Neurobehavioral deficits, histoarchitectural alterations, parvalbumin neuronal damage and glial activation in the brain of male Wistar rat exposed Landfill leachate

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Abstract

Concerns about inappropriate disposal of waste into unsanitary municipal solid waste landfills around the world has been on the increase; and this pose a public health challenge due to leachate production. The neurotoxic effect of Gwagwalada landfill leachate (GLL) was investigated in male adult Wistar rats. Rats were exposed to 10% concentration of GLL for 21days. Control group received tap water for same period of experiment. Our results showed that neurobehavior, absolute body and brain weights and brain histomorphology as well as parvalbumin interneurons were severely altered, with consequent astrogliosis and microgliosis after 21 days of administrating GLL. Specifically, there was severe loss and shrinkage of purkinje cells, with their nucleus, and severe diffused vacuolations of the white matter tract of GLL exposed rat brains. There was severe cell loss in the granular layer of the cerebellum resulting to reduced thickness of the layer. Also. there was severe loss of dendritic arborization of the purkinje cells in GLL exposed rat brains, and damage as well of reduced populations of parvalbumin-containing fast-spiking GABAminergic interneurons in various regions of the brain. In conclusion, data from the present study demonstrated the detrimental effects of Gwagwalada landfill leachate on the brain which may be implicated in neuropsychological conditions.

Introduction

There is an increasing concern about inappropriate disposal of waste into unsanitary municipal solid waste landfills around the world (Li et al., 2006), especially in the developing countries due to projectile population growth resulting from industrial development in most major cities (Alimba et al., 2015; Usende et al., 2016; 2017, 2018a,b; 2020; 2022a). Landfills are known environmental threat and constitute a major problem of public health importance (Li et al., 2006; Alimba, 2013; Alimba et al., 2015) due to enormous amount of leachate and gas produced as a result of this waste (Alimba et al., 2015).

Leachates are water collected from landfill dumpsite and is posited to contain very high concentration of hazardous organic and inorganic chemicals, particulate matter, toxic heavy metals, radioactive substances and microorganisms (Oygard and Gjengedal, 2009; Eggen et al., 2010; Efuntoye et al., 2011; Alimba et al., 2015; Slack et al., 2005). Alimba and Bakare, (2012) and Alimba et al., (2012) have reported mixture of xenobiotics in leachates collected from Aba Eku and Olusosun landfills in Africa, Nigeria. These authors (Alimba and Bakare, 2012; Alimba et al., 2012; 2015) have shown that exposure to leachate can induce severe alterations in bosy weight gain, erythrocyte morphology and haematological indices, kidney and liver dysfunction (Alimba and Bakare, 2012; Alimba et al., 2012) in rat experimental model. Also, Li et al. (2006; 2010) posited that exposure to Xinguo, China leachate induced protein oxidation, lipid peroxidation as well as disturbances of antioxidant status in different organs including the brain, heart, liver, kidney and spleen.

There is scarcity of information on neurotoxic effect of leachate especially on brain functional and structural integrity. The brain among other organs is the most susceptible to lipid peroxidation and oxidative injury resulting from exposure to xenobiotics due to its very membranous high polyunsaturated

fatty acids concentration and high iron contents with reduced antioxidant status (Jayaraman et al., 2008, Todorich et al., 2011). Indeed, some evidences showed that each chemical in the landfill leachate can alter the normal functionality of the nervous system resulting to increasing neurotoxic disorders in mammals including the human population (Wright et al., 2006; Neal and Guilarte, 2012; Alimba et al., 2015). Importantly, reports exist that regular neurotoxic assessment is key among health outcomes been suggested by the Agency for Toxic Substances and Disease Registry (ATSDR) to be critically monitored following exposure to any hazardous substances arising from sites of solid waste disposal (Schaumburg et al., 1983; Johnson, 1999). Herein, we evaluated the neurotoxic effects of Gwagwalada landfill leachates (GLL) in adult male Wistar rats. We posit that exposure to GLL induced severe neurobehavioral and structural alterations in brain tissue and alters body weight gain and brain weight.

Materials And Methods

Sampling Site and collection of Leachate

In this study, the sampling site was at the Federal Capital Development Authority (FCDA), Gwagwalada Abuja dumpsite, located at Gwagwalada Area Council, one of the five area councils in Abuja, Nigeria. Abuja is the Federal Capital Territory (FCT) of Nigeria with a population of above two million people. The centrality of the FCT in relation to other states makes it influential and important in various socioeconomic activities (Ishaya and Abaje 2009, Usende et al., 2017; 2022a). This dumpsite is not equipped with a leachate collection and treatment system; thus, leachate produced is freely discharged into the surrounding aquatic and terrestrial environmental media. We selected this dumpsite due to high polluting status of this environment via landfill gas and leachate production, which may increase public health risk via exposure to microorganism and landfill chemicals (Alimba, 2013, Efuntoyr *et al.*, 2011) to the human pollution living in this area. The method for collection of leachates was as described by Alimba and Bakare (2016). Briefly, raw leachates were obtained from seven (7) separate leachate wells of the landfills and thoroughly mixed together. The homogenous leachate sample gotten was immediately transferred to Neuroscience unit laboratory of the Department of Veterinary Anatomy, University of Abuja, in pre-cleaned plastic 10-liter container. In the laboratory, the leachate obtained was filtered in a biosafety hood using glass wool and Whatman No.42 filter paper to eliminate any suspension particles. The filtered leachate sample was then centrifuged at 3000rpm for 10minutes and supernatant was collected, labelled Gwagwalada landfill leachate (GLL) and stored at 4^{o}C until time of used. The processed GLL was considered a stock sample (100%).

Physical and chemical analysis of leachate

Nitrate, chloride, ammonia, sulphate, phosphate, total hardness, total alkalinity, chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total solids (TS) contents of the GLL leachates were assessed according to APHA (2005). Also, heavy metals including iron (Fe), copper (Cu), lead (Pb), manganese (Mn), arsenic (As), chromium (Cr), nickel (Ni) and cadmium (Cd) concentrations were analyzed in the GLL leachates in conformity with APHA (2005) and USEPA (2006).

Animal experimental design

Twenty-four (24) Wistar albino male rats weighing 80g ± 0.68g was used for this study. The rats were procured from Core Animal Facility of the University of Nigeria, Nsukka, at 8-weeks old and transported in ventilated cages to the Animal Core facility of the Neuroscience Unit of the Department of Veterinary Anatomy, University of Abuja. Animals were acclimatized for 14-days before start of experimental procedure, and were strictly maintained in a 12- hour/12-hour light/dark cycle during the period of experiment. Food and clean tap water were provided *ad libitum*. The experiments were performed under strict veterinarian assistance, thus minimizing the number of animals used and also avoiding animal suffering. Animal experiments received ethical acceptance from University of Abuja Ethics Committee for Animal Use (UAECAU/2021/0012) and according to ethical standard of National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023) and European Communities Council Directive of November 24, 1986 (86/609/EEC).

At the start of experiment, the animal were 10 weeks old and weighed 100.3 ± 0.40g. The 24 animals used were randomly split into two experimental groups as follows:

Group 1: Control group had 12 male Wistar rats as group members. They were allowed access to clean tap water *per os ad libitum*. Feed (rat chow) were also administered *ad libitum*.

Group 2: The Gwagwalada landfill leachate (GLL) exposed group. This group also had 12 male Wistar rats as members and were allowed access *ad libitum* to 10% GLL as drinking water and feed with rat chow. Both control and GLL exposed groups received treatment for 21-days period.

Neurobehavioral Test

After 21days treatment, the effects of exposure to Gwagwalada landfill leachate on neurobehavior of the male Wistar rats was accessed using open field test, Modified Morris Water Maze test, and Forelimb Grip Strength test (Hanging wire test).

Open Field Test

For open field test, we used a modified method of Usende et al., (2016) and was carried out on the day of animal sacrifice. Briefly, open field arena made of glossy white plywood cage of 64 cm x 64cm and 30cm height was used. A green marker was used to draw lines of sixteen 16x16cm squares on the arena floor. A central square (16cmx16cm) was also drawn in the open field (Brown et al., 1999, Usende et al., 2016) using a red permanent marker. A video camera positioned at 65cm above the centre of the open arena floor was used in capturing the entire arena. For the behavioral test, individual animal was placed at the center of the open arena and by pressing space bar key on the computer keyboard, the test manually starts and ends. Each rat was allowed to explore the open field for 5minutes before they were removed from the open field arena and the arena thoroughly cleaned with 70% ethanol, allowed to air dry before testing of a next rat.

In this experiment, parameters studied are as reported by Brown et al. (1999) and modified by Usende et al., (2016) and include:

i. Line Crossing: indicating the number of times rat crossed each grid line with all four (4) paws.
ii. Centre Square Entries: indicating the number of times rat crossed each red line into the central square with all four (4) paws.
iii. Centre Square Duration: indicating the length of time in central square spent by the rat.
iv. Rearing: indicating the number of times rat stood on their hind legs in open field arena.
v. Grooming: indicating length of time spent by the rat scratching or licking itself while being stationary.
vi. Urination: indicating number of urine puddles or streaks.
vii. Defecation: indicating total faecal boli number.

