Original Research Article

Effect of smoke from wood harvested from crude oil polluted site on liver enzymes and lungs histopathology

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The study evaluated the influence of air polluted by smoke of crude oil contaminated firewood on the liver enzymes and lungs histology. Seven groups of adult female albino wistar rats (five per group) were used. Group one was exposed to ambient air, groups two to four were exposed to smoke generated from non crude oil imparted wood for a period of 10, 30 and 60 minutes respectively while groups five to seven were exposed to smoke generated from the test wood for a period of 10, 30 and 60 minutes respectively. The exposure lasted for 28 days. Polycyclic aromatic hydrocarbon (PAH) and heavy metals (mercury, lead, arsenic, nickel, zinc and cadmium) were determined in Rhizophora racemosa stem harvested from crude oil polluted environment. Carbon monoxide (CO), Oxides of Nitrogen (NO\textsubscript{x}), Oxides of Sulfur (SO\textsubscript{x}), Hydrogen sulfide (H\textsubscript{2}S), Methane (CH\textsubscript{4}), Volatile Organic Compound (VOCs), Ammonia (NH\textsubscript{3}) and Particulate Matter (PM) (1, 2.5, 10) were determined in the smoke generated. Alanine transaminase (AST), alkaline phosphatase (ALP), aspartate transaminase (AST) and histopathology of the lungs were evaluated in wistar rat exposed to smoke from this wood. The total PAH level of the test wood was 8.85 mg/kg. The heavy metal concentration was mercury (0.001mg/kg), arsenic (0.274mg/kg), zinc (52.182mg/kg), lead (69.923mg/kg), cadmium (0.596mg/kg), nickel (14.548 mg/kg). The value of air pollutants in this smoke wood were; CO (112.3ppm), SO\textsubscript{2} (0.61ppm), VOC (3.5ppm), NH\textsubscript{3} (2.63ppm), PM\textsubscript{1} (0.11ppm), PM\textsubscript{2.5} (0.16ppm), PM\textsubscript{10} (0.27ppm). The liver enzyme AST was significantly increased (p≤ 0.05) in groups 6 and 7 while ALP showed significant increase (p≤ 0.05) in group 4 and 7. These results reveals that smoke of firewood from an oil impacted environment have the potential to elicit a more sever toxic effects on the liver and lungs of experimental animals.

Key word: Air pollutant, Liver enzyme, histopathology.

INTRODUCTION

Environmental pollution has been a problem to the world since the invention of technology. It is the introduction of pollutants to the environment to an extent that the physical and biological components are negatively affected. Twenty percent of the world's disease conditions are caused by environmental pollution. Most at risk are the 1.3 billion people living in the developing countries of sub Saharan Africa and south East Asia (Pimentel et al., 1998). Despite the efforts made over the years to remediate the environment of pollution, it still remains a problem. This is because the rate of unregulated industrial emission, poor waste management and biomass burning are increasingly practiced in most countries (Sexton et al., 2005; Bernard et al., 2001). Wood burning is also a major contributor to air pollution and this has more negative effect because of the fact that most people believe that since wood smoke is a natural substance, it has to be devoid of harmful substance. But it has been established that wood smoke from firewood
stove, agricultural fires etc emit considerable quantities of known health harmful pollutants, including several carcinogenic compounds like CO, NO₂, benzene, polycyclic aromatic hydrocarbon (PAHs), and particulate matter (Rani et al., 2011). In African culture as well as other cultures of the world women are saddled with the responsibility of cooking for the family, this exposes them more to the dangers that come with smoke inhalation.

Firewood is the main source of fuel for rural dwellers which they use in cooking, catching warmth during cold seasons, preservation of fresh foods etc. firewood smoke like other smoke is produced as a result of an incomplete combustion (Lee, 2005). Many factors affect the composition of smoke. First the nature of the biomass being burnt followed by the combustion temperature and condition. For example, small amount of smoke are produced when biomass is burnt in high temperature and oxygen. Biomass which contain halogen such as chlorine may lead to produce hydrogenchloride, chloromethane, bromomethane and other halocarbon (Fardell, 1998). When it contains metals, then the smoke is likely to contain metal oxide or inorganic salt, example ammonium sulfate, ammonium nitrate, or sodium chloride (Smith et al., 2012).

