Microbial and Heavy Metal Evaluation of Solutions of Ash Produced From Unripe Plantain Peels and Oil Palm Fruit Bunch Sold in Market Outlets within Afikpo South L.G.A. in Ebonyi State

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Abstract: Studies were carried out to evaluate the microbial and heavy metal levels in ash solutions of unripe plantain peel and palm fruit bunch samples sold in market outlets within Afikpo South L.G.A, in Ebonyi State, using standard biochemical procedures and instrumentation. The microbes investigated were isolated using nutrient and potato-dextar agar for the bacteria and fungi respectively. The pH of the ash sample solutions of the plants were determined using pH meter while the investigated metals were analyzed using spectrophotometric technique. The pH of the ash sample solutions of the unripe plantain peels and palm fruit bunch were 11.29 and 12.73 respectively, thus provided an unhealthy environment for the micro organism to thrive. The coliform count of the isolated micro organisms (Klebsiella pneumoniae, Staphylococcus aureus and Bacillus cereus) were far within the WHO recommended limit. The mean levels of the studied metals in the ash sample solutions of the unripe plantain peels were, 43.17 ± 5.32, 0.54 ± 0.11, 22.05 ± 1.60, 0.35 ± 0.09, 8.82 ± 0.52 and 35.91 ± 17.14μg/g for Zn, Cd, Cu, Pb, Cr and Fe respectively while the mean levels of the studied metals in the ash sample solution of the palm fruit bunch were, 70.02 ± 3.66, 0.78 ± 0.40, 65.09 ± 2.55, 0.62 ± 0.18, 6.06 ± 0.79 and 19.11 ± 3.42μg/g for Zn, Cd, Cu, Pb, Cr and Fe respectively. Fe, Cu and Zn were found to be of higher values in the ash solutions of the two plant samples while Pb and Cd were of lesser values. Only Cd in the ash sample solutions of the two plants and Pb in the ash sample solution of palm fruit bunch were present at toxic levels. The mean levels of the studied metals in the ash sample solutions of the unripe plantain peels and palm fruit bunch differed significantly at p < 0.05.

Keywords: Heavy metals, Pathogenic microorganisms, Ash solution of unripe plantain peels and Ash solution of palm fruit bunch, pH and Market outlets.

1. Introduction

Potash has gained a domestic and world-wide use in the flat glass food chemical pulp and paper sectors (Babayemi et al., 2010). It is used in the preparation of African dishes and production of local soap. The simplicity of potash chemistry and the easily available local and improved potash production technology has lent the ash-derived potash a promising future as a sustainable source of raw material for a wide range of applications (Ankrah, 1974). It has diverse traditional uses. According to Alaibe et al., (2019), potash is often used as a tenderizer in cooking, an ingredient in certain foods and medicinal preparations, a mordant in dyeing and a purgative in drinking water for livestock.

Ash solutions of certain vegetable matter or agricultural waste have been used locally in the production of an instant emulsion called ncha used in preparing dishes such as Nkwobi, isi-ewu, kpomo, ugba, abacha and otong (Uzodimma et al., 2014).

Olabanji et al., (2012) stated that agricultural wastes such as unripe banana peels, unripe plantain peels, maize cob and palm fruit bunch contains a good percentage of potash alkali especially for a wide range of uses comparable to the conventional and inorganic potash salt locally called akanwu in igbo or kawu in Yoruba language.

Palm fruit bunch and unripe plantain peels are not only regarded as cheaper but are also thought to be safer than kawu or akanwu, the conventional potash. Most local women normally after ashing the unripe plantain peels or palm fruit bunch, soaks it in water and sieve it before selling the filtrate as ‘potash’ solution for various uses, some of which have been earlier mentioned. The sources of the water used by the local processors in processing ‘potash’ solution locally most times followed anthropogenic microbial and heavy metal contamination.

According to Okeke et al., (2019), unhygienic practices such as sneezing, coughing during processing stages of food products, not washing of hands after defecation, spitting, using dirty water and containers are the surest ways of introducing microbes and other contaminants into food products prepared for human consumption.

