INTRODUCTION

The World Health Organization (WHO) estimates that 1 billion men and 250 million women currently smoke cigarettes and ranks tobacco smoking among the 10 greatest risks to health (WHO, 2002). The global burden of deaths attributable to tobacco use at present, as estimated, is approximately 5 million people. If current smoking patterns continue, it will cause some 10 million deaths by year 2025. Most of the increase is in the Third World, where the annual number of deaths from smoking is predicted to rise to 7 million (UNDESA, 2004).

Several chemical classes, such as polyaromatic hydrocarbons, specific nitrosamines and heavy metals, are present in tobacco smoke and have long been associated with various diseases (Pappas et al., 2006). Since even essential metals can become toxic if their concentration in cells gets too high, homeostatic mechanisms exist to control intracellular essential metal ion concentrations.

Manganese (Mn) is a biologically essential trace element for humans (Pine et al., 2005), but becomes toxic at high concentrations, thus leading to adverse health effects, predominantly in the central nervous system (CNS). Concerns involving potential adverse health effects from even sub-microgram metal exposures have increased and frequently been discussed (Barceloux, 1999b; Elsner and Spangler, 2005; Finley, 2004). Cobalt (Co) is an essential trace element but however when inhaled in excess, it produces serious health problems (Lison et al., 2001; Barceloux, 1999a). Some 59Co derivatives have been shown to be confirmed carcinogenic for human beings (IARC, 2003).

Evaluation of trace metals in tobacco of local and imported cigarette brands used in Pakistan by spectrophotometer through microwave digestion

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(Received March 1, 2008; Accepted May 16, 2008)

ABSTRACT — Uncontrolled exposure of active and passive smokers to trace metals causes increase in health risks. The primary objective of this study was to determine whether local and imported cigarette brands used in Pakistan, have elevated levels of metals or not. Six metals manganese (Mn), cobalt (Co), copper (Cu), cadmium (Cd), lead (Pb) and zinc (Zn) were determined in tobacco of twenty cigarette brands (local and imported) used in Pakistan by flame atomic absorption Spectrophotometry. To overcome contamination chances and for complete digestion of analytes a microwave digester was used. The analytical results showed highest concentration of Mn (84.78 μg/g dry weight), Cd (0.525 μg/g dry weight) and Zn (14.34 μg/g dry weight) metals in imported brands in relation to counterparts from the local brands. Certain elevated levels were observed for Co (3.344 μg/g dry weight), Pb (14.16 μg/g dry weight) and Cu (7.889 μg/g dry weight) metals in local brands. The inter-metal relationships in the tobacco of local and imported cigarette brands showed some integrated variation in the selected metal levels. In view of health risk associated with the above metals, there should be a strict quality control over monitoring of heavy metals during growing, processing and smoking of tobacco. Therefore, it is prudent to minimize exposure to toxic substances whenever possible because smoking and exposure to cigarette smoke is a confounder to be taken into account when carrying out epidemiological studies on human exposure to metals.

Key words: Trace metal, Cigarette, Pakistan, Microwave digestion, Spectrophotometer

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Copper (Cu) and zinc (Zn) are also trace elements and play an essential role for the function of various enzymes and other cellular proteins, but excessive intracellular accumulation is related to toxicity (Dock and Vahter, 1999). Cu becomes toxic in case of excessive intracellular accumulation playing a role in initiating the generation of detoxification of reactive oxygen species (ROS) and apoptotic processes (Medici et al., 2002; Santon et al., 2004). Zn becomes toxic in the case of excessive intake playing a role in induction of the pathological conditions that have been associated with oxidative stress (Yanagisawa et al., 2004).

The environmentalist, government agencies and health practitioners are much worried about the presence of non-essential and highly toxic metals cadmium and lead. Lead is potentially hazardous and carcinogenic. In human beings it causes chronic neurological disorders especially in foetuses and children (Awofolu et al., 2005). In the general population tobacco smoking is one of the most important sources of cadmium exposure (Adnan et al., 2005). Several metals, including cadmium contribute to toxicological noncancer indices of health risks for respiratory and cardiovascular diseases such as peripheral artery disease (Fowles and Dybing, 2003; Navas-Acien et al., 2004).

