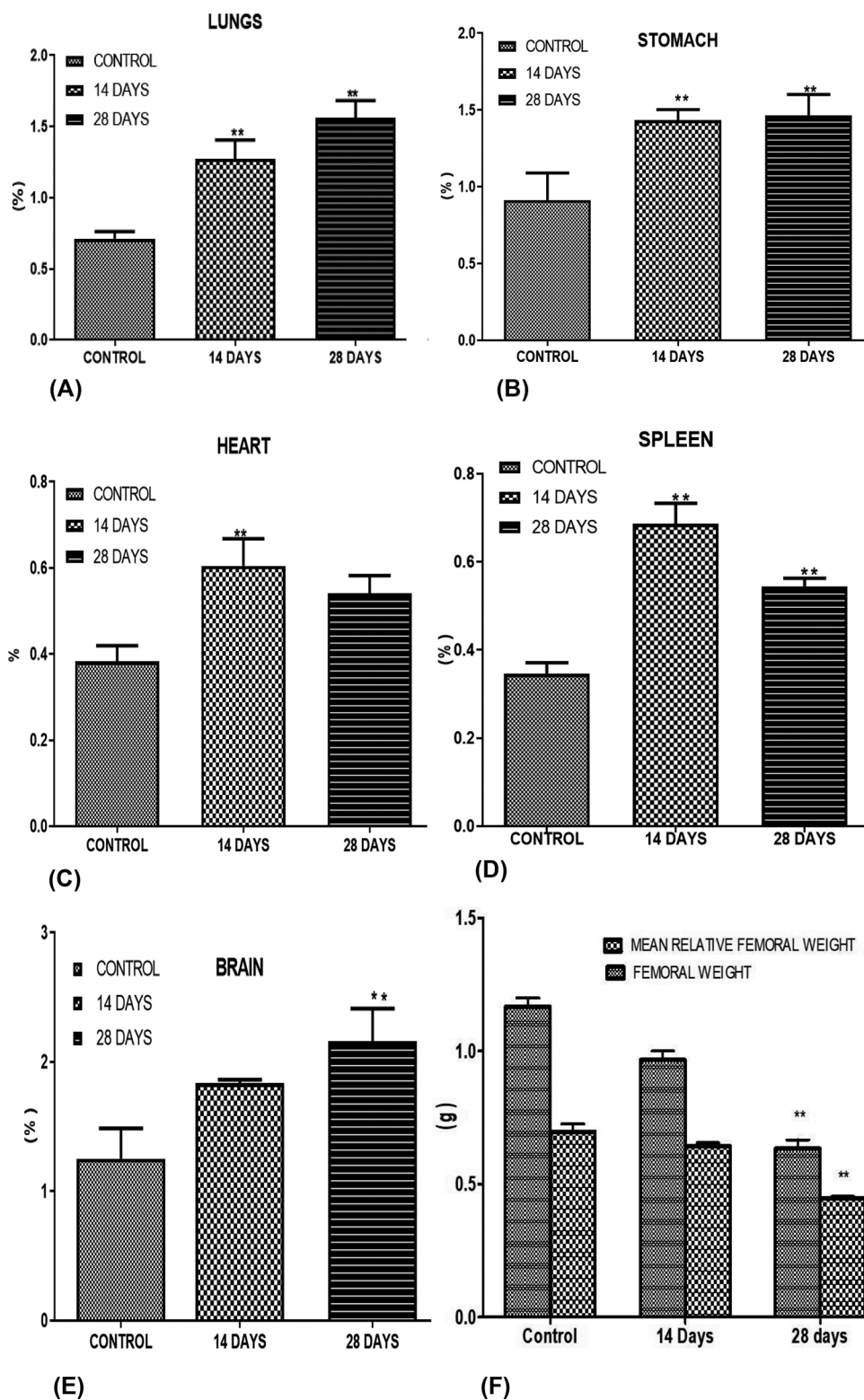


Fig. 3. A and B: Lung tissue showing normal architecture of control animals. C and D: Lung tissue showing alveolar congestion and hemorrhage (Fig. 3C brown arrow), Alveolar septal thickening with congestion, hemorrhages, diffuse mild type II pneumocyte proliferation and infiltration of mononuclear cell infiltration (Fig. 3D; black arrow) of 14-day Exposed animal. E and F: Lungs showing effect of cement dust on pulmonary tissue with evidence of severe alveolar type II pneumocyte cell hyperplasia (Fig. 3E green arrow) and mononuclear cell infiltration (Fig. 3F black arrow) and medial hypertrophy of muscular arteries and fibrinoid necrosis of 28-day exposed animals. Mag: X400. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** A Effect of cement dust on relative lungs weight. \*\* Significant when compared with the control. B: Effect of cement dust on relative stomach weight. \*\* Significant when compared with the control. C: Effect of cement dust on relative heart weight. \*\* Significant when compared with the control. D: Effect of cement dust on relative spleen weight. \*\* Significant when compared with the control. E: Effect of cement dust on relative brain weight. \*\* Significant when compared with the control. F: Effect of cement dust on femoral weight and mean femoral weight. \*\* Significant when compared with the control.

relative weights of the brain (F-value = 5.323, p-Value = 0.0298) and femur (F-value = 50.33, p-Value = 0.0002) was significantly higher only in the 28-day group while that of the heart increased significantly only in the 14-day exposed group when compared with the control at  $p < 0.05$ . Lung, stomach and brain tissues shows somewhat similar pattern of response to cement dust exposure. While other organs (Heart and Spleen) show a mitrhitadic reduction in the 28-day group when compared with the 14-day group, stomach, lungs and brain tissues in

Fig. 4A, B and E respectively depicted a persistent increase in weight from the 14-day to the 28-day.