Modified Morris Water Maze test (Swimming Test)

The modified Morris water maze used was as described by Folarin et al., (2016). In brief, the Morris water maze used was a circular pool of water (120cm diameter and 40cm height) and a circular platform for escape (14cm diameter) (hidden) which location, the rat must learn using visual and contextual cues (Folarin et al., 2016). The test was used to access hippocampal memory and dependent spatial learning in rodents (Shabani et al., 2012). The principle of the test is that rodents (including rats) are driven to escape from water environment by the quickest direct route (Folarin et al., 2016). The pool used was marked East, West, South, and North with the hidden platform positioned in a specific location. Each rat was gently introduced into the pool and anticipated to quickly find the hidden platform. The duration it takes for the rat to locate the platform was then recorded. The rats were directed to the hidden platform if they do not find it after 60seconds, and allowed there for 15seconds before removal. For two successive days, all rats in each group were trained through three (3) trials per day. To test for memory, on the fourth day, we performed a one probe trial hiding the platform. Rats' memory of the location of escape platform was assessed by recording the time it takes to locate the hidden platform where it was originally located. We used a video camcorder, Nikon coolpix L341 (Nikon Imaging, USA) for video capturing of this neurobehavior and was positioned 90cm above the centre of the circular water pool. The complete arena of the pool in the area of the view of the camera was captured from this location. The camcorder was connected to Acer laptop (Aspire 5, A517-53-54N6 | NX.K64EK.007; China) to record this behaviour. The behavior observation tests started at 9.00am each day and were performed by pressing space bar key on the computer keyboard. The test for each rat started and ended manually.

Forelimb Grip Strength Test

This test was used to access the animal's muscular strength and balance (Folarin et al., 2016). We performed this test according to the protocol described by Folarin et al., (2016). Briefly, the forepaws of each rat were placed on a horizontally suspended wire (2mm diameter and 1.0m length), and 1meter above a soft bedding-filled area for landing. The latency to fall, indicated by the length of time the rat was able to stay suspended before falling off the wire was recorded with an electronic stopwatch by two

experimenters blinded to the groups. Each rat from both groups had two trials and a time of 3minutes was used for each rat before removal.

Animal sacrifice, Dissection, Brain weight measurement and Histopathological examinations

On the day of animal sacrifice, rats from individual group were transcardially perfused with normal saline to flush out the blood and then with 4% PFA. Complete fixation was ascertained by stiffness of the muscles and paleness of liver and kidney. Brains were then carefully harvested out and weight on a bench top sensitive weighing balance (Lark electronic balance; LP 502A, China). The sensitivity of balance was 0.1 to 5kg (Oyelowo et al., 2017) before post-fixation in same 4% PFA for 24 hours before processing for histopathological examination using the routine Haematoxylin and Eosin stain and immunohistochemistry. For the H&E and immunohistochemical staining, we explored the protocol that we have described in our previous studies (Usende et al., 2013; 2022b). For immunohistochemical staining we used HRP/DAB detection kit-micro-polymer. Briefly, all prepared brain slides (serial sections from the olfactory bulb through the hypothalamus, and the cerebellum) were collected in series of every sixth section. Collected sections were air-dried and well labelled using pencil, deparaffinized by first, baking for 25mins at 60°C before two changes in xylene. Sections were then rehydrated in ethanol (decreasing percentage) and retrieval of antigen was done for 25mins in 10mM citrate buffer (pH = 6.0). Peroxidase quenching was achieved in hydrogen peroxide for 10 min before protein blocked (abcam, ab236469) for 10 min in humidity chamber (room temperature). Sections were then probed with anti-parvalbumin (Abcam, ab11427), anti-GFAP (Abcam, ab7260) and anti-Iba1 (Abcam, ab153696) polyclonal rabbit antibodies following manufacturer instructions overnight using humidity chamber and at 4°C. Bound antibodies detection and chromogen reaction was performed using rabbit specific HRP/DAB detection IHC Detection kit-Micro-polymer (Abcam, Ab236469) according to manufacturer's protocol and counterstain was done using hematoxylin solution (Mayer's modified, Abcam 220365) and slides were subsequent dehydration in ethanol. Stained slides were mounted with permount, coverslipped, allowed to dry before capturing of images using light microscope (Olympus CX31 light microscope) connected to a laptop computer (Hp, China) with digital camera.

Quantitative analyses

Cell counts and Analysis of Area of stained soma of PV+, IBA1 + and GFAP + cells in the Cerebellum, Prefrontal and Somatosensory cortexes and Hippocampus

To quantity PV+, microglia and astrocytes cell populations, quantitative analyses were done blinded to experimental conditions following a modified protocol described by Usende et al., (2022b), with a bright field microscope (Leica DM 300) connected to Excelis HDS (1080P) Camera and Monitor and equipped with Image J (NIH, Bethesda, MD, USA) software at X10 magnification, using the four brains each from GLL treated and control groups. Unbiased counts of labeled PV immunoreactive neurons as well as IBA-1 microglia and GFAP astrocytes immunoreactive cells were performed in sections regularly spaced throughout the rostro-caudal extent of these brain (at 10-µm intervals) in the cerebellum prefrontal and

somatosensory cortexes and hippocampus. Cell counting was performed following a modified protocol described by Usende et al., (2022b) and in line with earlier protocol described by Gerashchenko et al., (2001) and Gaykema and Goehler (2009), using grid with some modifications following the specific region of interest (ROI). The optical fractionator method described by West, (1993) was followed. Each region selected for PV+, GFAP + and IBA1 + cell counts were divided into 100 counting frames (100µm by 100µm counting frame size) (Usende et al., 2022b). The counting unit was an immunopositive cell (PV+, GFAP + and IBA1+) profiles counted only when a cell was entirely within the counting frame (Palomba *et al.*, 2015).

Also, PV+, GFAP + and IBA1 + cells soma from the purkinje cell layer of the cerebellum, prefrontal and somatosensory cortexes and hippocampus, were viewed with the bright field microscope (Leica DM 300) connected to Excelis HDS (1080P) Camera and Monitor using X40 (zoom factor 1.9) and images were captured. To evaluate the area of soma of each cell in these regions, a circle was drawn round the parvalbumin, astrocytes or microglia cell body using the Image J computer software. The circle covered the entire cell body as much as possible and figures were automatically generated and recorded. Six (6) parvalbumin, astrocytes or microglia cell bodies from 5 slices from 4 rat brains per group were averaged to obtain mean ± SEM and the numbers of analyzed brain slices were considered as n for statistical analysis.

Statistical Analysis

All numerical data obtained were evaluated using Graph-pad prism version 9.0 software for statistical significance. Descriptive statistics of the groups were expressed as mean ± SEM and were compared using Student t test. P value of $\alpha < 0.05$ was taken as statistically significance.

Results

Here, we investigated the neurotoxic effects of Gwagwalada landfill leachates (GLL) exposure on male Wistar rats *in vivo*. We showed that the neurobehavior, body weight gain, absolute brain weight and histomorphology and parvalbumin interneurons were severely altered, with consequent astrogliosis and microgliosis after 21-days of administration of GLL.

Physicochemical and heavy metals characteristics

To ascertain the physicochemical and heavy metals characteristics of the GLL, we performed a physical and chemical analysis of collected raw leachate and our findings are presented in Table 1. Our data showed that ammonia, nitrate, phosphate, sulphate and chloride values obtained from GLL were far above permissible limits. Similarly, the COD and BOD were above permissible limits. Also, the heavy metal concentrations including Fe, V, Pb, Se, Cd, Mn, Zn and Cr in GLL were high above the permissible limit. Only the concentration of Cu was seen to be below the permissible limit in GLL.

Parameter	GLL	USEPA
Ammonia	76.8	0.02
Nitrate	56.3	10
Phosphate	200.6	-
Sulphate	284.2	250
Chloride	2161.7	250
COD	601.6	410
BOD	431.9	-
Fe	61.5	0.3
V	7.5	0.001
Pb	11.9	0.015
Se	6.2	0.05
Cd	4.3	0.05
Mn	18.1	0.05
Zn	20.4	5.0
Cu	0.10	1.3
Cr	3.2	0.1

Source; www.epa.gov/safewater/mcl.html. All values are in mg/L.

Effects of GLL on Neurobehavioral Assessment

To evaluate neurobehavioral deficits associated with GLL exposure, we used three paradigms; forelimb grip, a test of balance and muscular strength (Folarin et al., 2016), open field test of anxiety-like behavior and emotionality (Usende et al., 2016), and water maze test, a test of hippocampal dependent spatial learning as well as memory (Shabani et al., 2012). These batteries of neurobehavioral test detected some impairment of motor and memory functions for the GLL exposed group relative to control. Specifically, a statistically significant reduction ($P < 0.01$; Fig. 1A) in latency to fall using forelimb grip test was recorded in the GLL exposed group compared to control. Upon exposure of the rats to five minutes exploration of the open field arena, behavioral deficits occurred in the GLL exposed group on all parameters taken compared to control. Specifically, a statistically significant deficits on line crossing ($P < 0.0001$; Fig. 1B), rearing ($P < 0.01$, Fig. 1C), centre square entries ($P < 0.05$, Fig. 1D) and grooming ($P < 0.0001$, Fig. 1E) were seen in the leachate exposed group compared to control. However, no significant difference was noticed in faecal drops (Fig. 1F), and urination (Fig. 1G) comparing GLL treated to control group. Concerning the

Morris water test of memory, latency to find hidden platform (indicative of quick learning abilities) was significantly longer in the GLL exposed relative to control ($P < 0.05$, Fig. 1H).

Effects of GLL on Body weight gain and Brain weight

Concerning the body weight gain and brain weight, our data showed a time dependent decreasing body weight gain in GLL treated group when compared to control (Fig. 2A). Also, after 21 days exposure to GLL a significant decrease brain weight was seen in the exposed group relative to control ($P < 0.01$, Fig. 2B).