The present study is aimed to explore concentrations of six trace metals, i.e. Co, Mn, Cu, cadmium (Cd), lead (Pb) and Zn in tobacco of local and imported cigarette brands used in Pakistan. Microwave digestion technique was used for digestion of samples, so that accuracy could be attained because in old methods chances of contamination were huge and also there was no assurance of the complete digestion of analyte, as most part of the analyte in the form of residues remained undigested.

**MATERIALS AND METHODS**

A total of twenty cigarette brands (10 local and 10 imported) commonly available in local markets of Pakistan were purchased. Their names along with code numbers are given in Table 1. All reagents used were analytical grade in quality and purchased from E-Merck (Darmstadt, Germany). All the glassware was soaked in 10% HNO₃ for 24 hr and then cleaned with deionized water and dried carefully to avoid any contamination (Adnan et al., 2005). Stock standard solutions (1,000 mg/l) of metals were used procured from E-Merck (Darmstadt, Germany).

**Preparation of Tobacco Samples**

The average weight of each cigarette brand was determined by weighing 5 sticks of each brand before and after removing the filters using Sartorious Analytical Balance (ISO 9001). Composites of tobacco of each brand were selected from a pack of 20 (Nnorom et al., 2005). The samples were dried in an oven (FS – TYPEOVEN. Model FS –63D. TOYO SEISAKUSHO CO., LTD., Chiba, Japan) at a temperature of 80°C for 12 hr and allowed to cool in a desiccator (Elinder et al., 1983). 0.5 g of sample (tobacco) of each brand was transferred into the acid washed and oven dried digestion vessel, appropriate aliquots of acids (10 ml 65% HNO₃ + 5 ml 60% HClO₄) were

| Table 1. Names of Local and Imported cigarette brands |
|---------------------------------|---------|-----------------|---------|
| Gold leaf                       | L1      | Marlboro (USA)  | I1      |
| Diplomat                        | L2      | Rothmans (King size, UK) | I2 |
| K 2 (King size filter)          | L3      | Benson & Hedges (UK) | I3 |
| Embassy (Kings)                 | L4      | Mild Seven (Japan) | I4 |
| Red & white                     | L5      | Pine (Menthol lights, USA) | I5 |
| Morven Gold (King size filter)  | L6      | More (International, Japan) | I6 |
| Royals Filter                   | L7      | Business club (UK) | I7 |
| Gold Flake                      | L8      | Dunhill (International, UK) | I8 |
| Park Lane (Special)             | L9      | Pine Lights (USA) | I9 |
| Capstan (International)         | L10     | Fisher (USA) | II0 |

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added to the digestion vessel and loaded in to the micro-
wave oven (CEM MARS –5). Then the following pro-
gramme was applied with two turns (Table 2).

RESULTS AND DISCUSSION

Atomic absorption spectrometry has been successful-
ly used for the measurement of six trace metals, i.e. Zn,
Mn, Cu, Cd, Pb and Co in twenty cigarette brands com-
monly used in Pakistan. Metal levels of local brands are
given in Table 3 and that of imported brands in Table 4.
For the accuracy of the analytical results by AA 240 FS
Fast Sequential Atomic Absorption Spectrometer (Varian
Australia Ltd., Mulgrave, Australia) at least one reagent
blank was analyzed. A verification of the samples prepa-
ration and measurements was also carried out by analysis
of lyophilized blood samples (Batch 1701-1703) provid-
ed by Jasper Kristiansen (AMI, Denmark). The standard
deviations for metals mostly reflected the variability of
metal levels in the samples and in different batches of the
cigarettes. Data was analyzed by Basic descriptive statis-
tics as minimum, maximum, mean and standard deviation
(S.D.). The coefficient of correlations between various
metals in tobacco of local and imported cigarette brands

Table 2. Digestion Program

<table>
<thead>
<tr>
<th>Power (Watt)</th>
<th>maximum (%)</th>
<th>Time (min)</th>
<th>Temperature (ºC)</th>
<th>Hold Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200</td>
<td>100</td>
<td>20.00</td>
<td>150</td>
<td>10</td>
</tr>
<tr>
<td>600</td>
<td>50</td>
<td>20.00</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3. Concentration of Metals in Tobacco of local Cigarette Brands Mean ± S.D. (μg/g Dry Weight ) n = 30