### 3.4. Heavy metals analysis

Heavy metals analysis as found in Table 5 above represents a considerable output. Analysis of the heavy metal content of the various visceral organs shows that the lung tissue has a significantly high level of



**Table 3**

Mean relative femoral weight of experimental animal following exposure to cement dust.

GROUPS	TBW(g)	MFW(g)	MRFW
CONTROL	168.7 ± 4.1	1.07 ± 0.03	0.7 ± 0.03
14-DAY	150.7 ± 2.4*	0.97 ± 0.03*	0.64 ± 0.01
28-DAY	141.0 ± 5.51*	0.73 ± 0.13*	0.51 ± 0.07*

Only values of the MRFW are presented in mean ± SEM.

TBW- Terminal body weight; MFW; mean Femoral Weight; MRFW; mean Relative Femoral Weight.

\* p < 0.05 are significant when compared with control.

**Table 4**

Weekly percentage mean body weight change induced by 4 weeks exposure to cement dust.

GROUPS	WEEK 1	WEEK 2	WEEK3	WEEK 4
CONTROL	165 ± 26	257 ± 40	373 ± 50	463 ± 58
14-DAY	98 ± 31*	68 ± 22*		
28-DAY	96 ± 22*	87 ± 26*	152 ± 45*	166 ± 52*

Values are presented in percentages.

\* p < 0.05 is significant when compared to the control.

Ni at 14-day and 28-day groups, Cr and Co showed significantly high level only at 28-day while Cd was significant only at the 14-day exposed group when compared with the control. The stomach tissue has significantly high Pb, Fe at both exposed groups (14-day and 28-day exposure), while Cr and Ni were significantly high only at the 28-day exposed group when compared with the control. The brain tissue as shown by the 14-day and 28-day groups had significantly high levels of Pb, Cd, Mn, and Ni while Cr and Fe were only significantly higher in the 28-day group compared to the control. The spleen at both 14-day and 28-day groups depicted a significantly high level of Pb, Cr, Co, Ni while Cd and Mn were only significantly high at the 28-day group when compared with the control.

The values of Fe in the respective organs (brain, spleen, lungs, and stomach) are significantly higher in the test groups when compared with the control. Moreover, values of this metal in the 14-day exposed group approximately twice as found in the 28-day exposed group. This is

**Table 5**

Heavy metal analysis of the various tissues (mg/L) of experimental animals exposed to cement dust.

PARAMETERS		ORGANS			
HEAVY METAL	GROUPS	LUNGS	BRAIN	SPLEEN	STOMACH
Pb	CONTROL	0.03 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.03 ± 0.01
	14-DAY	0.95 ± 0.07*	1.05 ± 0.06*	1.29 ± 0.14*	1.02 ± 0.04*
	28-DAY	1.02 ± 0.07*	1.08 ± 0.33*	1.43 ± 0.24*	1.10 ± 0.03*
Cr	CONTROL	0.02 ± 0.00	0.06 ± 0.03	0.07 ± 0.01	0.03 ± 0.01
	14-DAY	0.17 ± 0.04*	0.11 ± 0.00	0.12 ± 0.01*	0.12 ± 0.00*
	28-DAY	0.26 ± 0.08*	0.15 ± 0.00*	0.17 ± 0.01*	0.35 ± 0.12*
Cd	CONTROL	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
	14-DAY	0.35 ± 0.05*	0.05 ± 0.02*	0.05 ± 0.01*	0.06 ± 0.01
	28-DAY	0.12 ± 0.07*	0.05 ± 0.01*	0.27 ± 0.03*	0.05 ± 0.01
Co	CONTROL	0.02 ± 0.00	0.05 ± 0.00	0.01 ± 0.00	0.11 ± 0.01
	14-DAY	0.16 ± 0.05*	0.44 ± 0.17*	0.20 ± 0.07*	0.39 ± 0.02*
	28-DAY	0.84 ± 0.15*	0.45 ± 0.13*	0.74 ± 0.13*	0.30 ± 0.03*
Mn	CONTROL	0.05 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.07 ± 0.01
	14-DAY	0.13 ± 0.01*	0.06 ± 0.00	0.04 ± 0.01	0.06 ± 0.01
	28-DAY	0.98 ± 0.00*	0.08 ± 0.0*	0.18 ± 0.08*	0.06 ± 0.01
Fe	CONTROL	0.02 ± 0.00	0.16 ± 0.02	0.04 ± 0.00	0.06 ± 0.01
	14-DAY	0.59 ± 0.07*	1.18 ± 0.13*	0.14 ± 0.01*	1.81 ± 0.27*
	28-DAY	1.15 ± 0.00*	2.52 ± 0.22*	0.25 ± 0.02*	3.76 ± 0.33*
Ni	CONTROL	0.05 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	14-DAY	0.14 ± 0.01*	0.14 ± 0.01*	0.05 ± 0.02*	0.06 ± 0.01
	28-DAY	0.24 ± 0.01*	0.18 ± 0.01*	0.15 ± 0.02*	0.40 ± 0.08*