Effects of GLL on Histomorphology, fast spiking GABAminergic interneurons (parvalbumin neurons) of the cerebellum, Prefrontal (cingulate) and somatosensory cortexes and hippocampus

Upon histological examination of the brains of the rats, we examined the purkinje cell and the granular cell layers, and the white matter tract of the cerebellum and observed that 21 days exposure to GLL was linked with dramatic loss and shrinkage of purkinje cells, and their nucleus, with severe diffused white matter tract vacuolations. There was also severe loss of cells in the granular layer of the cerebellum resulting to reduced thickness of the layer in GLL exposed compare to control. We also noticed severe loss of the dendritic arborization of the purkinje cells in GLL exposed group compare to control (Fig. 3; 4D, 4H).

To be specific, next we explore to see if the affected purkinje cells of the cerebellum are of the fast-spiking GABAminergic interneurons type using immunohistochemistry (Fig. 4). We also explored to know the effects of GLL on these fast-spiking GABAminergic interneurons types in other brain regions. Our data showed destructions (especially of dendritic ramifications) (Fig. 4D, 4H), and reduced population of these interneurons in the purkinje cell layer of the cerebellum in GLL exposed rats. These interneurons in control group were apparently normal with elaborate ramifications (Fig. 4B, 4F). Also, the somatosensory and prefrontal cortexes and hippocampus of GLL treated rat brains had reduced populations of parvalbumin-containing fast-spiking GABAminergic interneurons compared to matched control. We next performed stereological cell counts of these cells population and our data showed a significant reduced cell number of the fast-spiking GABAminergic interneurons in the purkinje layer of the cerebellum (****$P < 0.0001$, Fig. 4K), prefrontal (***$P < 0.001$, Fig. 4I) and somatosensory (**$P < 0.01$, Fig. 4J) cortexes and in the hippocampus (***$P < 0.001$, Fig. 4L) of GLL treated rat brains compared to the untreated group. Also, our data on immunolabelling of dendrites and neuropil of GLL treated rat brains showed a decreased staining compared to those of untreated group. Interestingly, we also showed that the soma area of these parvalbumin-containing fast-spiking GABAminergic interneurons of GLL treated rat brains appeared significantly reduced in the prefrontal (*$P < 0.05$, Fig. 4M) and somatosensory (*$P < 0.05$, Fig. 4N) cortexes, cerebellum (****$P < 0.0001$, Fig. 4O) and hippocampus (****$P < 0.0001$, Fig. 4P) and less stained compared to the untreated group.

Effects of GLL on astrocytes and microglia activation

To investigate a possible relationship between exposure to GLL and neuroinflammation, we analyzed GFAP-labeled astrocytes and IBA1-labeled microglia by immunoperoxidase. Our data showed activation of astrocytes (Fig. 5B) which was characterized by increase in number of these cells (GFAP positive astrocytes) and astrocytic hypertrophy in different brain regions Specifically, we studied the central portion of the cerebellum, prefrontal and somatosensory cortexes and in the hippocampus. To quantify the difference in astrocytes number in the different brain regions in the two groups, we performed a regional stereological cell count. Astrocytic count in the cerebellum showed a significant increased (***$p < 0.001$, Fig. 5E) in the GLL treated group compared to control. The same pattern was seen in the prefrontal (****$p < 0.0001$, Fig. 5C) and somatosensory (***$p < 0.001$, Fig. 5D) cortexes and in the hippocampus (*$p < 0.05$, Fig. 5F) of GLL treated rat compared to control match. We also showed that the area of the GFAP positive cells soma were significantly increased in the prefrontal (****$p < 0.0001$, Fig. 5G) and somatosensory (*$p < 0.05$, Fig. 5H) cortexes, cerebellum (****$p < 0.0001$, Fig. 5I) and in the hippocampus (*$p < 0.05$, Fig. 5J) indicative of hypertrophy of astrocytes in these regions of the brain of GLL treated rat compared to control match

Similarly, and in the same regions of the brains of GLL treated rats was microglial cells activation characterized by increased IBA1 immunostaining and hypertrophy of microglial cell body and processes (Fig. 6B). To quantify, we also performed stereological cell count and our results showed a statistically significant increase in number of IBA1 positive immunostained microglia cells in prefrontal (**$p < 0.01$, Fig. 6C) and somatosensory (****$p < 0.0001$, Fig. 6D) cortexes, cerebellum (**$p < 0.01$, Fig. 6E), and in the hippocampus (****$p < 0.001$, Fig. 6F) of GLL treated rat compared to control match. Similar to our results o

Discussion

Currently, research has posited that uncontrolled recycling industries and sites including landfill leachate, a quick method for disposal of solid waste (Li et al., 2006; Alimba et al., 2015) releases large molecules of hazardous and deleterious chemicals to groundwater through leachate (Farombi et al., 2010). In this current study, we showed elevated levels of heavy metals and physiochemical concentration in Gwagwalada landfill leachate (GLL) and this finding is an indication of these parameters leachability from the waste in the GLL. Similar findings of elevated toxic metals concentration and physiochemical parameters have been reported by Alimba et al., (2015) in Olusosun and Aba-Eku landfill leachates. Of note, the high toxic heavy metals concentration seen in the GLL is capable of inflicting deleterious effects of the biological system especially the nervous system. Specifically, the toxic metals in landfill leachate may possess adverse effects on the morpho-physiology of the nervous system (Wright et al., 2006; Alimba et al., 2015). Herein, we have shown also that exposure to landfill leachate induced reduction of body and brain weight gain and caused neurobehavioral deficits and neuropathological disordered characterized by destruction of neuronal cell population of purkinje cell layer, parvalbumin-containing fast-spiking GABAminergic interneurons, a class of neuron sensitive to oxidative stress (Usende et al., 2022b) and glia activation in Wistar rat model. These lesions perhaps may be due to oxidative stress and neuroinflammation resulting from leachate intoxication (Li et al., 2006; Alimba et al., 2015).

Herein, we showed that Wistar rats exposed to GLL had a significant decreased body weight gain and brain weight. Similar findings have been reported by Alimba et al. (2015) after exposure to Olusosun and Aba-Eku landfill leachates on body weight gain, but not brain weight. They (Alimba et al., 2015) linked the observed reduced body weight to the deleterious effects of landfill leachate on biological system. However, Li et al. (2006) have reported no significant difference on body weight of adult rats exposed to leachate compared to those not exposed. Interestingly, Alimba et al., (2015) reported an increased brain weight after 7 days exposure to Olusosun and Aba-Eku landfill leachates contrary to our present report of reduced brain weight gain. They (Alimba et al., 2015) argued that that alterations in brain weight (brain weight gain) was due to brain damage, notably oedema (Alimba et al., 2015) resulting from heavy metals present in the leachate (Bailey et al., 2004; Lanning et al., 2002; Alimba et al., 2015) majority of which are known neurotoxicants (Jablonska et al., 1994; Lidsky and Scneider, 2003; Ngwa et al., 2014; Usende et al., 2016; 2022b). While this is true for some toxic metals (Bailey et al., 2004; Lanning et al., 2002), others such as vanadium induced reduced brain weight gain (Azeez et al., 2016; Usende et al., 2016). The mechanism of increased or reduced brain weight gain following exposure to landfill leachate therefore remains to be further investigated. The weight of organs, the brain inclusive usually provides a useful risk assessment in experimental studies (Seller *et al.*, 2007).

Our present finding on neurobehavior is the first to evaluate neurobehavioral deficits following exposure to leachate and we explored several neurobehavioral testing. In this study, we explored the hanging wire paradigm to evaluate muscular or grip strength. We report herein a significantly decreased muscular strength due to the intake of GLL. We hypothesized that this could be as a result of the high concentration of heavy metals in leachate (Alimba et al., 2015; Akintunde and Oboh, 2015) as we reported herein. Several reports have also shown directly that exposure to heavy metals including vanadium resulted to reduced muscular strength (Adebiyi et al., 2016; Azeez et al., 2016; Todorich et al., 2011; Usende et al., 2016) possibly due to interfere with motor functions leading to decreased motor activities. This is true due to our present report following the observation of exploratory activities of these rats in open field maze.

In the open field test, when the exploratory neurobehavior and general activities (both qualitative and quantitative), including fear (anxiety) (Folarin et al., 2016) and locomotor activities of the control and GLL rats were evaluated, we showed that rats in the GLL exposed group exhibited a great deal level of hypoactivity in all the parameters measured excepting urinations and fecal bolus number. Moreover, a high performance was seen in grooming activity. Similar report has been recorded by Torres-González et al., (2021), Usende et al., (2016) and Betharia and Maher (2012) following exposure to heavy metals such as vanadium, mercury and lead, thus confirming our hypothesis. When animals are exposed to a novel environment, the stress of the environment encourages them to remain close to the walls of the maze while their innate sense of inquisitiveness pushes them to explore their surroundings (Betharia and Maher, 2012), a neurobehavioral deficit we herein reported.

Furthermore, we conducted a test known as “Morris water test” to check the ability of their remembrance/memory skills as they are supposed to recall their escape route haven learn that in

previous trial exposure. We reported herein, a significant increase latency to find hidden platform in the GLL exposed to compared to control. Also, similar reports have been seen following exposure of mice to heavy metals including mercury and vanadium (Betharia and Maher, 2012; Folarin et al., 2016). Also, reports have shown reduced memory scores in form of impairment in memory function after cadmium exposure in a dose dependent manner (Haider et al., 2015). Furthermore, a significant longer escape latency have been documented in rat exposed to manganese relative to control (Lu et al., 2014). These are all reports on heavy metals exposure individually. This is the first report on memory deficits using Morris water maze after leachate exposure.