**Danger of Wood Smoke to the Lungs**

Wood smoke contains tiny particles and gases that can cause serious health challenge when inhaled (Barn et al., 2008). Wood smoke is a mixture of solids, gases, and liquids. Much like cigarette smoke, wood smoke produces hundreds of air pollutants that can cause cancer and other health problems (Gustafson et al., 2007). The highly potent among these pollutants which is of great concern is the fine particles which are tiny solids and liquids that results from partial burning of wood. When air with wood smoke is inhaled, the fine particles travel deep into the lungs. The particles contain toxic substances that can remain in the lungs for months, causing changes that lead to diseases and structural damage (Gustafson et al., 2007). These particles are so small that they get past the respiratory tract's defenses and reach the deepest areas of the lungs alveoli and the blood (Molnár et al., 2005). These fine particles even at low concentration may harm the human system and studies have shown that firewood burning increases the concentration of these fine particles in the environment (Chuang et al., 2007)

**Toxic Chemicals in Wood Smoke**

People who do not indulge in cigarette smoking are faced with the same risk when they are exposed to wood smoke. This is because a number of toxic chemical present in cigarette smoke are also found in wood smoke. Examples are particulate matter, benze, benzo(a)pyrene, dibenz(a,h) anthracene etc. Environmental Protection Agency (EPA) estimates that wood smoke produces more particulate matter which poses 12 times more cancer risk than that of cigarette smoke. At Louisiana State University an experimental study showed that the free radicals produced by wood smoke are more chemically active than those from cigarette. So when they are inhaled, they will harm the body for a longer duration. Secondly, it has been established that more pollutants are generated from firewood smoke due to the proportion of wood burnt per time. It has been estimated that for a simple fire place that burnt for an hour are capable of releasing 4,330 times more carcinogenic substance than 30 sticks of cigarette.

The liver is the largest organ/gland in the body that helps keep the body safe from harmful or toxic substance (Maton et al., 1993). The primary role of the liver includes the detoxification of foreign compounds (Qatanani and Moore, 2005). It also detoxifies toxic substances produced by the body. The principal behind the detoxification by the liver is due to its ability to convert lipid soluble toxins that are difficult to excrete to water soluble substance which are easy to excrete (Gandhi and Mancera 2008). The liver is able to do this due to the presence of some enzyme. For a complete detoxification/excretion of a toxicant it must pass through three phases. The first phase is to convert a nonpolar toxin to a polar substance. This is made possible through oxidation, reduction and hydrolysis reactions (Gamage et al., 2005). Cytochrome P450 enzymes play key role in this phase. The second phase is where the metabolite from phase one is made even more polar by the addition of an indigenous metabolite to the functional groups introduced in phase 1. The third phase is called efflux or excretion. In this phase the water soluble phase 2 product is excreted out of the body through sweat, bowels, urine and through exhale, saliva or hair thereby completing the detoxification process.

There is dearth of literature on the effect of smoke from crude oil polluted wood as it affects the liver and lungs; hence this study is designed to investigate the effect of smoke from crude oil polluted wood on the liver enzymes and lungs histology in Wistar rats.

**MATERIALS AND METHODS**

**Collection of the wood**

*Rhizophora racemosa* a popular firewood among the oil rich area (Niger Delta) of Nigeria were collected from an abandoned artisan refinery site in Bonny local government area of Rivers state (polluted site) while the control wood (non-polluted *Rhizophora racemosa*) was collected from a non oil polluted site.

**Animals**

Female wistar rats (weighing 100-150g at the start of the study) were obtained from the Department of Veterinary Medicine, University of Nigeria Nsukka, Enugu State. Studies were conducted in compliance with applicable laws and regulations for handling experimental animals.
animals were weighed and sorted into seven groups of five animals each, so that the average weights were approximately equal. The animals were housed in individual cages and acclimatized for 1 week on growers mesh and water fed ad libitum.

**Heavy Metals Analysis**

An absorption spectrometry was used to determine the concentration of the analyte. To determine the heavy metal 1g of the sample was weighed into a proceluin crucible container and then introduced into a furnace to derive the ash for 8 hours (at 550°C) then cooled. After cooling, 5ml of 1M Trioxonitrate (V) acid (HNO₃) was added to the left over ash, evaporated to dryness. Hydrochloric acid (15ml) was then added to the ash dissolve it and the solution was filtered into a volumetric flask and made up to the 100ml mark with deionized water. End determination of metal was performed using Atomic Absorption Spectroscopy (AAS).