Values are presented as mean ± SEM; \*p < 0.05 are significant when compared with control.

consistent with all the assessed organs. Values of Ca in the test groups (14- and 28-day) in the respective organs are significantly higher than in the control. However, the 28-day group produced multiple folds of value as found in the 14-day except for the stomach sample where the 14-day is approximately half. Values of Pb and Cr are also significantly higher in the test group when compared with the control. Similarly, almost none of the values presents a double or multiple fold of the other. The 28-day group only show a marginal increase when compared with the 14-day group. With respect to the data from the lungs, Pb and Cr show a marginal increase over time as found in the comparison between the 14-day and the 28-day groups, Cd decreased, Co and Mn increased in multiple (5 and 7 times respectively). Only, Fe and Ni doubly increased in line with what might be expected considering the double length of exposure. In the brain tissue, only Fe increased doubly while other metals appear about the same level at both the 14- and 28-day groups. In the spleen, however, Pb and Cr increase are similar to the findings in the lungs, whereas Cd, Co, Mn, Fe, and Ni all increased 5, 3, 4, 2, and 3 times respectively, showing substantial accumulation from 14 to 28 day of exposure. Available data at the 14- and 28-day exposed groups particularly for the lungs and stomach are interesting when compared with the

**Table 6**

Observed pulmonary histomorphometry of rats exposed to cement dust.

ALTERATIONS/GROUPS	CONTROL	14 DAY	28 DAY
Alveolar type II Pneumocyte Hyperplasia	-	++	+++
Alveolar mononuclear cell infiltration	-	++	+++
Focal Hemorrhage	-	++	++
Alveolar Septal thickening	-	+-	++
Emphysema	-	++	++
Periarteritis	-	++	++
Medial hypertrophy of muscular arteries	-	+-	+++
Eosinophilic Substance	-	+-	++
Fibroblast tissue proliferation	-	++	++
Fibrinoid Necrosis of blood vessels	-	++	+++
Oedema	-	+-	++

The observed morphological alteration of the erythrocyte was assessed using the grade below:

- : morphological change absent in animals in a group.
- + -: morphological change rarely found in animals in a group.
- +: morphological change found in some animals in a group.
- ++: morphological change common to all animals of a particular group.
- +++ : morphological change common to most animals in the exposed groups.

control. There was a substantially significant difference between the treated and control groups. A more interesting difference is observable between the 14-day and 28-day groups. Asides from Co and Mn, all other heavy metals assessed in this study follow a similar pattern of bioaccumulation in the two tissues.

#### 4. Discussion

This study presents a standardized form of the earlier presented and deployed exposure model [24]. The morphology, operation, and modification of the model were presented in Fig. 1A, B, and Table 2. It meets the need to simplify, substantiate and normalize the suitability of the model in the assessment of the systemic effect of inhalable/particulate matters. It was deployed in this study to assess the effect of cement dust on the respiratory tract; the foremost points of call for investigation in aerosol-mediated toxicity. The primitive exposure chamber was fabricated and immediately deployed for use. Initially, it was not clear if the chamber would fill the gap of the experimental toxicological evaluation model particularly with respect to occupational/residential scale of exposure or not. However, subsequent results emanating from the study featured a number of pathological manifestations that closely mimic those earlier reported in the literature on human subjects in many conditions of case reports, questionnaires, interviews, etc. hence, the need to standardize the exposure chamber became overtly expedient. The “modification” column of Table 2 outlines the advancement of the chamber with a view to standardizing it. For the present study, the dust generation rate was stabilized at 0.2 g/hr.

Cement particles have been reported to consist of toxic metals in varied proportions [28,29] depending particularly on the raw materials used. Nigeria cement dust particles contain very high concentrations of heavy metals known to be lethal even at small doses such as Cd, Pb, and mercury (Hg) [30]. The significantly high levels of Pb, Cd, Cr, Co, Ni, Mn, and Fe observed in the stomach, lungs, heart, and brain tissues of the exposed animals confirm intoxication with heavy metallic components of the dust. There is a considerable siamese relationship between the pattern of heavy metals deposition at the lungs and stomach tissues as shown by Table 5. Almost all the heavy metals show a similar trend of bioaccumulation, particularly when the test groups are compared. This similarity is probably borne out of the anatomical relationship between the lungs and the gastrointestinal tract (GIT) as established by the mucociliary escalator. The GIT is susceptible to the toxicosis of any toxicant found within the upper respiratory tract. Germs, particles, dust, and other pollutants in the inspired air are trapped by mucus before the mucociliary escalator moves them up and out of the lungs. These materials can then be removed from the body via coughing or swallowing. The latter makes the GIT become vulnerable to the debris moved out of the lungs. In the case of this study, the heavy metal-loaded dust removed from the lungs may have found its way to the GIT where they stimulate similar pathophysiological mechanisms. Similar evidence was recently reported by [31] who found significantly high levels of heavy metal in visceral organs of rats co-exposed to cadmium and lead, and [32] who similarly found significantly high levels of heavy metals in various tissues of snails picked around cement factory. Also, in plant physiology, similar evidence has been reported where heavy metallic constituents of cement dust have been demonstrated to leach into the soil around cement factory site causing a significantly higher proportion of toxic metal within the factory neighborhood [2,33,34]. Recently, our laboratory reported an increase in plasma concentration of heavy metals following exposure to cement particles [24]. The present study provides the first evidence using experimental animals that cement dust could trigger heavy metal bioaccumulation in living tissues. After heavy metal intoxication; sequestration and bioaccumulation precede a number of pathological manifestations [35] as they are not easily metabolized or excreted [36]. Apart from Cd, Pb, and Hg that are known to elicit systemic toxicities even at low concentrations [37,38], most divalent cations have been implicated in effective cytophysiology as they participate