We hypothesized that neurobehavioral deficits including the memory and locomotor deficits we herein reported may be due to severe loss of neuron in memory center of the brain (including the hippocampus) and in the cerebellum of the GLL exposed group of rats. To investigate this hypothesis, we process the brain tissue for histopathological examination. First, we stained with H&E stain for routine examination and second, we performed immunohistochemical staining for parvalbumin-containing fast-spiking GABAminergic interneurons. Histopathological examination of tissues is critical and very sensitive end point used to detects specific lesions induced on tissues by xenobiotics (Lanning et al., 2002; Travlos et al., 1996). We report herein that oral exposure of rats to GLL is associated with severe loss and shrinkage of purkinje cells and their nucleus; severe loss of the dendritic arborization of the purkinje cells, with severe diffused vacuolations of the white matter tract; severe loss of cells in the granular layer and reduction in the thickness of the layer. The neurologic lesions seen in the cerebellum of the GLL exposed group suggest neurotoxic damage of this leachate. Also, the severe vacuolation seen in the cerebellum of GLL exposed group is similar to that reported by Usende et al., (2016) following exposure of rats to vanadium. It has been suggested that vacuolations in the brain might be caused by the massive movement of heavy metals across the blood brain barrier (BBB) with consequent accumulation in the brain tissue (Wojcik et al. 2006; Hyman 2011). Specifically, purkinje cells neuronal necrosis observed in the treated rats is associated with disruption of both functional and structural integrities of purkinje cell membrane. Interestingly, we also showed that this damaged purkinje cells are parvalbumin positive cells. Parvalbumin neurons belongs to the class of fast spiking interneurons of GABA cells subpopulation controlling basic output of other principal neurons (Cabungcal et al., 2013; Usende et al., 2022b) and are necessary for fast rhythmic neuronal synchrony facilitating information processing in times of cognitive task (Sohal et al., 2009; Whittington et al., 2011; Cabungcal et al., 2013). These destructions reported herein accounts for the deficits reported in the behavioural test experiment. Of note, the fast-spiking nature of these PV interneurons forces a high metabolic demand with increased mitochondrial density on them, thereby causing this class of neurons to be highly sensitive to oxidative stress (Cabungcal et al., 2013). We herein report for the first time the effects of leachate on this class of interneurons and posit that exposure to leachate severe damage these interneurons. Interestingly, several experimental reports have shown that vanadium and lead, two major metals found to be very high in the GLL used for this experiment are associated with increased oxidative stress levels through the generation of reactive oxygen species (ROS) (Todorich et al., 2011; Azeez et al., 2016; Usende et al., 2016; 2018a, b; 2022b). Corroborating well with the increased oxidative stress and generation of ROS pathway, our current report

concerning the damaging effects of GLL on cerebellar PV immunopositive cells are similar to earlier reports of Grillo et al. (2003), Schiavone et al. (2009), and Hu et al. (2010) on PV cells after exposure to severe environmental stress conditions. It is also possible that pro-oxidant metals such as Cu in the GLL may have reacted with lipid hydroperoxides of the brain cell membrane and this may have elicited significant increase in malondialdehyde (MDA) formation in the GLL exposed rats. Of course, if this pathway is true, then the produced MDA interfered with the integrity of the neuronal membrane (Chattopadhyay et al., 2002; Jomova et al., 2010; Knaapen et al., 2004) leading to the loss of these cells due to either necrosis or apoptosis. Loss and damage of these Purkinje cells may affect impulse/signal conduction (poor signal transmission) from the cerebellum to higher centre that will enhance motor coordination of the body (Gartner and Hiatt, 2001) hence the deficits in our neurobehavioral results. Interestingly, neuro-psychological features were also reported in school-aged children raised near hazardous waste sites (Wright et al., 2006). We also posit herein that exposure to GLL elicited astrogliosis and microgliosis. Astrocytes on one end, are known for their role in neuronal microenvironment regulation, defending the brain against possible and intending toxic and oxidative insults (Sofroniew and Vinters, 2010; Heller and Rusakov, 2015) therefore, their activation herein reported in GLL exposed brains is a clear suggestion of oxidative insults correlating with the damage and loss of PV immunopositive purkinje neuronal populations sensitive to oxidative stress (Usende et al., 2022b). Astrogliosis have previously been reported in lead and vanadium neurotoxicity (Azeez et al., 2016; Usende et al., 2016; 2022b). Microglia on the other end are known mediators of neuroinflammatory processes (Hanisch and Kettenmann, 2007; Azeez et al., 2016; Usende et al., 2022b) and we showed in the present study intense microglia activation in brains of GLL leachate exposed group compared to their control match, confirming neuroinflammation together with the astrogliosis. Studies have posited that microglia activation is neurotoxic (Jha et al., 2016; Azeez et al., 2016; Usende et al., 2022b) and that astrocyte regulates the recruitment of microglia (Skripuletz et al., 2013; Gudi et al., 2014), corroborating our current findings of astrocytes and microglia activation in GLL exposed rat brains.

In conclusion, data from the present study have demonstrated the detrimental effects of Gwagwalada landfill leachate on some brain parameters. We have shown that continuous oral exposure to Gwagwalada landfill leachate exerts severe adverse effects on neurobehavior, body and brain weight gain and damage/loss of purkinje cells as well as parvalbumin-containing fast-spiking GABAminergic interneurons (both its nucleus and dendritic arborization) with astrogliosis and microgliosis which could lead to neuropsychological impairment.

Declarations

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Competing Interests

The authors have no financial or non-financial interests to disclose.

Author Contributions

All authors contributed to study design and conception. Material preparation, data collection and analysis were performed by Usende Ifukibot Levi, Osinachi Chinonyerem Daniella, Azeez Mariam Adedamola, Oyelowo Fatima Oyenike, Adikpe Oluwa Agbonu, Beselia Gela Smart I, Mbagwu and Edem Edem E. The first draft of the manuscript was written by Usende Ifukibot Levi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Funding was acquired by Usende Ifukibot Levi and Connor James.

Ethics approval

Animal experiments received ethical acceptance from University of Abuja Ethics Committee for Animal Use (UAECAU/2021/0012) and according to ethical standard of National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023) and European Communities Council Directive of November 24, 1986 (86/609/EEC)

Data availability

All data of this work will be made available to anyone upon request.

References

1. Adebiyi OE, Olopade JO, Olayemi FO (2016) Neuroprotective Effect of *Grewia carpinifolia* extract against Vanadium induced Behavioural Impairment. Folia Vet 60 (4): 5–13. https://doi.org/10.1515/fv-2016-0031
2. Alimba CG (2013) DNA and systemic damage induced by landfill leachates and health impacts of human exposure to landfills in Lagos and Ibadan, Nigeria. Dissertation, Department of Zoology, University of Ibadan, Nigeria
3. Alimba CG, Bakare AA (2012) Municipal landfill leachates induced alterations in haematological indices and erythrocyte morphology of rats. Int J Chem Environ Eng Syst 3:84–100.
4. Alimba CG, Bakare AA (2016) In vivo micronucleus test in the assessment of cytogenotoxicity of landfill leachates in three animal models from various ecological habitats. Ecotox, 25:310–319. https://doi.org/10.1007/s10646-015-1589-3

5. Alimba CG, Bakare AA, Aina OO (2012) Liver and kidney dysfunction in Wistar rats exposed to municipal landfill leachate. Resour Environ 2:150–63. https://doi:10.5923/j.re.20120204.04
6. Alimba CG, Saliu JK, Ubani-Rex OA (2015) Cytogenotoxicity and histopathological assessment of Lekki Lagoon and Ogun River in *Synodontis clariasi* (*Linnaeus*, 1758). Toxicol Environ Chem 97:221–234. https://doi.org/10.1080/02772248.2015.1041956
7. American Public Health Association (APHA) (1998). Standard methods for the examination of water and wastewater. 20th ed. Washington, DC: American Public Health Association (APHA, AWWA, WPCF).
8. Akintunde JK, Oboh G (2015) Subchronic exposure to leachate activates key markers linked with neurological disorder in Wistar male rat. Environ Sci Pollut Res 22:18541–18553. https://doi.org/10.1007/s11356-015-5327-4
9. Azeez IA, Olopade F, Laperchia C, Andrioli A, Scambi I, Onwuka SK, Bentivoglio M, Olopade JO (2016) Regional myelin and axon damage and neuroinflammation in the mouse brain after long-term postnatal vanadium exposure. J Neuropathol Exp Neurol 75, 843–854. https://doi.org/10.1093/jnen/nlw058.
10. Bailey SA, Zidell RH, Perry RW (2004) Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? Toxicol Pathol 32:448–66. https://doi.org/10.1080/01926230490465874
11. Betharia S, Maher TJ (2012) Neurobehavioral effects of lead and manganese individually and in combination in developmentally exposed rats. NeuroTox 33: 1117–1127. https://doi.org/10.1016/j.neuro.2012.06.002
12. Brown RE, Corey SC, Moore AK (1999) Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. Behav Genet 29: 263–271. https://doi:10.1023/A:1021694307672
13. Cabungcal J, Steullet P, Morishita H, Kraftsik R, Cuenod M, Hensch TK, Do KQ (2013) Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proc. Natl. Acad. Sci. U. S. A.* 110 (22): 9130 – 1935 https://doi.org/10.1073/pnas.1300454110.
14. Chattopadhyay S, Bhaumik S, Nag CA, Das GS (2002) Arsenic induced changes in growth development and apoptosis in neonatal and adult brain cells in vivo and in tissue culture. Toxicol Lett 128:73–84. https://doi.org/10.1016/S0378-4274(01)00535-5
15. Efuntoye MO, Bakare AA, Sowunmi AA (2011) Virulence factors and antibiotic resistance in Staphylococcus aureus and Clostridium perfrigens from landfill leachate. Afr J Microbiol Res 5:3994–3997
16. Eggen T, Moeder M, Arukwe A (2010) Municipal landfill leachates: a significant source for new and emerging pollutants. Sci Total Environ 408:5147–5157. https://doi.org/10.1016/j.scitotenv.2010.07.049
17. Farombi EO, Adedara IA, Ebokaiwe AP, Teberen R, Ehwerhemuepha T (2010) Nigerian Bonny light crude oil disrupts antioxidant systems in testes and sperm of rats. Arch Environ Contam Toxicol