<table>
<thead>
<tr>
<th>Brands</th>
<th>Co</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>2.816 ± 0.176</td>
<td>5.666 ± 0.189</td>
<td>50.88 ± 0.176</td>
<td>23.53 ± 0.425</td>
<td>0.260 ± 0.036</td>
<td>14.16 ± 1.041</td>
</tr>
<tr>
<td>L2</td>
<td>3.883 ± 0.355</td>
<td>7.283 ± 0.153</td>
<td>47.33 ± 0.104</td>
<td>7.066 ± 0.257</td>
<td>0.308 ± 0.010</td>
<td>13.33 ± 1.258</td>
</tr>
<tr>
<td>L3</td>
<td>3.416 ± 0.076</td>
<td>11.266 ± 0.293</td>
<td>41.36 ± 0.36</td>
<td>8.116 ± 0.115</td>
<td>0.530 ± 0.017</td>
<td>14.33 ± 0.764</td>
</tr>
<tr>
<td>L4</td>
<td>3.65 ± 0.304</td>
<td>8.366 ± 0.076</td>
<td>34.05 ± 0.150</td>
<td>9.316 ± 2.983</td>
<td>0.508 ± 0.028</td>
<td>12.16 ± 0.764</td>
</tr>
<tr>
<td>L5</td>
<td>1.933 ± 0.115</td>
<td>8.066 ± 0.325</td>
<td>42.96 ± 0.076</td>
<td>4.650 ± 0.278</td>
<td>0.541 ± 0.043</td>
<td>10.5 ± 2.179</td>
</tr>
<tr>
<td>L6</td>
<td>3.750 ± 0.278</td>
<td>9.383 ± 0.189</td>
<td>36.56 ± 0.176</td>
<td>6.033 ± 0.161</td>
<td>0.515 ± 0.049</td>
<td>17.16 ± 3.055</td>
</tr>
<tr>
<td>L7</td>
<td>3.266 ± 0.379</td>
<td>9.250 ± 0.250</td>
<td>47.78 ± 0.252</td>
<td>9.383 ± 0.252</td>
<td>0.618 ± 0.028</td>
<td>11.50 ± 1.000</td>
</tr>
<tr>
<td>L8</td>
<td>3.800 ± 0.350</td>
<td>7.233 ± 0.225</td>
<td>36.85 ± 0.300</td>
<td>5.583 ± 0.301</td>
<td>0.560 ± 0.028</td>
<td>27.33 ± 2.754</td>
</tr>
<tr>
<td>L9</td>
<td>4.066 ± 0.252</td>
<td>5.616 ± 0.375</td>
<td>66.15 ± 0.250</td>
<td>7.216 ± 0.325</td>
<td>0.566 ± 0.015</td>
<td>10.16 ± 2.082</td>
</tr>
<tr>
<td>L10</td>
<td>2.866 ± 0.202</td>
<td>6.766 ± 0.375</td>
<td>46.40 ± 0.200</td>
<td>4.850 ± 0.300</td>
<td>0.605 ± 0.013</td>
<td>13.33 ± 1.041</td>
</tr>
</tbody>
</table>

Table 4. Concentration of Metals in Tobacco of imported Cigarette Brands Mean ± S.D. (μg/g Dry Weight) n = 30