Although water is necessary for life, it could also be channel for transmitting diseases and death. Water sources with
Toxicity of heavy metals refers to the harmful effects that result from exposure or consumption of excessive amounts or more than the daily recommended limits (Onakpa et al., 2018). Although individual metals exhibit specific signs of toxicity, the general signs associated with Cd, Pb, As, Hg, Zn, Cu and Al poisoning include gastrointestinal disorders, diarrhea, stomatitis, tremor, hemoglobinuria, ataxia, paralysis, vomiting, convulsion, depression, pneumonia and cancer etc (Jaishankar et al., 2014). Metal bioaccumulation in plants is really of great concern as their concentration/dosage may exceed WHO recommended safety levels thereby posing health hazards to man and his environment.

Equally, unhygienic practices inadvertently could introduce contaminants such as pathogens and heavy metals into food materials etc. solutions of ash (potash) produced from unripe plantain peels and palm fruit bunch, which have been used mainly for the preparation of instant-emulsion (ncha) for preparation of Nigerian delicacies (such as abacha, nkwoibi, isu ewu or ubga) than their use in soap making by the local people in Nigerian societies for a long time now.

Therefore, studies was conducted to assess the microbial and heavy metal levels in solutions of ash produced from unripe plantain peels and palm fruit bunch sold in market outlets within Afikpo South L.G.A. in Ebonyi State.

2. Materials and Methods

The samples comprising ash solutions from unripe plantain peels and palm fruit bunch were respectively purchased from market outlets within Afikpo South LGA in Ebonyi State, stored in very clean plastic containers and immediately taken to the laboratory for analysis.

Preparation of media

The media for culturing was especially prepared according to the established procedures and autoclaved at 121°C for 15mins.

Serial dilution and culturing

2ml of the sample solution were pipettes into a beaker containing 10ml of distilled water in a ration of 2:10 and was swirled. A tenfold serial dilution was carried out as described by (Inetianbor et al., 2014). The procedure was carried out for each of the sample solution studied. Bacteria were grown in nutrient agar at 37°C for 20hrs. Pure cultures of different isolates were obtained and stored in a nutrient broth slant. For fungi isolates, the inocula were grown in a potato dextro agar for 96hrs at room temperature. Cultural and morphological characterization of the microbes were determined as described by Harrigan and Mc Cance, (2006).

Biochemical test and gram staining

The biochemical test and gram staining was carried out in accordance with the procedures of Chessbrough, (2006).

Determination of pH

The pH of the samples was determined following the method described by AOAC, (2000).

Heavy metal analysis

About 100ml of the sample solution was transferred into a beaker and 5ml of Conc. HNO₃ was added. It was warmed slowly and allowed to evaporate to 20ml in a fume cupboard. Few drops of Conc. HNO₃ was added and then...
heated until a light coloured, clear solution was observed. The beaker wall was washed with de-ionized water and then filtered. The filtrate was transferred to a 100ml volumetric flask, allowed to cool and made up to mark with de-ionized water. The same process was repeated for each sample solutions being studied.

The sample digests were analyzed for possible heavy metal (Zn, Cd, Cu, Pb, Cr and Fe) contamination using UNICAM 969 atomic absorption spectrophotometer.

**Statistical analysis**
The data obtained were expressed in means and standard deviations and subjected to one-way analysis of variance (ANOVA) using SPSS version 22.0 at 5% level of confidence.

### 3. Results and Discussions

| Table 1: Mean pH of the ash sample solutions of unripe plantain peels and palm fruit bunch |
|---------------------------------------------|-------------|
| Sample                                      | pH          |
| Ash solution of unripe plantain peels       | 11.29       |
| Ash solution of palm fruit bunch            | 12.73       |

Results of Table 1 shows that the pH of the ash sample solutions of unripe plantain peels and palm fruit bunch were, 11.29 and 12.73 respectively. The result therefore indicated that the ash sample solution of palm fruit bunch was more alkaline and could therefore contain more components of alkaline metallic salts than the ash sample solution of unripe plantain peels. The age of these two sample vegetable matter and their assess to mineral nutrients in the soil could have significantly account for their varying pH levels.