59.1: 166–174. https://doi.org/10.1007/s00244-009-9443-3

18. Folarin O, Olopade F, Onwuka S, Olopade J (2016) Memory deficit recovery after chronic vanadium exposure in mice. *Oxid Med Cell Longevity* 2016, 1–7. https://doi.org/10.1155/2016/4860582
19. Gartner LP, Hiatt JL (2001) Colour textbook of histology. 2nd ed. New York Saunders.
20. Gaykema RP, Goehler LE (2009) Lipopolysaccharide challengeinduced suppression of Fos in hypothalamic orexin neurons: their potential role in sickness behavior. Brain Behav. Immun 23:926–930. https://doi.org/10.1016/j.bbi.2009.03.005.
21. Gerashchenko D, Kohls MD, Greco MA, Waleh NS, Salin-Pascual R, Kilduff TS, Lappi DA, Shiromani PJ (2001) Hypocretin-2-saporin lesions of the lateral hypothalamus produce narcoleptic-like sleep behavior in the rat. J. Neurosci 27:7273–7283. https://doi.org/10.1523/JNEUROSCI.21-18-07273.2001.
22. Grillo CA, Piroli GG, Rosell DR, Hoskin EK, Mcewen BS, Reagan LP (2003) Region specific increases in oxidative stress and superoxide dismutase in the hippocampus of diabetic rats subjected to stress. Neurosci 121 (1): 133–140. https://doi.org/10.1016/S0306-4522(03)00343-9.
23. Gudi V, Gingele S, Skripuletz T, Stangel M (2014) Glial response during cuprizoneinduced de- and remyelination in the CNS: lessons learned. Front Cell Neurosci 8, 73. https://doi.org/10.3389/fncel.2014.00073.
24. Haider S, Anis L, Batool Z, Sajid I, Naqvi F, Khaliq S, Ahmed S (2015) Short term cadmium administration dose dependently elicits immediate biochemical, neurochemical and neurobehavioral dysfunction in male rats. Metab Brain Dis 30:83–92. https://doi.org/10.1007/s11011-014-9578-4
25. Hanisch UK, Kettenmann H, (2007) Microglia: Active sensor and versatile effector cells in the normal and pathologic brain. Nat. Neurosci. 10: 1387–1394. https://doi.org/10.1038/nn1997.
26. Heller JP, Rusakov DA (2015) Morphological plasticity of astroglia: understanding synaptic microenvironment. Glia 63:2133–2151. https://doi.org/10.1002/glia.22821.
27. Hu W, Zhang M, Czeh B, Flügge G, Zhang W (2010) Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. Neuropsychopharmacol 35 (8): 1693 1707. https://doi.org/10.1038/npp.2010.31.
28. Hyman MS (2011) Neurodegeneration with brain iron accumulation- clinical syndromes and neuroimaging. Biochim Biophys Acta 1822(3):350–360
29. Ishaya S, Abaje IB (2009) Assessment of bore wells water quality in Gwagwalada Town of FCT. J Ecol Nat Environ 1(2):032–036
30. Jablonska L, Walski M, Rafalowska U (1994) Lead as an inductor of some morphological and functional changes in synaptosomes from rat brain. Cell Mol. Neurobiol. 14:701–709. https://doi.org/10.1007/BF02088678.
31. Jayaraman T, Kannappan S, Rivichandran MK, Anuradha CV (2008) Impact of Essentiale L on ethanol-induced changes in rat brain and erythrocytes. Singapore Med J 49:320–7. PMID: 18418525

32. Jha MK, Lee WH, Suk K (2016) Functional polarization of neuroglia: Implications in neuroinflammation and neurological disorders. Biochem Pharm 103:1–16. https://doi.org/10.1016/j.bcp.2015.11.003.
33. Johnson BL (1999) Impact of hazardous waste on human health. New York: CRC press Inc.
34. Jomova K, Vondrakova D, Lawson M, Valko M (2010) Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem 345:91–104. https://doi.org/10.1007/s11010-010-0563-x
35. Knaapen AM, Borm PJA, Albrecht C, Schins RPF (2004) Inhaled particles and lung cancer, part A: mechanisms. Int J Cancer 109:799–809. https://doi.org/10.1002/ijc.11708
36. Lanning LL, Creasy DM, Chapin RE, Mann PC, Barlow NJ, Regan KS, et al. (2002) Recommended approaches for the evaluation of testicular and epididymal toxicity. Toxicol Pathol 30:507–520. https://doi.org/10.1080/01926230290105695
37. Li G, Sang N, Wang Q (2006) Oxidative damage induced in brains and livers of mice by landfill leachate. Ecotoxicol Environ Saf 65:134–9. https://doi.org/10.1016/j.ecoenv.2005.06.011
38. Li H, Han M, Hou L, Li G, Sang N (2010) Landfill leachate ingestion induces protein oxidation and DNA–protein crosslinks in mouse viscera. J Hazard Mater 174:54–58. https://doi.org/10.1016/j.jhazmat.2009.09.015
39. Lidsky TI, Schneider JS (2003) Lead neurotoxicity in children: basic mechanisms and clinical correlates. Brain 126:5–19. https://doi.org/10.1093/brain/awg014.
40. Lu CL, Tang S, Meng ZJ, He YY, Song LY, Liu YP, Ma N, Li XY Guo SC (2014) Taurine improves the spatial learning and memory ability impaired by sub-chronic manganese exposure. J Biomed Sci 21.1: 51. https://doi.org/10.1186/1423-0127-21-51
41. Neal AP, Guilarte TR (2012) Mechanisms of heavy metal neurotoxicity: lead and manganese. J Drug Metab Toxicol S5:1–13. https://doi:10.4172/2157-7609.S5-002
42. Ngwa HA, Kanthasamy A, Jin H, Anantharam V, Kanthasamy AG (2014) Vanadium exposure induces olfactory dysfunction in an animal model of metal neurotoxicity. Neurotox 43:73–81. https://doi.org/10.1016/j.neuro.2013.12.004.
43. Oyelowo F, Usende IL, Abiyere E, Adikpe A Ghaji A (2017) Comparative Gross Morphology and morphometric investigations on the alimentary tract of three age groups of barn owl (Tyto alba) found in North-Central Nigeria. Inter J Vet Sci, 6(1): 7–12.
44. Oygard JK, Gjengedal E (2009) Uranium in municipal solid waste landfill leachate. Int J Environ Res 3:61–8.
45. Shabani M, Nazeri M, Parsania S et al., (2012) Walnut consumption protects rats against cisplatin-induced neurotoxicity. Neurotox 33(5): 1314–1321. https://doi.org/10.1016/j.neuro.2012.08.004
46. Schaumburg HH, Spencer PS, Joseph C, Arezzo JC (1983) Monitoring potential neurotoxic effects of hazardous waste disposal. Environ Health Perspect 48:61–64. https://doi.org/10.1289/ehp.834861
47. Schiavone, S., Sorce, S., Dubois-Dauphin, M., Jaquet, V., Colaianna, M., Zotti, M., Cuomo, V., Trabace, L., Krause, K., (2009). Involvement of NOX2 in the development of behavioral and pathologic

alterations in isolated rats. Biol. Psychiatry 66 (4): 384–392. https://doi.org/10.1016/j.biopsych.2009.04.033