**Determination of Polycyclic Aromatic Hydrocarbon (PAH)**

The extraction was carried out according to TNRCC TX method 1005 (1997). In this method the sample were extracted with dichloromethane (DCM) and subjected to gas chromatographic analysis.

**Air quality sampling and testing**

During the smoke exposure air quality was monitored. The meteorological parameters monitored included; carbon monoxide, nitrogen IV oxide, sulphur IV oxide, hydrogen sulphide, volatile organic compounds, ammonia, methane and particulate matter (1, 2.5, and 10 micron). This was done using an Industrial Scientific Corporation IBRID MX6 Multi-Gas monitor. Measurement was done by placing the sensor in the cages and reading was recorded at stability.

**Mode of Smoke Exposure**

The smoke exposure was carried out in a locally constructed smoke chamber following the guideline of Fire Toxicology Committee of National Academy of Science for building smoke exposure chambers (Gann et al., 1994).

**Liver function test**

Determination of Aspartate Aminotransferase (AST)

Quantitative in vitro determination of AST in serum used the kinetic method according to the protocol of a commercial test kit (Randox Laboratories Ltd).

\[
\text{Oxoglutarate} + \text{L-alanine} \xrightarrow{\text{GOT}} \text{L-glutamate} + \text{pyruvate}
\]

Alanine aminotransferase is measured by monitoring the concentration of private hydrazine formed with 2,4 dinitrophenyl hydrazine.

Determination of Alanine Aminotransferase (ALT)

Quantitative in vitro determination of ALT in serum used the kinetic method according to the protocol of a commercial test kit (Randox Laboratories Ltd).

\[
\text{P-nitrophenyl phosphate} + \text{H}_2\text{O} \xrightarrow{\text{ALP}} \text{Phosphate} + \text{P-nitrophenol}
\]

Alkaline phosphate is measured by monitoring the concentration of phosphate from P-nitrophenyl phosphate.

**Statistical Analysis**

All data were subjected to statistical analysis. Values were reported as mean ± standard error mean (SEM), while one way ANOVA was used to test for significance using statistical package for social sciences (SPSS). The results were considered significant at values of less than 0.5 that is 95% confidence level (p ≤ 0.05).

**RESULTS**

The result of the concentration of some heavy metals present in the wood collected from crude oil polluted environment (Test wood) and that of those collected from non-crude oil polluted environment (Control wood) are summarized and presented in Table 1. The heavy metals measured include mercury (Hg), arsenic (As), zinc (Zn), lead (Pb), cadmium (Cd) and nickel (Ni) and the result showed that all of these metals were present in the test wood at varying concentration. The most abundant metal was lead (69.9 mg/kg) followed by zinc (52.18 mg/kg), nickel (33.74 mg/kg), cadmium (0.6 mg/kg) and mercury (0.28 mg/kg) and mercury (0.001 mg/kg). In the case of the control wood all the metals were not present at detectable limit. Mercury was not present in control wood. The highest metals present was lead 0.51 mg/kg followed by nickel...
### Table 1. Experimental Design

<table>
<thead>
<tr>
<th>Group Number</th>
<th>I.D</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>Exposed to ambient air</td>
</tr>
<tr>
<td>2</td>
<td>Test Control I</td>
<td>Exposed to smoke from non crude oil polluted firewood for 10 minutes</td>
</tr>
<tr>
<td>3</td>
<td>Test Control II</td>
<td>Exposed to smoke from non crude oil polluted firewood for 30 minutes</td>
</tr>
<tr>
<td>4</td>
<td>Test Control III</td>
<td>Exposed to smoke from non crude oil polluted firewood for 60 minutes</td>
</tr>
<tr>
<td>5</td>
<td>Treatment I</td>
<td>Exposed to smoke from crude oil polluted firewood for 10 minutes</td>
</tr>
<tr>
<td>6</td>
<td>Treatment II</td>
<td>Exposed to smoke from crude oil polluted firewood for 30 minutes</td>
</tr>
<tr>
<td>7</td>
<td>Treatment III</td>
<td>Exposed to smoke from crude oil polluted firewood for 30 minutes</td>
</tr>
</tbody>
</table>

### Result of Polycyclic Aromatic Hydrocarbon

(0.22 mg/kg), Zinc (0.14 mg/kg), Cadmium (0.07) and arsenic (0.06mg/kg). The heavy metal concentration of the test wood were above WHO’s permissible limit of heavy metals in plant while those of the control metals were below.