in most intracellular activities such as oxidative phosphorylation, enzymatic activities, nucleic acid, and protein synthesis, membrane stabilization, and transport. However, regulation of their relative biological abundance and bioavailability is a requirement for the effective functioning of the cell. Asides from the sole toxicities of heavy metals, their influx into the cell as typified by cement dust exposure is deleterious as they compete for transporters with essential trace elements especially when they exceed the homeostatically tolerable limit [39,40]; thereby leading to loss of function as a result of deficiencies of essential minerals [41].

Clinical presentations are often indicators of systemic toxicity. The observed clinical signs following exposure in this study confirm the systemic toxicity of cement dust. The animals exhibited vigour at the initial stage of the study exploring every accessible point within the apparatus including the top of the subchamber. The fabricated chamber differs significantly in internal morphology and space from the plastic cages they were housed. This answers for the rambunctious behavior initially exhibited by the animals. However, towards the end of the experiment, they appeared docile, hypoactive, and anorexic constantly maintaining a stationary position. This could be due to the stress accumulation occasioned by the exposure to the dust or perhaps due to anorexia. The mortality of one animal which occurred while the exposure was ongoing may be due to multi-organ failure caused by the acute effect of the heavy metal in the dust. The observed laboured breathing and frequent sneezing are suggestive of respiratory distress induced by cement dust.

The source of bodily functions, regulation, and integration is the central nervous system (CNS) being principally composed of the brain and the spinal cord. It gathers information from far and near extremities for coordination and control. Maintenance of gait, posture, and coordination of movement right from thought to execution are all the functions of the CNS. Dyskinesia and other poor movement coordination resembling hemiballism, abnormal posture, and hypoactivity as observed in this study following the exposure to cement dust indicate a central nervous disorder. The dermal route is one of the major routes of exposure to cement aerosol; others being inhalation and gastrointestinal routes. Since cement dust is an airborne toxicant, the skin, by virtue of its large surface area remains the most affected. It quickly settles on the skin and exerts topical effects with toxicoses yet to be studied in detail. In this study, exposure to cement dust may be responsible for the integumentary degradation as marked by increased fur lability. It was evident that more than one animal showed signs of loss of fur at the cephalic region at different times during the study.

Abnormal body weight change is considered a veritable toxicity index [42–44]. Similarly, organ weight is an indicator of the physiological or pathological condition of experimental animals [45,46]. There was a significant reduction in weekly body weight gain which is in contrast with an increase in the relative visceral organs (stomach, spleen, lungs, and brain) weights of the exposed groups when compared with the control (Fig. 4A-F). Although daily food intake was not assessed, the exposed and the control were equally allowed free access to food and water. The weight discrepancies observed in this study may either be due to anorexia or the direct systemic toxicity induced by the dust. The foregoing is expected since visceral organs are directly exposed to the deleterious effect of toxicants [47]. Most organs have the ability to sequester heavy metals following entry into the body [35]. This finding is in concert with several other findings [48–50]. Also, [51] in their study involving exposure of experimental animals to silica found significantly higher lung weight of the exposed animals when compared with the control. The condition of organomegaly observed in this study is an indication that the exposed rats bioaccumulated the heavy metals. The latter which is a condition typical of heavy metal toxicity remains an inevitable precursor to pathological manifestations especially during carcinogenesis [52].

Bone density changes have been shown to inversely correlate with heavy metal toxicity (Hee-Sook et al., 2016). Bone has been regarded as



one of the major target sites for heavy metal toxicosis [53–55]. Heavy metals such as Pb and (Cd) which accumulate in the bone matrix can store up significantly and displaced calcium, leading to bone demineralization, and in the process makes the bone susceptible to osteoporosis. Even though the comprehensive and holistic explanation is not yet available in the literature, exposure to a higher concentration of Cd alone has been strongly linked to lower bone densities, decrease trabecular number and decrease thickness [53,55,56]. Co-exposure to Pd and Cd stimulates bone histopathological damage [57]. As the largest bone in the body, the decrease in femoral density observed in this study suggests the possibility of chelation of essential minerals like calcium from the bone matrix causing mineral imbalance which may eventually predispose the bone to osteoporosis. Low bone density observed in this study does not only corroborate but is also suspected to be responsible for the low body weight gain observed in the exposed groups.