Olabanji et al., (2012) reported a higher pH value of 12.88 for ash solution of unripe plantain peels than what was gotten in this research. Differences in geographical location of the plants, age and nutrient content in the soils where they were planted could have accounted for this variation.

### Table 2: Biochemical characteristics of bacterial and fungal isolates

<table>
<thead>
<tr>
<th>Cultural characteristics</th>
<th>Cellular morphology</th>
<th>Gram staining</th>
<th>Glucose</th>
<th>Indole</th>
<th>Coagulase</th>
<th>Catalase</th>
<th>Citrate</th>
<th>Methyl red</th>
<th>Most probable identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellowish-orange and slimy</td>
<td>Cocci in pairs</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Pink, smooth, flat and irregular</td>
<td>Rods in single pairs and clusters</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td>Pink, round into smoothy, shiny surface</td>
<td>Rods in clusters, spores present and flagellated</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Bacillus cereus</td>
</tr>
</tbody>
</table>

Table 3 shows that two microbial isolates comprising of a bacteria and a fungi were identified in the ash sample solution of the unripe plantain peels while only one microbial isolate which composed of a bacterium was identified in the ash sample solution of the palm fruit bunch. The mean microbial count in the ash sample solution of the unripe plantain peel samples were 15.4 and 21.6 (Cfu/g) for *Staphylococcus aureus* and *Bacillus cereus* respectively. The mean microbial count in the ash sample solution of the palm fruit bunch was 9.3 Cfu/g for *Klebsiella pneumoniae*.

Although, the bacteria and fungal isolates identified in the study can be classified as contaminants in the ash sample solutions however their mean counts were far within the WHO permissible limits. The food and water borne diseases associated with these fungal and bacterial isolates is already well documented in literature (Okeke et al., 2019). It can be equally deduced that the high alkalinity of the ash sample solutions could not have aided the growth and thriving of the isolated pathogenic organisms.

According to Ohmain and Izah (2013), the capacity of pathogenic micro-organisms to survive and grow at alkaline pH values is of widespread importance in their epidemiology in remediation and industrial settings.

Hence, micro-organisms (bacteria and fungi) must maintain a cytoplasmic pH that is compatible with the optimal, functional and structural integrity of the cytoplasmic proteins that supports their growth and this is usually between 5.5-8.5.

According to Padan et al., (2005) most non extremophilic micro organisms (such as *S.aureus*, *E.coli*, *B. cereus*, *Streptococcus spp.*, *Aspergillus spp.*, and *Klebsiella pneumoniae* among others) grow over a broad range of external pH values from 5.5-9.0 and usually maintain a cytoplasmic pH values that lies within the narrow range of pH 7.4-7.8.

Forster, (2000) reported that a shift to an alkaline environment like a shift to an acid environment is stressful to micro-organisms as shown by *E.coli* response in an alkaline medium. Most micro-organisms are neutrophilic and grow at a pH range of 5-8.0, hence do not fare well in a strongly acidic or basic medium. Example of such neutrophilic micro-organisms are *S.aureus*, *B.cereus*, *salmonella spp.*, *Aspergillus spp.* etc. Usually, hydrogen bonds holds strands of the DNA of micro-organisms break up at high pH values. Panda et al., (2005) observed
that changes in pH modifies the ionization of amino acid functional groups in the micro-organisms and disrupt hydrogen bonding which in turn promotes changes in the folding of the molecule, promoting deactivation and destroying activity. Vassa et al., (2001) reported that an increase in alkalinity increases the lag phase of micro-organisms, especially when the pH is adjusted from 9.5-11.0.