**Result of Polycyclic Aromatic Hydrocarbon**

The results of the concentrations of the polycyclic aromatic hydrocarbon (PAHs) present in the wood collected from crude oil polluted environment and of those of the control are shown in Figure 1. The total PAHs present in the test wood was 8.85 mg/kg and that of the control wood was 5.55mg/kg. benzo(k) fluoranthene and dibenz(a,h)anthrenes were absent in the control wood but were present in test wood.

**Result of the wood smoke components**

The smoke generated from the combustion of the test wood was analyzed of air pollutants. The results were high when compared to (World Health Organization) WHO permissible limit and to that generated by the control wood. VOC was 3.5 ± 0.23ppm, NH₃ was 2.63±0.35ppm, SO₂ was 0.61±0.04ppm, PM₁₀, PM₂.₅, PM₁₀ was 0.11±0.01, 0.16±0.02, 0.27±0.01 respectively in test wood and those obtained from the control wood was 2.2±0.12ppm, 0.73±0.15ppm, 0.00±0.0, 0.1±0.00, 0.12±0.00, 0.12±0.00.
Table 2. Heavy metal concentration

<table>
<thead>
<tr>
<th></th>
<th>Hg (mg/kg)</th>
<th>As (mg/kg)</th>
<th>Zn (mg/kg)</th>
<th>Pd (mg/kg)</th>
<th>Cd (mg/kg)</th>
<th>Ni (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control wood</td>
<td>0.000</td>
<td>0.063</td>
<td>0.140</td>
<td>0.512</td>
<td>0.078</td>
<td>0.22</td>
</tr>
<tr>
<td>Test Wood</td>
<td>0.001</td>
<td>0.280</td>
<td>52.18</td>
<td>69.90</td>
<td>0.60</td>
<td>33.72</td>
</tr>
<tr>
<td>FAO/WHO permissible limit</td>
<td>0.002*</td>
<td>-</td>
<td>0.6*</td>
<td>2.00*</td>
<td>0.02*</td>
<td>10.0*</td>
</tr>
</tbody>
</table>

Table 3. Major Air Pollutants measured in Ambient Air, Test Wood and Control Wood Smoke

<table>
<thead>
<tr>
<th></th>
<th>Ambient air (ppm)</th>
<th>Smoke of Control wood (ppm)</th>
<th>Smoke of Test wood (ppm)</th>
<th>WHO acceptable limit (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₂</td>
<td>0.0±0.00</td>
<td>0.0±0.00</td>
<td>0.0±0.00</td>
<td>0.04</td>
</tr>
<tr>
<td>CO</td>
<td>0.00±0.00</td>
<td>48.67±2.6</td>
<td>112.3±8.7</td>
<td>10</td>
</tr>
<tr>
<td>VOC</td>
<td>0.9±0.20</td>
<td>2.2±0.12</td>
<td>3.5±0.23</td>
<td>3</td>
</tr>
<tr>
<td>CH₄</td>
<td>0.0±0.00</td>
<td>0.0±0.00</td>
<td>0.0±0.00</td>
<td>-</td>
</tr>
<tr>
<td>NH₃</td>
<td>0.57±0.22</td>
<td>0.73±0.15</td>
<td>2.63±0.35</td>
<td>-</td>
</tr>
<tr>
<td>SO₂</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.61±0.04</td>
<td>0.5</td>
</tr>
<tr>
<td>H₂S</td>
<td>0.0±0.00</td>
<td>0.0±0.00</td>
<td>0.0±0.00</td>
<td>-</td>
</tr>
<tr>
<td>PM₁</td>
<td>0.00±0.00</td>
<td>0.1±0.00</td>
<td>0.11±0.01</td>
<td>-</td>
</tr>
<tr>
<td>PM₂.₅</td>
<td>0.00±0.00</td>
<td>0.12±0.00</td>
<td>0.16±0.02</td>
<td>0.25</td>
</tr>
<tr>
<td>PM₁₀</td>
<td>0.00±0.00</td>
<td>0.12±0.00</td>
<td>0.27±0.00</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Note: Values are means ± SEM (SEM). Values with the different superscript are statistical significant at (p< 0.05). Superscript (a,b) compared the ambient air with all the smoke generated. Superscript (c,d) compared the smoke produced from test wood and control wood (Dike et al., nd).

respectively. This result shows that people exposed to smoke emanating from crude oil polluted wood stand a higher chance of developing health challenges associated with exposure to polluted air.