<table>
<thead>
<tr>
<th>Brands</th>
<th>Co</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>I1</td>
<td>2.116 ± 0.289</td>
<td>6.116 ± 0.333</td>
<td>81.23 ± 0.176</td>
<td>13.10 ± 0.218</td>
<td>0.525 ± 0.018</td>
<td>5.333 ± 1.756</td>
</tr>
<tr>
<td>I2</td>
<td>2.883 ± 0.236</td>
<td>6.350 ± 0.278</td>
<td>61.80 ± 0.218</td>
<td>11.46 ± 0.431</td>
<td>0.243 ± 0.016</td>
<td>2.500 ± 1.323</td>
</tr>
<tr>
<td>I3</td>
<td>1.600 ± 0.200</td>
<td>4.700 ± 0.350</td>
<td>72.23 ± 0.202</td>
<td>24.08 ± 0.225</td>
<td>0.560 ± 0.023</td>
<td>11.17 ± 2.082</td>
</tr>
<tr>
<td>I4</td>
<td>3.066 ± 0.293</td>
<td>6.383 ± 0.225</td>
<td>69.30 ± 0.312</td>
<td>15.01 ± 0.275</td>
<td>0.608 ± 0.024</td>
<td>8.000 ± 2.000</td>
</tr>
<tr>
<td>I5</td>
<td>1.233 ± 0.236</td>
<td>5.983 ± 0.153</td>
<td>172.1 ± 0.153</td>
<td>22.95 ± 0.350</td>
<td>0.551 ± 0.033</td>
<td>14.00 ± 1.732</td>
</tr>
<tr>
<td>I6</td>
<td>2.766 ± 0.247</td>
<td>6.083 ± 0.252</td>
<td>61.45 ± 0.100</td>
<td>9.050 ± 0.300</td>
<td>0.610 ± 0.028</td>
<td>10.00 ± 3.500</td>
</tr>
<tr>
<td>I7</td>
<td>1.816 ± 0.029</td>
<td>5.000 ± 0.278</td>
<td>49.80 ± 0.477</td>
<td>12.03 ± 0.425</td>
<td>0.455 ± 0.028</td>
<td>9.333 ± 2.255</td>
</tr>
<tr>
<td>I8</td>
<td>3.233 ± 0.202</td>
<td>4.700 ± 0.312</td>
<td>76.66 ± 0.104</td>
<td>14.41 ± 0.161</td>
<td>0.630 ± 0.026</td>
<td>9.333 ± 1.756</td>
</tr>
<tr>
<td>I9</td>
<td>3.883 ± 0.419</td>
<td>5.466 ± 0.465</td>
<td>133.9 ± 0.278</td>
<td>19.11 ± 0.252</td>
<td>0.613 ± 0.026</td>
<td>8.666 ± 3.329</td>
</tr>
<tr>
<td>I10</td>
<td>2.150 ± 0.328</td>
<td>5.850 ± 0.350</td>
<td>69.40 ± 0.180</td>
<td>2.266 ± 0.382</td>
<td>0.795 ± 0.028</td>
<td>9.166 ± 2.887</td>
</tr>
</tbody>
</table>
was also calculated by using Microsoft Excel software.

The average concentration of Mn in tobacco of local brands was found to be 45.03 μg/g (DW) and for imported brands 84.78 μg/g (DW). Highest concentration of Mn recorded for ITS (172.1 μg/g DW) in imported brands and LT9 (66.5 μg/g DW) for local brands. On the whole imported brands reflected greater concentration of Mn in relation to the counterparts from the local brands.

Co contents in local brands ranged from 1.933-4.066 μg/g (DW) with an average of 3.344 μg/g (DW) and in case of imported brands 1.233-3.833 μg/g (DW) with an average of 2.474 μg/g (DW). Certain elevated levels were observed in sample LT9 (4.066 μg/g) (DW) for local and IT9 (3.833 μg/g) (DW) for imported brands.

The average concentration of Pb in tobacco of local brands observed was 14.39 μg/g (DW) (range 10.16-7.33 μg/g DW). In counter case for the imported brands the mean Pb concentration was 8.749 μg/g (DW) ranged from 2.500-14.00 μg/g (DW) in tobacco.

Relatively higher value was obtained for Zn metal of imported cigarette brands i.e. 14.34 μg/g (DW) ranging from 2.266-24.08 μg/g (DW) as compared to local cigarette brands, 8.574 μg/g (DW) with a range 4.650-23.53 μg/g. Maximum value was found in sample of LT1 (23.53 μg/g) (DW) of local cigarette brands and IT (324.08 μg/g) of imported cigarette brands.

The average concentration of Cu in tobacco of local brands was found to be 7.889 μg/g (DW) having range 5.616-11.26 μg/g (DW) and for imported brands 5.713 μg/g (DW) with a range 4.700-6.383 μg/g (DW). Certain elevated levels were observed in sample LT3 (11.26 μg/g) for local and IT4 (6.383 μg/g) in case of imported brands. We found significantly elevated Cu level in tobacco of local brands in relation to the counterparts from the imported brands.