48. Sellers RS, Mortan D, Michael B, Roome N, Johnson JK, Yano BL, Perry R, Schafer K (2007) Society of Toxicologic Pathology Position Paper: Organ Weight Recommendations for Toxicology Studies. Toxicol Path. 35(5):751–755. doi:10.1080/01926230701595300
49. Skripuletz T, Hackstette D, Bauer K, Gudi V, Pul R, Voss E, Berger K, Kipp M, Baumg¨artner W, Stangel M, (2013) Astrocytes regulate myelin clearance through recruitment of microglia during cuprizone-induced demyelination. Brain 136:147–167. https://doi.org/10.1093/brain/aws262.
50. Slack RJ, Gronow JR, Voulvoulis N (2005) Household hazardous waste in municipal landfills: contaminants in leachate. Sci Total Environ 337:119–137.
51. Sofroniew MV, Vinters HV, (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7–35. https://doi.org/10.1007/s00401-009-0619-8
52. Sohal VS, Zhang F, Yizhar O, Deisseroth K (2009) Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 459:698–702. https://doi.org/10.1038/nature07991
53. Todorich B, Olopade JO, Surguladze N, Zhang X, Neely E, Connor JR (2011) The mechanism of vanadium-mediated developmental hypomyelination is related to destruction of oligodendrocyte progenitors through a relationship with ferritin and iron. Neurotox. Res 19:361–373. https://doi.org/10.1007/s12640-010-9167-1.
54. Torres-González OR, Sánchez-Hernández IM., Flores-Soto ME, Chaparro-Huerta V, Soria-Fregozo C, Hernández-García L, Padilla-Camberos E, Flores-Fernández JM (2021) Landfill Leachate from an Urban Solid Waste Storage System Produces Genotoxicity and Cytotoxicity in Pre-Adolescent and Young Adults Rats. Int. J. Environ. Res. Public Health 18: 11029. https://doi.org/10.3390/ijerph182111029
55. Travlos GS, Morris RW, Elwell MR, Duke A, Rosenblum S, Thompson MB (1996). Frequency and relationships of clinical chemistry and liver and kidney histopathology findings in 13- week toxicity studies in rats. Toxicology, 107:17–29.
56. United States Environmental Protection Agency (USEPA) (2006). Drinking water contaminants.Washington, DC, USA, 2006. Available from: <http://water.epa.gov/drink/contaminants/index.cfm#List> Accessed 9 July 2021
57. Usende IL, Okafor CL, Adaka N, Onyiche ET, Nwaogu IC, Ezeasor DN (2013) Gross and Histomorphometric changes in the small intestine of Anak and Marshal broiler hybrids. Indian J. Vet. Anat 25 (2):76–78.
58. Usende IL, Leitner DF, Neely E, Connor JR, Olopade JO (2016) The deterioration seen in myelin related morpho-physiology in vanadium exposed rats is partially protected by concurrent iron deficiency. Niger. J. Physiol. Sci 31:11–22.
59. Usende I.L, Emikpe BO, Olopade JO (2017) Heavy metal pollutants in selected organs of African giant rats from three agro-ecological zones of Nigeria: evidence for their role as an environmental

specimen bank. Environ. Sci. Pollut. Res 24:22570–22578. https://doi.org/10.1007/s11356-017-9904-6.

60. Usende IL, Alimba CG, Emikpe BO, Bakare AA, Olopade JO (2018a) Intraperitoneal sodium metavanadate exposure induced severe clinicopathological alterations, hepato-renal toxicity and cytogenotoxicity in African giant rats (Cricetomys gambianus, Waterhouse, 1840). Environ. Sci. Pollut. Res 25:26383–26393. https://doi.org/10.1007/s11356-018-2588-8.
61. Usende IL, Olopade JO, Emikpe BO, Oyagbemi AA, Adedapo AA (2018b) Oxidative stress changes observed in selected organs of African giant rats (Cricetomys gambianus) exposed to sodium metavanadate. Inter J. Vet. Sci. Med 6:80–89. https://doi.org/10.1016/j.ijvsm.2018.03.004.
62. Usende IL, Olopade JO, Emikpe BO, Allam AMN (2020) Biochemical and ultrastructural changes in kidney and liver of African Giant Rat (Cricetomys gambianus, Waterhouse, 1840) exposed to Intraperitoneal sodium metavanadate (vanadium) intoxication. Environ. Toxicol. Pharm 79:103414 https://doi.org/10.1016/j.etap.2020.103414.
63. Usende IL, Oyelowo FO, Adikpe AO, Emikpe BO, Nafady AHM, Olopade JO (2022a) Reproductive hormones imbalance, germ cell apoptosis, abnormal sperm morphophenotypes and ultrastructural changes in testis of African giant rats (Cricetomys gambianus) exposed to sodium metavanadate intoxication. Environ. Sci. Pollut. Res 29: 42849–42861. https://doi.org/10.1007/s11356-021-18246-z
64. Usende IL, Olopade JO, Azeez IA, Andrioli A, Bankole MO, Olopade FE, Nafady AA, Bentivoglio M (2022b) Neuroecotoxicology: Effects of environmental heavy metal exposure on the brain of African giant rats and the contribution of vanadium to the neuropathology. IBRO Neurosci Reports 13: 215–234. https://doi.org/10.1016/j.ibneur.2022.08.008
65. West MJ (1993) New stereological methods for counting neurons. Neurobiol. Aging 14:275–285. https://doi.org/10.1016/0197-4580(93)90112-O.
66. Whittington MA, Cunningham MO, LeBeau FE, Racca C, Traub RD (2011) Multiple origins of the cortical γ rhythm. Dev. Neurobiol. 71 (1):92–106. https://doi.org/10.1002/dneu.20814.
67. Wojcik D, Godfrey ME, Christie D, Haley BE (2006) Mercury toxicity presenting as chronic fatigue, memory impairment and depression: diagnosis, treatment, susceptibility and outcomes in a New Zealand general practice setting (1994–2006). Neuro Endocrinol Lett 27(4): 415–423
68. Wright RO, Amarasiriwardena C,Woolf A, Jim R, Bellinger DC (2006) Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. Neurotox 27:210–216. https://doi.org/10.1016/j.neuro.2005.10.001

Figures

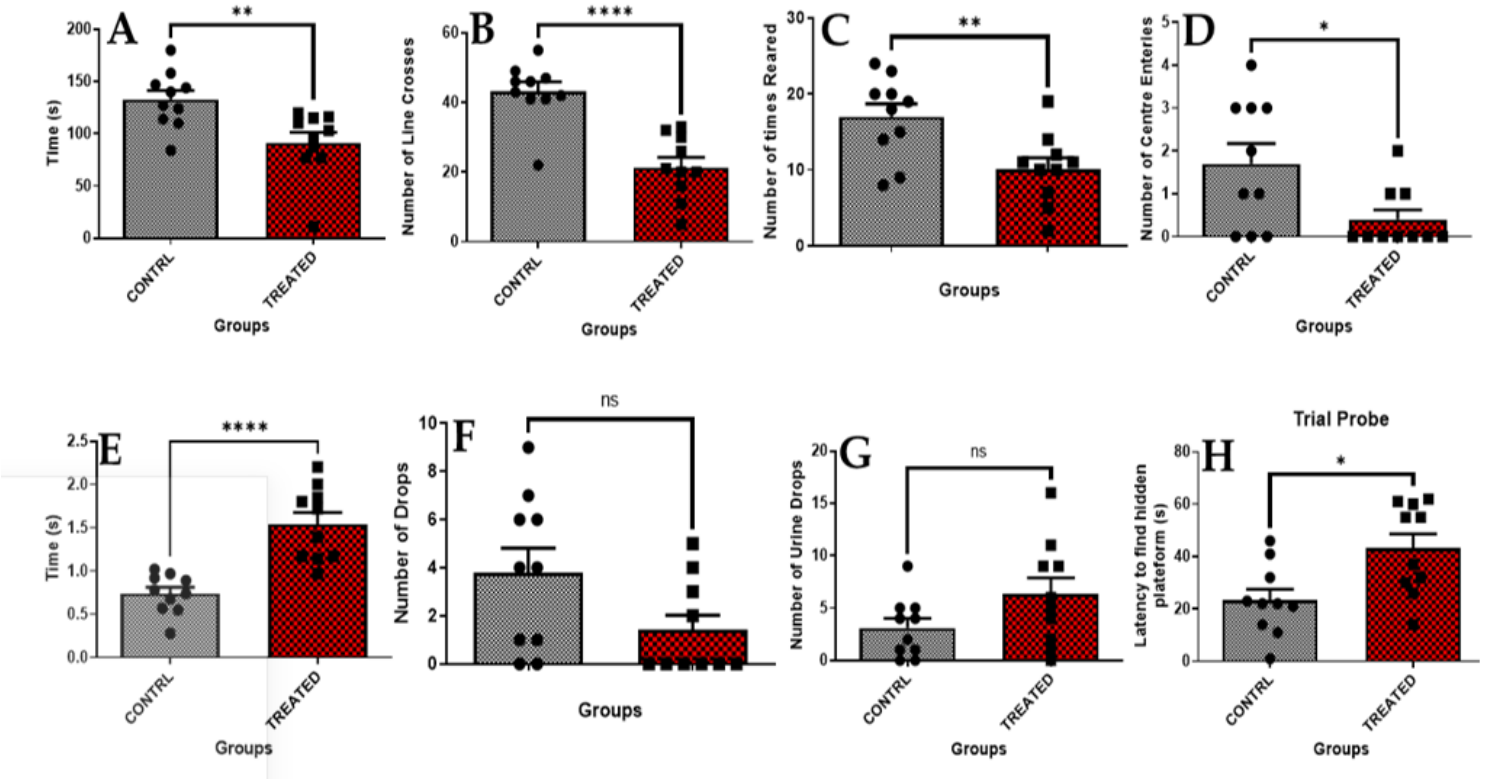

Figure 1

Bar graphs of the results of behavioral tests in Gwagwalada landfill leachate (GLL) treated rats and matched controls for the hanging wire test (A), open field test (B–G) and Morris water maze test (H) after 21-days of exposure. The hanging wire test showed a statistically significant reduction in latency. Note the significant alterations in line crosses (B) number of times reared (C), center square enteries (D), and grooming time (E) after GLL exposure. The Morris water maze test (H) showed a statistically significant increased latency to find hidden platform. Values are expressed as means±SD and were analyzed using the Student t-test. * $p<0.05$, ** $p<0.01$, **** $p<0.0001$ (Student t-test).