Result of the wood smoke components

Liver Enzyme Result

The result from the liver function test showed a significant increase (p<0.05) in the serum AST and ALP levels of the animal group exposed to the highest smoke duration (60 minutes) with more effect observed in those exposed to 60 minutes of test wood. This supports the fact that the dangers of smoke exposure increases proportionally with exposure time and as the level of pollution increases. AST is found mainly in the mitochondrial and cytoplasm of the liver. It is also partly found in the heart, kidney, brain and skeletal muscle. But the increase in the serum level is mostly because of damage in the liver. ALP is a liver cytosolic enzyme which is more specific to the liver. Its increase in serum is an indication of liver damage or disease (Poli et al., 1987)

Histopathological Effect of Crude Oil Polluted Wood Smoke on Lungs

In the histopathologic discovery no microscopic distortion was observed in the lungs of the control rat and those exposed to 10, 30 and 60 minutes of smoke produced by control wood. Similar result was found in those exposed to 10 minutes of smoke produced by wood harvested from crude oil polluted environment. There were normal alveolar spaces, interalveolar septa with capillaries and bronchiole. For group 6 and 7 rats exposed to 30 and 60 minutes of smoke from wood harvested from crude oil polluted environment, histological distorted lungs were observed. There were thickened interalveolar septa which is an indication of inflammatory in the lungs.

DISCUSSION

Plant has ability to picks up pollutants from the soil through their root (salt et al., 1995). This is the principle used in intentional phytoremediation; a process whereby plants are used to clean up a polluted environment (Baltrenaite et al., 2010). When this is done intentionally the polluted plant is harvested and dumped in a well-designed dump site far away from human dwelling. The same is not what is seen in unintentional phytoremediation which occurs when there is an oil spill in farmlands, swamps and creeks instead the plants woods are harvested and use as firewood (energy) for cooking which is the case seen in Niger Delta area of Nigeria. This they do with little or no knowledge on the health effect this may have. The preliminary study done
on a popular wood plant among the Niger Delta area of Nigeria collected from two different areas shows that those collected from oil polluted site had more concentration of pollutants than those from non crude oil polluted site which was seen in the level of its heavy metal and polycyclic aromatic hydrocarbon concentrations as can be seen in Figure 1 and Table 2. Smoke generated during the cause of using this polluted wood as firewood also showed that those collected from crude oil imparted environment had high level of pollutants which can endanger the lungs and other internal organs as shown in Table 3. In the lungs the interalveolar septa which separate the alveolar sacs are meant to be slim to enable proper exchange of air as can be seen in Figure 2 to Figure 6. Thickened interalveolar septa
are seen during inflammation of the lungs and in other disease conditions. Thickened interalveolar septa were observed in the lungs cells of groups exposed to the polluted wood smoke for a longer period of time as can be seen in Figure 7 and 8. According to studies pollutants no matter the form affects the lungs in similar ways, the inflame the linings of the lungs which makes them work harder and this can lead to inflammation that produces mucus, coughing.
Figure 6: Histological micrograph of Group 5 Lungs
A representative photomicrograph of group 5 lung tissue showing histological normal lungs (H&E. mag x400)

Figure 7: Histological micrograph of Group 6 Lungs
A representative photomicrograph of group 6 lung showing thickened interalveolar septa (arrowed) histological distorted lungs (H&E. mag x400)

and serious breathing trouble (Walsh, 2011). The liver is the largest organ in the human system. Its primary function is to help detoxify harmful substances in the body. This makes the liver almost the first point of damage to these
hazardous compounds (Maton et al., 1993). The liver is able to do this detoxification work due to the presence of some unique enzymes. In case of damage to the liver these enzymes finds their way to blood streams in elevated levels. The detection of these enzymes in elevated concentration is blood stream is used as a pointer to liver damage (Johnston, 1999). This elevation of serum liver enzymes was what was observed in the experimental animals exposed to the smoke from woods collected from crude oil polluted environments as can be seen in Table 4.

CONCLUSION

The results obtained from this study suggest that wood collected from an environmentally polluted area such as crude oil spill site can affect the level of pollutant present in the wood and in the smoke generated by such wood. The study also revealed that the pollutant in this smoke has the ability to increase liver enzyme concentration in serum and alter the histological features of the lungs in experimental animals and this effect increase as the exposure time increases. This
suggest possible health danger to women that are exposed to firewood smoke generated from woods collected from polluted environment.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

**REFERENCES**