Zinc is an essential trace element for plants, animals and human beings as it is associated with many enzymes and with certain proteins. The major health concern for zinc in general is marginal or deficient zinc intake rather than toxicity (Olabanji et al., 2012). Zinc is considered as being of a low toxicity due to the wide margin between usual environmental concentrations and toxic levels.

According to Iwegbu et al., (2011) high levels of zinc are undesirable as it may lead to copper deficiency by inhibiting copper absorption. Table 4 shows that the mean levels of Zinc in the ash sample solutions of unripe plantain peels and palm fruit bunch were, 43.17 ± 5.32 and 70.02 ± 3.66μg/g respectively. The mean levels of the metal in the ash sample solutions were statistically significant and equally within the recommended permissible limit. The ash sample of palm fruit bunch was found to have higher mean level of Zn than the ash sample of unripe plantain peels which was attributed to the differences in the soil chemistries where the plant samples where grown. The nutrient availability, anthropogenic activities within the environments were the plants grew, the age of the plants, and water sources.

Alaribe et al., (2019) reported a lower value of 1.766 ± 0.001μg/g for Zn in the ash of unripe plantain peels than what was obtained for the metal in this study.

Cadmium is a highly mobile element and can be easily transported through the shoots of plants and usually uniformly distributed throughout the affected plant (Sekara et al., 2005). Table 4 shows that the mean levels of Cd in the ash sample solutions of unripe plantain peels and palm fruit bunch were, 0.54 ± 0.11 and 0.78 ± 0.40μg/g respectively. The mean levels of the metal in the ash sample solutions differed significantly and were both above the WHO recommended permissible limit for the metal. World Health Organization as a health regulatory and supervisory body provided a policy document on the permissible limits of various pollutants in consumable food substances to safeguard the health of the people from undue exposure to toxic substances (WHO, 2005). Research has shown that exposure to Cd at toxic levels can result in reproductive failure, stomach pains, diarrhea, severe vomiting, bone fracture, liver and kidney damage, cancer development and alteration of central nervous system (Ogbonna et al., 2015).

Table 4: Mean heavy metal levels (μg/g) in the ash sample solutions of unripe plantain peels and palm fruit bunch sold in market outlets in Afikpo South L.G.A. in Ebonyi State.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Ash sample solution of unripe plantain peels (μg/g)</th>
<th>Ash sample solution of palm fruit bunch (μg/g)</th>
<th>P value</th>
<th>F test</th>
<th>WHO STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>43.17 ± 5.32</td>
<td>70.02 ± 3.66</td>
<td>0.01</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.54 ± 0.11</td>
<td>0.78 ± 0.40</td>
<td>0.02</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>22.05 ± 1.60</td>
<td>65.09 ± 2.55</td>
<td>0.00</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.35 ± 0.09</td>
<td>0.62 ± 0.18</td>
<td>0.01</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>8.82 ± 0.52</td>
<td>6.06 ± 0.79</td>
<td>0.02</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>35.91 ± 7.14</td>
<td>19.11 ± 3.42</td>
<td>0.00</td>
<td>300</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Bar chart representation of the mean bacterial and fungal counts in the ash solutions of the unripe plantain peels and palm fruit bunch.

Figure 3: Bar chart representation of the mean levels of Zn (μg/g) in the ash sample solutions of unripe plantain peels and palm fruit bunch.

Figure 4: Bar chart representation of the mean levels of Cd (μg/g) in the ash sample solutions of unripe plantain peels and palm fruit bunch.
Copper
Table 4 shows that the mean levels of Cu in the ash sample solutions of the unripe plantain peels and palm fruit bunch were, 22.06 ± 1.60 and 65.09 ± 2.22(μg/g) respectively. Ash sample solution of palm fruit bunch was found to have a higher mean level of Cu that the ash sample solution of unripe plantain peels. The variation in the ages of the plants samples, soil chemistries where the plants grew and anthropogenic activities among others as earlier indicated could have been the reason.

The levels of Cu in the ash sample solutions of unripe plantain peels and palm fruit bunch were statistically significant. Equally, the mean levels of Cu in the ash sample solutions of the two plants were within the recommended permissible limit of 100μg/g.