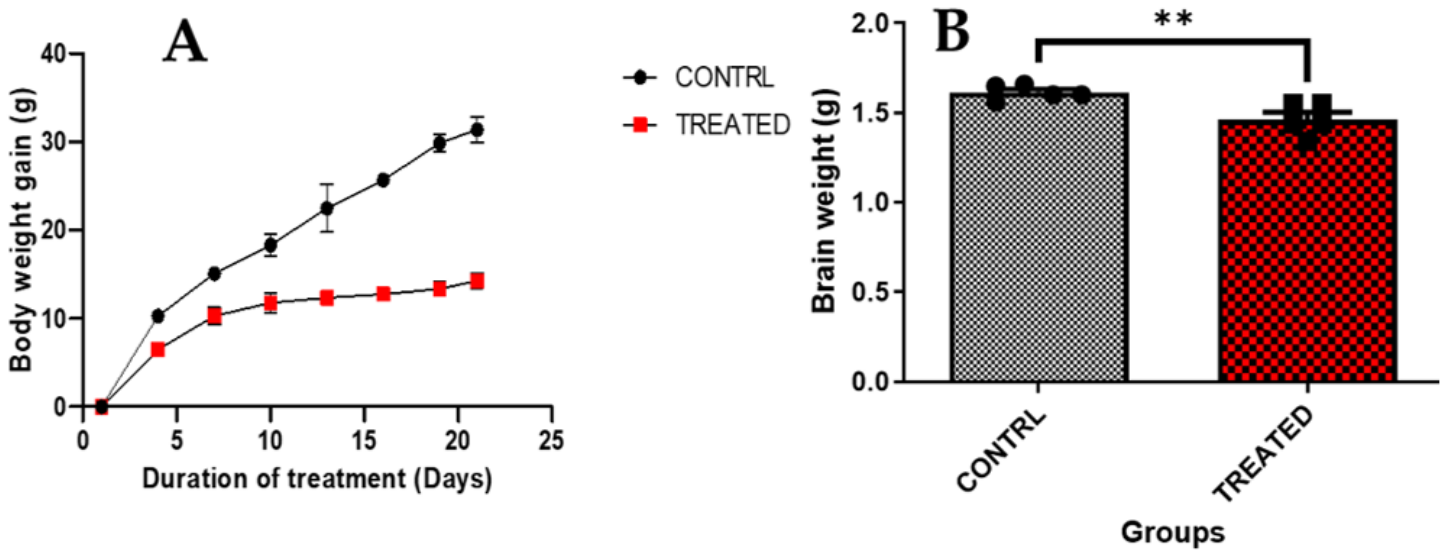

Figure 2

Body weight gain (A) and brain weight (B). (A) Exposure to Gwagwalada landfill leachate leads to reduced body and brain weight gain compared to matched controls. Values are expressed as means±SD and were analyzed using the Student t-test.

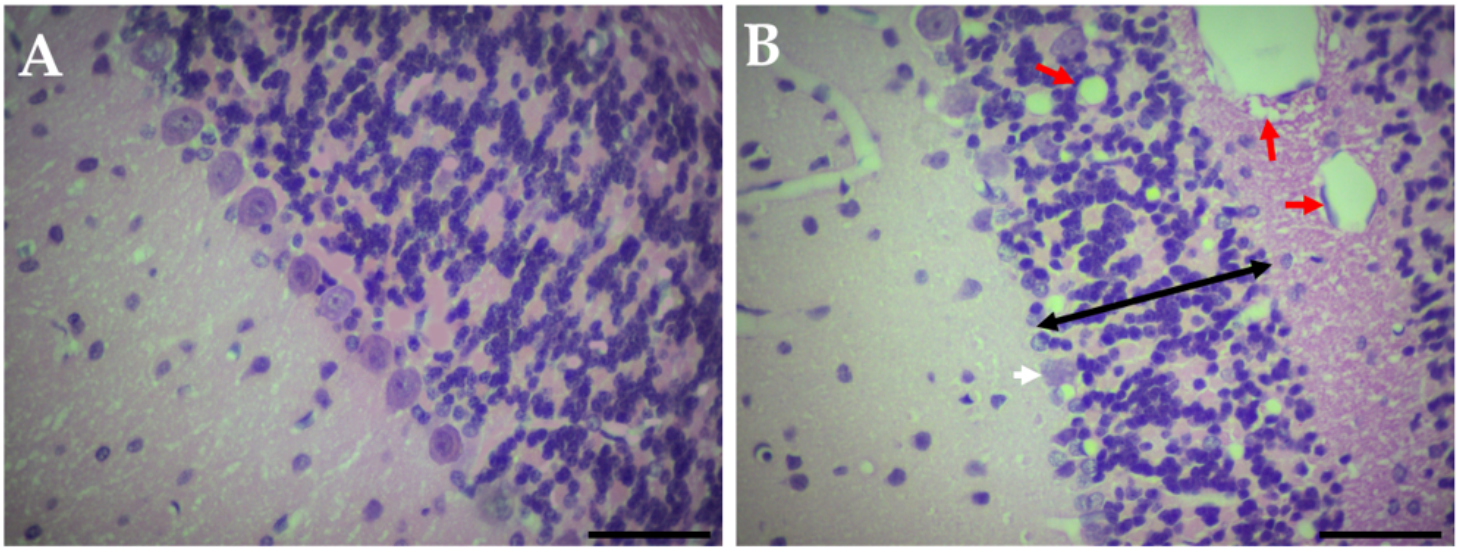

Figure 3

Photomicrograph of control (A) and Gwagwalada landfill leachate (GLL) treated rat groups. In the control group (A), the purkinje neuronal population appeared normal and evenly distributed. The granular cell layer also has numerous cell population. In the GLL treated group (B), there was dramatic loss and shrinkage of purkinje cells, and their nucleus (white arrow), with severe diffused white matter tract vacuolations (reed arrows). There was also severe loss of cells in the granular layer of the cerebellum resulting to reduced thickness of the layer (black two arrow heads).

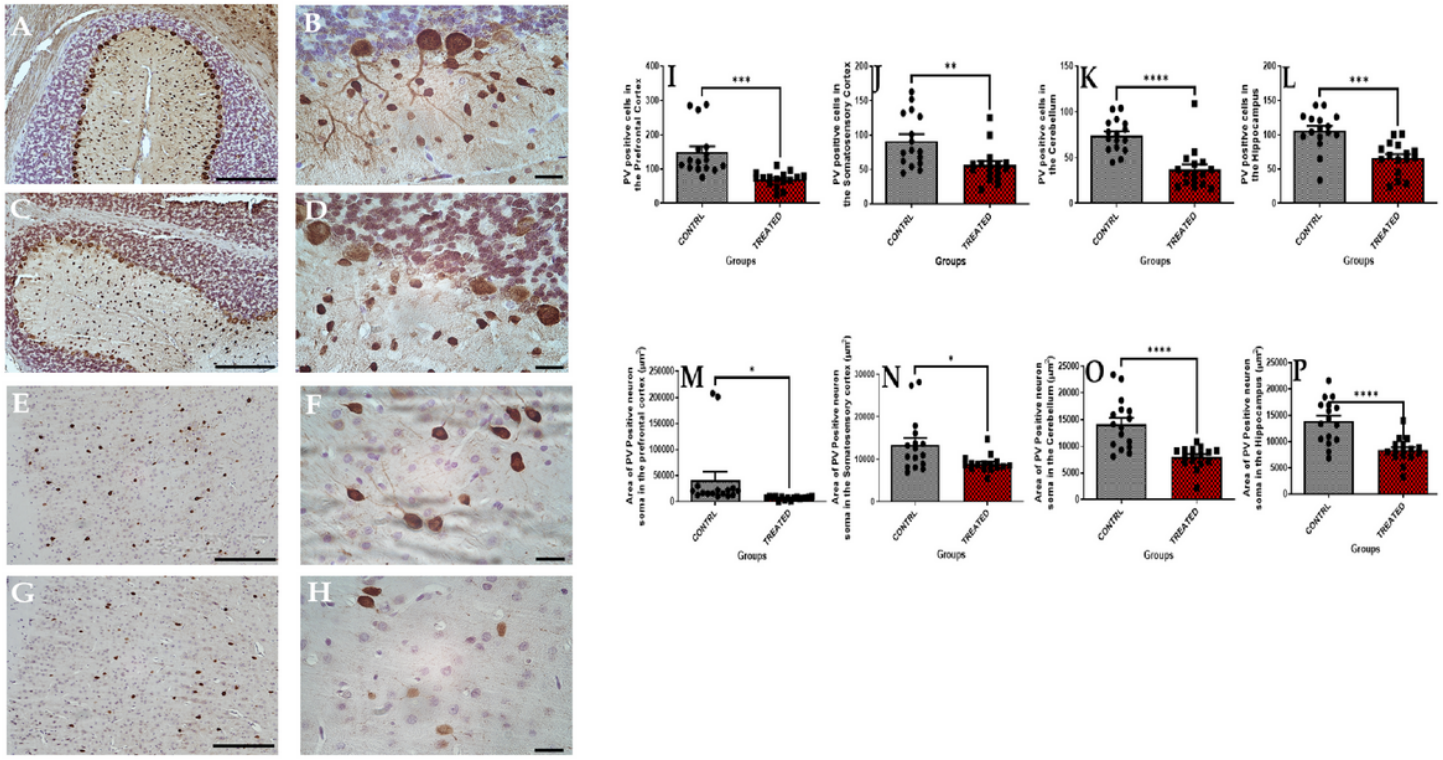

Figure 4

Images of immunoreactivity of fast-spiking inhibitory parvalbumin (PV) interneurons in the cerebellum (A-D) and Somatosensory cortex (E-H) of control (A, B and E, F respectively) compared to Gwagwalada landfill leachate (GLL) treated (C, D and G, H respectively) group. In the control group (A, B and E, F), the purkinje PV+ neuronal population appeared normal and evenly distributed with fine dendritic arborization. In the GLL treated group (C, D and G, H), there was loss and shrinkage of purkinje cells soma with severe loss of dendritic arborization. Scale bar: 50μm in A, C, E and G and 20μm in B, D, F and H.