Pulmonary tissue reaction to dust particles is known to be dependent on a number of factors such as the composition of the dust, the length of exposure, and the immunological status of the exposed [58]. Of more significance is the composition of cement dust owing to its multi-heavy metallic composition. Pneumoconiosis was grossly observed in the lung tissue of the exposed groups (Fig. 2D-F) when compared to the control (Fig. 2C). The topical pulmonary effect of the dust culminates the observed pneumoconiosis; the fibrogenic tendency of the dust is thereby suspected. The onset of the pneumopathology may be the stimulus for the respiratory distress observed in some of the exposed animals as they show irregular and laboured breathing. The clinical signs of toxicity observed in this study show a wide range of semblance with the pathological manifestations that accompany occupational exposure to cement dust [12,16,59,60]. In addition, the serosal surface of the lungs shows signs of infarction (Fig. 2F). This is suggestive that exposure to cement dust may significantly affect blood supply at the organ level. Cytoarchitectural investigations play a significant role in establishing pathological alterations at the tissue level following exposure to toxicants. It gives reliable information about the extent of degradation in exposed tissues difficult to be observed macroscopically, cellularly, or even with the aid of subcellular biomarkers [61]. Pulmonary histopathological disruptions after exposure to cement dust had been earlier reported by a study of in-situ exposure [10] where inflammation, disrupted bronchiole and bronchus, and degenerated the epithelial lining were observed. Fig. 3A–F shows an array of histopathological alterations while Table 6 shows the frequency and severity of the observed alterations secondary to cement dust exposure. The black arrow in Fig. 3D shows infiltration of inflammatory cells. Inflammatory cells play significant roles in the development and healing of either chemical or topical injuries. Analogous to any condition of heavy metal intoxication, infiltration of inflammatory cells is considered a reliable yardstick for the assessment of the pathogenesis of heavy metal-induced toxicosis as they are known to produce and release pro-inflammatory cytokines, proteolytic enzymes, reactive oxygen, and nitrogen species [62]. Neutrophilic infiltration is known to precede the cascade of mechanisms that herald injuries on tissues. Hence, the histopathological changes observed in this study which feature inflammatory cell and mononuclear cell infiltration are indicators of the pro-inflammatory tendency of the dust. Meanwhile, according to Balduzzi and colleagues, crystalline silica elicits inflammatory cell production which ultimately leads to free radical generation [63]. The respiratory distress observed in the exposed animals may be due to the free radicals generated via the topical pulmonary effect of crystalline silica, a major compound constituent of cement dust, or by the inflammatory cell infiltration observed in the histoarchitectural alteration above. The “black arrow” in Fig. 4B depicts alveolar hyperplasia in the lung tissues. This condition is suggestive of the proliferative reaction of pulmonary tissue to the dust; a notable characteristic of the onset of carcinogenesis. Howbeit, [64] and [65] had established the positive correlations between cancer of the respiratory system and the length of cement dust exposure period, the literature has been devoid of laboratory-based support for the claim. Air space

enlargement is an early sign of emphysema.

## 5. Conclusion

This study provides a standardized laboratory-based experimental model of exposure for investigation on cement dust toxicity. It generally revealed heavy metal bioaccumulation and histoarchitectural alteration as organ damaging mechanisms with respect to the respiratory system. The exposure apparatus has been modified and standardized to mimic the cement factory environment and host communities of cement factories alike who are equally vulnerable to cement dust toxicities as occupationally exposed individuals. The results from this study add to the relatively few experimental-based data available on cement dust and therefore advance the existing claims of its toxicity. The pathogenesis of cement dust-induced toxicities is not limited to bioaccumulation of the heavy metal content of cement dust but also includes organomegaly and pneumopathological alterations. Further studies on the toxicosis of cement dust are hereby encouraged in order to validate the epidemiological reports in the literature on cement dust-induced pathologies and to incite policies geared towards the protection of occupationally and geographically exposed individuals.

## Authors' agreement

We the authors of this manuscript write to clearly state that there is no conflict of interest whatsoever in the conception, design and writing of this work.

## Declaration of Competing Interest

The authors report no declarations of interest.