Although copper is a major component of enzymes in iron metabolism, it exerts toxicity either at acute or chronic forms when taken in excess. Acute toxicity of copper manifest as nausea, vomiting, jaundice, liver necrosis and damages to the kidney (Ezeh et al., 2018). Wilson’s disease in man is a form of chronic copper toxicity that presents as mental alterations, motor abnormalities, dysphagia, ataxia and hepatic failure (Mc Dowells, 2003).

Babayemi et al., (2010) reported a higher mean value of 3.10μg/g for Pb in the ash solution of unripe plantain peels grown in Ogun state than what was obtained for the metal in this study.

![Figure 6: Bar chart representation of the mean levels of Pb (μg/g) in the ash sample solutions of unripe plantain peels and palm fruit bunch.](image)

Chromium
According to Broadhurst and Demenico, (2008), Chromium is biochemically very essential in maintaining blood glucose levels and equally widely used in diabetes medications. Table 4 shows that the mean levels of Cr in the ash solutions of unripe plantain peels and palm fruit bunch were, 8.32 ± 0.52 and 6.06 ± 0.79(μg/g) respectively. The mean levels of Cr in the ash sample solutions were within its recommended tolerable limit of 10μg/g. Equally the levels of Cr in the ash sample solutions were statistically significant at p<005.

![Figure 7: Bar chart representation of the mean levels of Cr (μg/g) in the ash sample solutions of unripe plantain peels and palm fruit bunch.](image)

Lead
Lead is considered as a potential carcinogen and is associated with the cardiovascular, kidney, blood and nervous diseases (Jarup, 2003). It interferes with the development of the nervous system and is therefore particularly toxic to children causing permanent learning and behavioural disorders. The permissible limit of Pb in food substances was put at 0.5(μg/g) (WHO, 2005).

Table 4 shows that the mean levels of Pb in the ash sample solutions of unripe plantain peels and palm fruit bunch were, 0.35 ± 0.09 and 0.62 ± 0.18μg/g respectively. The levels of Pb in the ash sample solutions were statistically significant. Only the mean levels of Pb in the ash solution of the unripe plantain peels was within the recommended permissible limit.

![Figure 5: Bar chart representation of the mean levels of Cu (μg/g) in the ash sample solutions of unripe plantain peels and palm fruit bunch](image)
Similarly, Khan et al., (2008) stated that exposure to chromium may result in liver, kidney and lung damage.

Iron

Table 4 shows that the mean levels of Fe in the ash sample solutions of unripe plantain peels and palm fruit bunch were, 35.19 ± 7.14 and 19.11 ± 3.42µg/g respectively. The metal was within its permissible limit in the ash sample solutions. The mean levels of Fe in the ash sample solutions of unripe plantain peels and palm fruit bunch were statistically significant. The ash sample solution of unripe plantain peels was found to contain a higher mean value of Fe than the ash sample solution of palm fruit bunch. The biochemical make-up of Musa spp., that makes them very rich in iron and the soil make-up where it was grown must have been the reason for having a higher level of Fe than the opposite plant sample (palm fruit bunch)

According to Kirmani et al., (2011) iron is an essential element that is very important in building red blood cells, oxygen transport, growth and development. It is equally involved in the transport of different substances, DNA synthesis and election transport chain. Deficiency of Fe in the body can result to goiter, anemia, high blood pressure, constipation stroke and ulcer (Afiukwa et al., 2009).

The six investigated metals (Zn, Cd, Cu, Pb, Cr and Fe) were found present in the ash sample solutions of the two plants. Only the mean levels of Cd in the ash sample solutions of the two plants and Pb in the ash sample solution of the palm fruit bunch were present at toxic levels.

Differences in anthropogenic activities and soil chemistries within the environments where the plant samples were grown and other unethical practices by the local processor of the “potash” could have accounted for some of the metals being present at above their recommended threshold limits.

References