Bar graphs (I-P) of results of stereological cell count (I-L) and area of soma (M-P) of fast-spiking inhibitory parvalbumin (PV) interneurons in the prefrontal (I and M respectively) and somatosensory (J and N respectively) cortexes, cerebellum (K and O respectively) and hippocampus (L and P respectively). There was significant loss of PV positive neurons and reduced area of soma in the GLL treated group compared to control. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ (Student t-test)

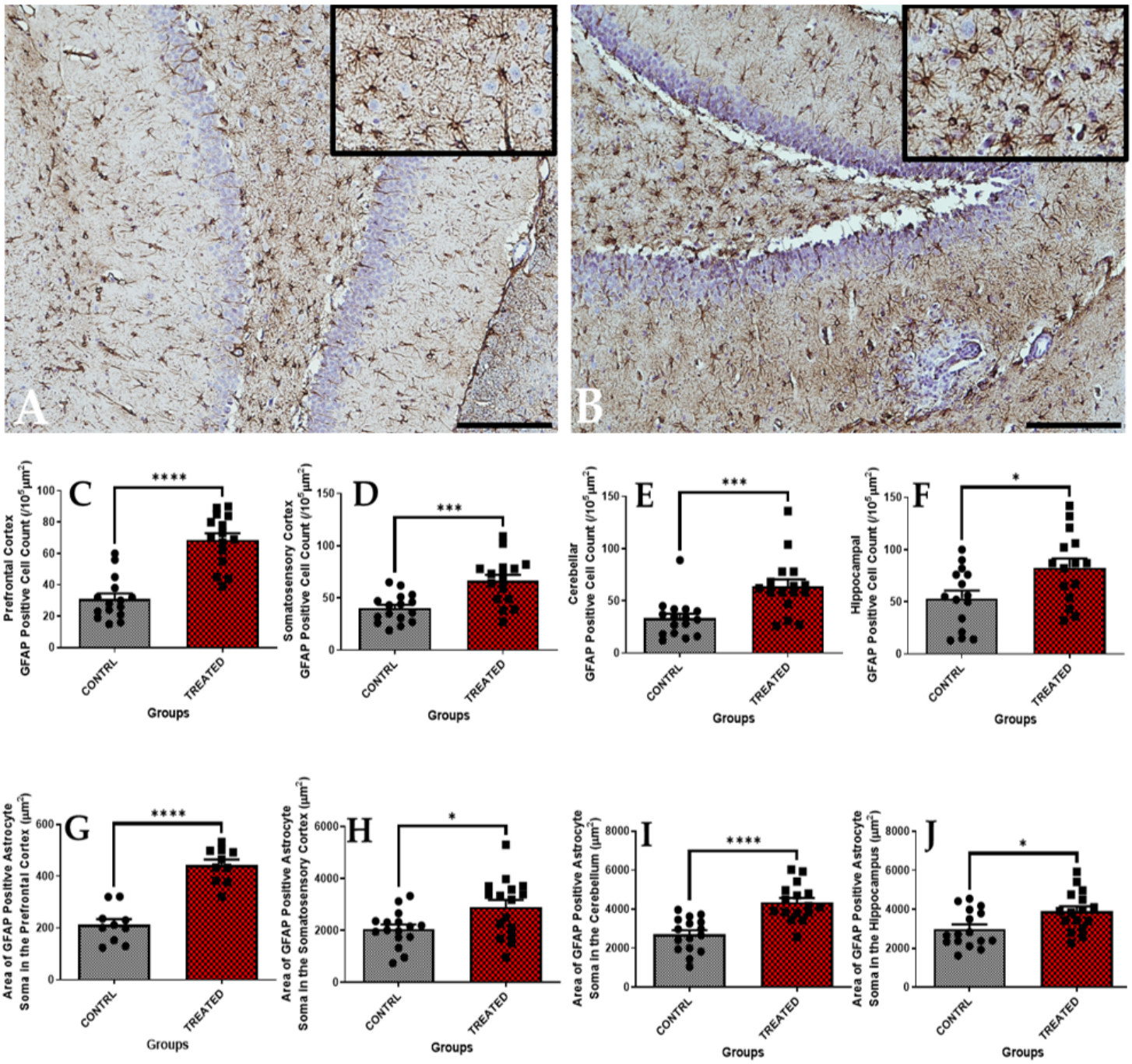

Figure 5

Images of GFAP-immuno-stained dentate gyrus of control (A) compared to Gwagwalada landfill leachate (GLL) treated (C) rat brains. Exposure to GLL resulted to astrocytic activation characterized by more extended cytoplasmic processes, increased cell number and thickened cell body (soma). Scale bar: 50µm

Bar graphs (C-J) of results of stereological cell count (C-F) and area of soma (G-J) of GFAP+ astrocytes in the prefrontal (C and G respectively) and somatosensory (D and H respectively) cortexes, cerebellum (E and I respectively) and hippocampus (F and J respectively). There was significant increased number of

cells and area of soma in the GLL treated group compared to control. * p<0.05, ** p<0.01, **** p<0.0001 (Student t-test)

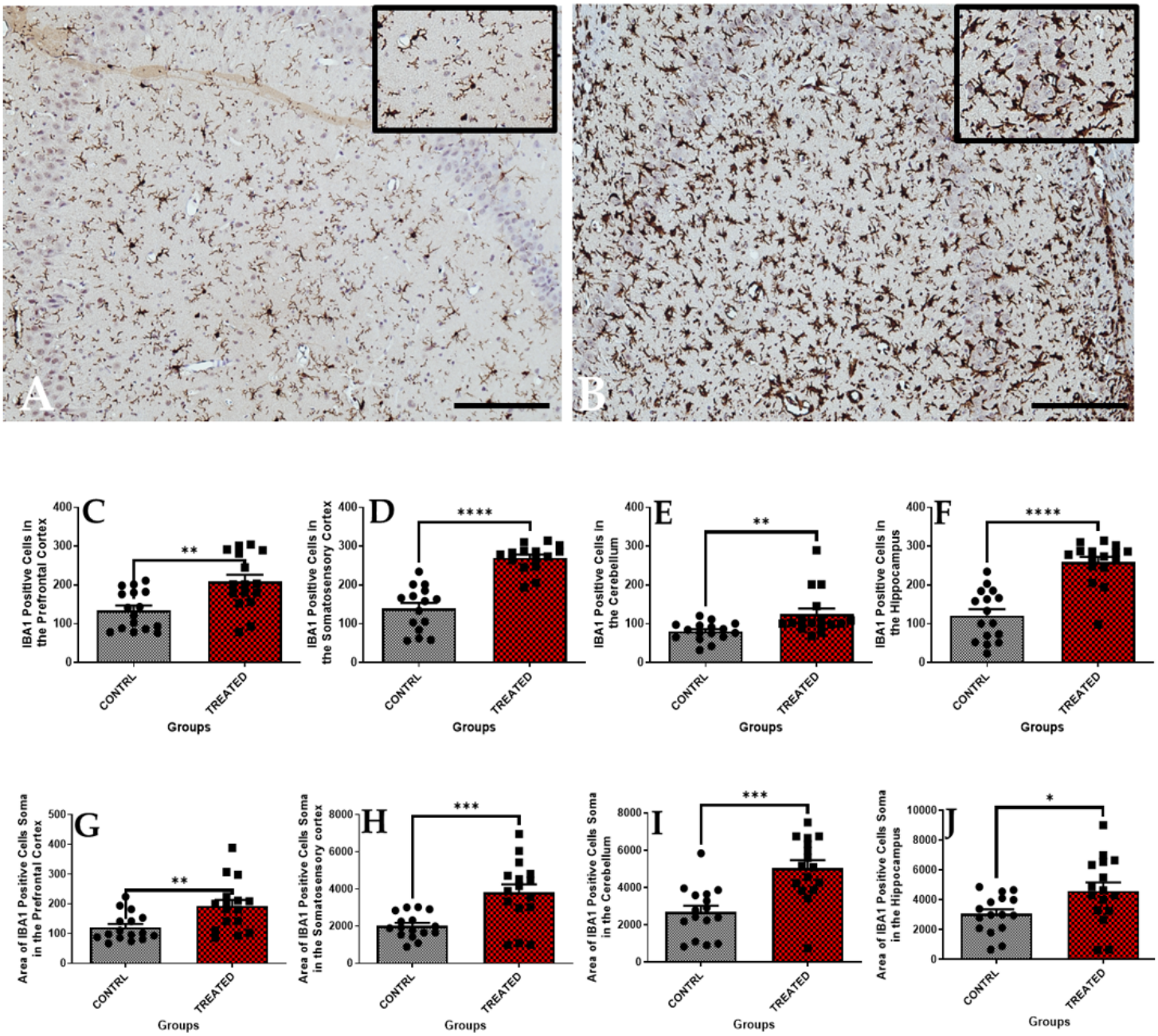

Figure 6

Images of IBA-1-immuno-stained hippocampus of control (A) compared to Gwagwalada landfill leachate (GLL) treated (C) rat brains. Exposure to GLL resulted to microglial activation characterized by enlarged cell body (soma) with short and thickened processes with increase in cell number. Scale bar: 50μm

Bar graphs (C-J) of results of stereological cell count (C-F) and area of soma (G-J) of IBA-1+ microglia in the prefrontal (C and G respectively) and somatosensory (D and H respectively) cortexes, cerebellum (E and I respectively) and hippocampus (F and J respectively). There was significant increased number of cells and area of soma in the GLL treated group compared to control. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ (Student t-test)