## References

- [1] N.O. Olatunbosun, B.A. Sawa, A. Jibrin, A.E. Ilori, Assessment of effect of cement dust from cement factory on elemental properties of some cultivated crops, Obajana, Kogi State, Nigeria, *J. Geogr. Environ. Earth Sci. Int.* 24 (1) (2020) 63–69.
- [2] R.E. Lamare, O.P. Singh, Effect of cement dust on soil physico-chemical properties around cement plants in Jaintia Hills, Meghalaya, *Environ. Eng. Res.* 25 (3) (2020) 409–417, <https://doi.org/10.4491/eeer.2019.099>.
- [3] M.D. Adak, S. Adak, K.M. Purohit, Ambient air quality and health hazards near min-cement plants, *Poll. Res.* 26 (3) (2007) 361–364.
- [4] Mehraj, Bhat, Cement Factories, Air Pollution and Consequences [Online], pp. 12.03.2016. Available from: 2013 [http://www.sciencepub.net/book/00065\\_book\\_1\\_65.pdf](http://www.sciencepub.net/book/00065_book_1_65.pdf).
- [5] S. Fairhurst, A. Phillips, Health & Safety Executive (Great Britain). Portland Cement Dust. Criteria for an Occupational Exposure Limit. Sudbury: HSE Books, 1994. ISBN 071760767631.
- [6] R. Schemback, Burning Our Health: Hazardous Waste Incineration in Cement Kilns in Mexico, Available at: <http://tcascenter.orgpublications/kiln.htm> (accessed 20 April 2021), 1998.
- [7] S.K. Gupta, J. Singh, Evaluation of mollusc as sensitive indicator of heavy metal pollution in aquatic system: a review, *IIOAB J.* 2 (1) (2011) 49–57.
- [8] S. Tak, G.A. Bhat, Assessment of epigeal invertebrate community in cement polluted and non-polluted areas, *J. Res. Dev.* 9 (2009) 45–52.
- [9] S.S. Mehraj, G.A. Bhat, H.B. Mehraj, T. Gul, Health risks for population living in the neighborhood of a cement factory, *Afr. J. Environ. Sci. Tech.* 7 (12) (2013) 1044–1052.
- [10] Y. Tajudeen, J. Okpuzor, T.F. Adedayo, Investigation of general effects of cement dust to clear the controversy surrounding its toxicity, *Asian J. Sci. Res.* 4 (2011) 315–325.
- [11] A. Fortune, Ethiopia: Cement Factory's Reign of Pollution, Addis Fortune, Addis Ababa, Ethiopia, 2011. <http://allafrica.com/stories/201104121072.html>.
- [12] J. Vestbo, F.V. Rasmussen, Long-term exposure to cement dust and later hospitalization due to respiratory disease, *Int. Arch. Occup. Environ. Health* 62 (1990) 217–220, <https://doi.org/10.1007/BF00379436>.
- [13] J. Mwaiselage, B. Moen, M. Bratveit, Acute respiratory health effects among cement factory workers in Tanzania: an evaluation of a simple health surveillance tool, *Int. Arch. Occup. Environ. Health* 79 (2008) 49–56, <https://doi.org/10.1007/s00420-005-0019-x>.
- [14] L. Coppeta, A. Pietrousti, A. Magrini, G. Somma, A. Bergamaschi, Prevalence and characteristics of functional dyspepsia among workers exposed to cement dust, *Scand. J. Work Environ. Health* 34 (5) (2008) 396–402.
- [15] S. Peters, Y. Thomassen, E. Fechter-Rink, H. Kromhout, Personal exposure to inhalable cement dust among construction workers, *J. Environ. Monit.* 11 (1) (2009) 174–180.