The average concentration of Cd in tobacco of local brands tested was 0.501 μg/g (DW) ranging from 0.260–0.618 (Table 3) and those of imported brands was 0.559 μg/g (DW) ranging from 0.243–0.795 (Table 4). Higher concentration of Cd was observed in imported cigarette brands as compared to local cigarette brands.

The inter-metal relationships in the tobacco of local and imported cigarette brands are shown as correlation coefficient matrix in Tables 5 and 6, wherein significant val-

<table>
<thead>
<tr>
<th>Cd</th>
<th>Co</th>
<th>Cu</th>
<th>Mn</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>-0.012</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.335</td>
<td>0.008</td>
<td>-0.582</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>-0.088</td>
<td>0.086</td>
<td>-0.582</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.031</td>
<td>0.311</td>
<td>0.022</td>
<td>-0.479</td>
<td>1</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.682</td>
<td>-0.144</td>
<td>-0.317</td>
<td>0.218</td>
<td>-0.107</td>
</tr>
</tbody>
</table>

Bold values are significant at $P < 0.01$

<table>
<thead>
<tr>
<th>Cd</th>
<th>Co</th>
<th>Cu</th>
<th>Mn</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.055</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>-0.252</td>
<td>0.033</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.140</td>
<td>-0.108</td>
<td>0.013</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.543</td>
<td>-0.443</td>
<td>-0.410</td>
<td>0.498</td>
<td>1</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.204</td>
<td>-0.183</td>
<td>-0.383</td>
<td>0.589</td>
<td>0.423</td>
</tr>
</tbody>
</table>

Bold values are significant at $P < 0.01$
ues are shown in bold at \( P < 0.01 \) where \( P \) stands for significance. Strong correlations were noteworthy between metal pairs, like Pb-Cd (0.543) and Zn-Mn (0.589), Pb-Mn (0.498) and Zn-Pb (0.423) cases of imported brands. This simple correlation study revealed some of the common origins of the selected metals in the tobacco of local and imported cigarette brands. On the whole, the correlation study showed some integrated variation in the selected metal levels. Correlation study is a clear indication of difference between tobacco quality of local and imported brands.

In cigarette manufacturing as many as 600-1,400 additives are used and many of them contain additives containing trace metals (Ebisike et al., 2004). Beside additives, the main sources of metals are the paper and the filter. The growing of tobacco involves the use of large amounts of fertilizers, herbicides and pesticides. Many of these are toxic and some contain known carcinogens (cancer causing agents). Depending on geographical location, industrial or mining activities and agronomic practices, certain levels of metals may be higher or lower in the tobacco grown in a certain location (Pappas et al., 2006). The monitoring of trace metals during growing, processing and smoking of tobacco therefore is essential for the protection of environment and our health.

Only about half of the deaths related to smoking are from cancer. It is also a major cause of heart diseases, aneurysms, bronchitis, emphysema, strokes and cataracts. Using tobacco can also damage a woman's reproductive health. The use of tobacco is linked with infertility, a higher risk of miscarriage, early delivery (premature birth), stillbirth, infant death, and a cause of low birthweight in infants. It has also been linked to sudden infant death syndrome (SIDS) (USDHHS, 2004). The risk of HIV transmission in people who smoke tobacco is higher than those who do not (Halsey et al., 1992).

The present study explored a baseline data on trace metal levels in cigarette brands commonly used in Pakistan to health authorities for necessary regulations to keep the environmental burden within tolerable limits. There was no sufficient data about trace metals in tobacco of cigarette brands used in Pakistan.

Moreover it is confirmed that smoking is a source of many toxic metals and quantitatively their distribution is clearly above the safer limit as laid down by WHO. Thus, increase in biological levels of at least these metals which are carcinogenic, represents health risks not only to the active smokers but to passive smokers as well. Therefore it is wise to take drastic measures to minimize exposure to toxic metals as much as possible and government has to play a vital role in this regard.

REFERENCES


Pappas, R.S., Polzin, G.M., Zhang, L., Watson, C.H., Paschal, D.C.