- [16] Z. Zeleke, B. Moen, M. Bratveit, Cement dust exposure and acute lung function: a cross shift study, *BMC Pulm. Med.* 10 (1) (2010) 19–26.
- [17] J.O. Ogunbileje, O.M. Akinosun, Biochemical and haematological profile in Nigeria cement factory workers, *Res. J. Environ. Toxicol.* (2011), <https://doi.org/10.3923/rjet.2011>. ISSN 1819-3420.
- [18] B. Isikli, T.A. Demir, T. Akar, Cadmium exposure from the cement dust emissions: a field study in a rural residence, *Chemosphere* 63 (2006) 1546–1552.
- [19] O. Oguntoke, A.E. Awanu, H.J. Annegarn, Impact of cement factory operations on air quality and human health in Ewekoro Local Government Area, South-Western Nigeria, *Int. J. Environ. Stud.* 2012 (2012) 1–12, <https://doi.org/10.1080/00207233.2012.732751>, iFirst Article.
- [20] J. Vestbo, K.M. Knudsen, E. Raffn, B. Korsgaard, F.V. Rasmussen, Exposure to cement dust at a portland cement factory and the risk of cancer, *Br. J. Ind. Med.* 48 (12) (1991) 803–807.
- [21] S.A. Meo, A.A. Muhammad, A.A. Qureshi, G.M. Ghori, A.M. Al-Drees, M.M. F. Subhan, Dose response effect of cement dust on respiratory muscles competence in cement mill workers, *Int. J. Environ. Health Res.* 16 (6) (2006) 439–447.
- [22] V. Karkhanis, J.M. Joshi, Cement dust exposure-related emphysema in a construction worker, *Lung Ind.* 28 (2011) 294–296.
- [23] T. Bamidele, B. Atolaye, Antioxidant status and hepatic lipid Peroxidation in Wistar rats exposed to cement dust, *Transnat. J. Sci. Technol.* 2 (4) (2011). May edition.
- [24] P.C. Nwafor, O.A. Odukanmi, A.T. Salami, M. Owonikoko, S.B. Olaleye, Evaluation of a Cement Dust Generation and Exposure Chamber for Rodents: Blood Heavy Metal Status, Haematological Variables and Gastrointestinal Motility in Rats, *Afr. J. Biomed. Res.* 22 (2019) 79–87.
- [25] J. Ilar, Guide for the Care and Use of Laboratory Animals, 8<sup>th</sup> edition, 1996.
- [26] S. Akram, N. Rahila, H.R. Ghazala, S.A. Abbas, Determination of heavy metal contents by atomic absorption spectroscopy (AAS) in some medicinal plants from Pakistani and Malaysian origin, *Pak. J. Pharm. Sci.* 28 (5) (2015) 1781–1787.
- [27] M.M. Haber, I. Lopez, Gastric histologic findings in patients with nonsteroidal anti-inflammatory drug-associated gastric ulcer, *Mod. Pathol.* 12 (1999) 592–598.
- [28] A. Dietz, H. Ramroth, T. Urban, W. Ahrens, H. Becher, Exposure to cement dust, related occupational groups and laryngeal cancer risk: results of a population-based case-control study, *Int. J. Cancer* 108 (2004) 907–911.
- [29] J.S. Swaran, P. Vidhu, Chelation in metal intoxication, *Int. J. Environ. Res. Public Health* 7 (7) (2010) 2745–2788.
- [30] J.O. Ogunbileje, V.M. Sadagoparamanujam, J.I. Anetor, E.O. Farombi, O. M. Akinosun, A.O. Okorodudu, Lead, mercury, cadmium, chromium, nickel, copper, zinc, calcium, iron, manganese and chromium (VI) levels in Nigeria and United States of America cement dust, *Chemosphere* 90 (2013) 2743–2749.
- [31] M. Andjelkovic, A.B. Djordjevic, E. Antonijevic, B. Antonijevic, M. Stanic, J. Kotur-Stevuljevic, V. Spasojevic-Kalimanovska, M. Jovanovic, N. Boricic, D. Wallace, Z. Bulat, Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney, *Int. J. Environ. Res. Public Health* 2019 (16) (2019) 274.
- [32] R.N. Ugbaja, M.A. Enilolobo, A.S. James, T.F. Akinhanmi, A.J. Akamo, D. O. Babayemi, O. Ademuyiwa, Bioaccumulation of heavy metals, lipid profiles, and antioxidant status of snails (*Achatina achatina*) around cement factory vicinities, *Toxicol. Ind. Health* 36 (11) (2020) 863–875.
- [33] A.F. Abimbola, O.O. Kehinde-Phillips, A.S. Olatunji, The sagamu cement factory, S. W. Nigeria: is the dust generated a potential health hazard? *Environ. Geochem. Health* 29 (2007) 163–167.
- [34] R. Devarajan, K.R. Hanumappa, N. Kuppan, The Study of change in physico-chemical properties of soil due to cement dust pollution-an hazardous terrorization to ecosystem, *Canadian J. Pure Appl. Sci.* 9 (1) (2015) 3193–3200. ISSN: 1920-3853; Print ISSN: 1715-9997 Available online at [www.cjpas.net](http://www.cjpas.net).
- [35] B. Olivier, J. Gregory, T. Michel, C. Marc, Effect of heavy metals on, and handling by, the kidney, *Nephron Physiol.* 99 (2005) 105–110.
- [36] Z. Cooper, R. Bringolf, R. Cooper, K. Loftis, A.L. Bryan, J.A. Martin, Heavy metal bioaccumulation in two passerines with differing migration strategies, *Sci. Total Environ.* 592 (2017) 25–32, <https://doi.org/10.1016/j.scitotenv.2017.03.055>.
- [37] J. Millichap, Lead neurotoxicity, *Pediatr. Neurol. Briefs* 9 (1995) 42.
- [38] B.O. Anyanwu, A.N. Ezejiiofor, Z.N. Igweze, O.E. Orisakwe, Heavy metal mixture exposure and effects in developing nations: an update, *Toxics* 2018 (6) (2018) 65, <https://doi.org/10.3390/toxics6040065>.
- [39] A.D. Dayan, A.J. Paine, Mechanism of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985 to 2000, *Human Exp. Toxicol.* 20 (2001) 439–451.
- [40] J. Liu, R.A. Goyer, M.P. Waalkes, Toxic effects of metals, in: C.D. Klaassen (Ed.), Casarett and Doull's Toxicology: The Basic Science of Poisons, seventh ed., The McGraw Inc., New York, 2008, pp. 931–979.
- [41] R. Singh, G. Neetu, M. Anurag, G. Rajiv, Heavy metals and living systems: an overview, *Indian J. Pharmacol.* 43 (3) (2011) 246–253.
- [42] S. Teo, D. Stirling, S. Thomas, A. Hoberman, A. Kiorpes, K. Vikram, A 90-days oral gavage toxicity study of D-methylphenidate and D, L- methylphenidate in sprague-dawley rats, *Toxicology* 179 (2002) 183–196.
- [43] C.D.S. Tomlin, The Pesticide Manual: a World Compendium, 14th ed., British Crop Production Council, Alton, 2006, p. 2006.
- [44] K. Toynton, B. Luukinen, K. Buhl, D. Stone, Permethrin Technical Fact Sheet, Available from: National Pesticide Information Center, Oregon State University Extension Services, 2009 <http://npic.orst.edu/factsheets/Permeth>.
- [45] Y.K. Vaghasiya, V.J. Shukla, S.V. Chanda, Acute oral toxicity study of Pluchea arguta Boiss Extract in Mice, *J. Pharmacol. Toxicol.* 6 (2) (2011) 113–123, <https://doi.org/10.3923/JPT.2011.113.123>. ISSN 1816-496X.
- [46] K. Dwivedi, D.K. Gupta, Concomitant influence of heavy metal intoxication on size of organs and body weight in albino rats, *IJPSR* 11 (3) (2020) 1417–1424.
- [47] E. Dybing, J. Doe, J. Groten, J. Kleiner, J. O'Brien, A.G. Renwick, J. Schlatter, A. Tritscher, R. Walker, M. Younes, Hazard characterization of chemicals in food and diet: dose response, mechanism and extrapolation issues, *Food Chem. Toxicol.* 42 (2002) 237–282.
- [48] H. Li, M. Han, L. Hou, G. Li, N. Sang, Landfill leachate inges-tions induced protein oxidation and DNA-protein crosslinks in mouse viscera, *J. Haz Mat.* 2010 (174) (2010) 54–58.
- [49] C.G. Alimba, A.A. Bakare, O.O. Aina, Liver and kidney dysfunction in wistar rats exposed to municipal landfill leachate, *Resour. Environ.* 2 (4) (2012) 150–163, <https://doi.org/10.5923/j.re.20120204.04>.
- [50] S.S.F. Kenston, H. Su, Z. Li, L. Kong, Y. Wang, X. Song, Y. Gu, T. Barber, J. Aldinger, Q. Hua, Z. Li, M. Ding, J. Zhao, X. Lin, The systemic toxicity of heavy metal mixtures in rats, *Toxicol. Res.* 7 (2018) 396–407.
- [51] D.W. Porter, A.F. Hubbs, R. Mercer, V.A. Robinson, D. Ramsey, J. McLaurin, A. Khan, L. Battelli, K. Brumbaugh, A. Teass, V. Castranova, Progression of lung inflammation and damage in rats after cessation of silica inhalation, *Toxicol. Sci.* 79 (2004) 370–380, <https://doi.org/10.1093/toxsci/kfh110>.
- [52] N.P. Okolie, A.U. Osagie, Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure, *Food Chem. Toxicol.* 37 (7) (1999) 745–750.
- [53] R. Honda, I. Tsuritani, Y. Noborisaka, H. Suzuki, M. Ishizaki, Urinary cadmium excretion is correlated with calcaneal bone mass in Japanese women living in an urban area, *Environ. Res.* 91 (2003) 63–70.
- [54] K. Theppeang, T.A. Glass, K. Bandeen-Roche, A.C. Todd, C.A. Rohde, J.M. Links, B. S. Schwartz, Associations of bone mineral density and lead levels in blood, tibia, and Patella in urban-dwelling women, *Environ. Health Perspect.* 116 (2008) 784–790.
- [55] X. Chen, G.Y. Zhu, C.L. Shao, T.Y. Jin, M.G. Tan, S.Z. Gu, Y.Y. Zhang, H.F. Xiao, Effects of cadmium on bone microstructure and serum tartrate-resistant acid phosphatase 5b in male rats, *Exp. Biol. Med.* 9 (2011) 1–8.
- [56] J.A. Staessen, H.A. Roels, D. Emelianov, T. Kuznetsova, L. Thijs, J. Vangronsveld, R. Fagard, Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study, *Public Health and Environmental Exposure to Cadmium (PheeCad) Study Group, Lancet* 353 (1999) 1140–1144.
- [57] H. Lu, G. Yuan, Z. Yin, S. Dai, R. Jia, J. Xu, X. Song, L. Li, C. Lv, Effects of subchronic exposure to lead acetate and cadmium chloride on rat's bone: Ca and Pi contents, bone density, and histopathological evaluation, *Int. J. Clin. Exp. Pathol.* 7 (2) (2014) 640–647. /ISSN:1936-2625/LJCEP1311073, [www.ijcep.com](http://www.ijcep.com).
- [58] D.E. Gardner, H.A. Wallace, A. John, in: Donald E. Gardner (Ed.), *Toxicology of the Lung*, 4th ed, Taylor and Francis, 2006.
- [59] A.S. Laney, E.L. Petsonk, M.D. Attfield, Pneumoconiosis among underground bituminous coal miners in the United States: is silicosis becoming more frequent? *Occup. Environ. Med.* 67 (2010) 652–656.
- [60] S. Aydin, S. Aydin, G. Croteau, I. Sahin, C. Citil, Ghrelin, nitrite and Paraoxonase/ Arylesterase concentrations in cement plant workers, *J. Med. Biochem.* 29 (2) (2010) 78–83.
- [61] L.L. Lanning, D.M. Creasy, R.E. Chapin, P.C. Mann, N.J. Barlow, K.S. Regan, D. G. Goodman, Recommended approaches for the evaluation of testicular and epididymal toxicity, *Toxicol. Pathol.* 2002 (30) (2002) 507–520.
- [62] T. Watanabe, K. Higuchi, K. Tominaga, Y. Fujiwara, T. Arakawa, Role of neutrophils in development, healing and recurrence of gastric ulcer in rats. Oxidative Stress and Digestive Diseases, Karger, Basel, 2001, pp. 41–50.
- [63] M. Balduzzi, M. Diociaiuti, B. De Berardis, S. Paradisi, L. Paoletti, In vitro effects on macrophages induced by noncytotoxic doses of silica particles possibly relevant to ambient exposure, *Environ. Res.* 96 (2004) 62–71.
- [64] H. Maier, U. Gewelke, A. Dietz, H. Thamm, W.D. Heller, H. Weidauer, Laryngeal cancer and occupation: results of the Heidelberg laryngeal cancer study, *H N O.* 40 (1992) 44–51.
- [65] H. Maier, M. Tisch, A. Dietz, C. Conradt, Construction workers as an extreme risk group for head and neck cancer, *HNO* 47 (1999) 730–736.