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# HEAVY METALS IN DIFFERENT INDIAN TOBACCO PRODUCTS AND THEIR HUMAN HEALTH IMPLICATIONS

**Shekhar Suman*, Moumita Bhaumik and Subrata Kumar Dey**

Department of Biotechnology, Centre of Genetic Studies, School of Biotechnology and Biological Sciences, Maulana Abul Kalam Azad University of Technology, BF-142, Sector I, Salt Lake, City, Kolkata, West Bengal, 700064, India.

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***Corresponding Author**
**Shekhar Suman**
Department of Biotechnology, Centre of Genetic Studies, School of Biotechnology and Biological Sciences, Maulana Abul Kalam Azad University of Technology, BF-142, Sector I, Salt Lake, City, Kolkata, West Bengal, 700064, India.

## ABSTRACT

Heavy metals are mandatory for most organisms in trace amounts, but they can quickly become toxic when in higher concentrations or when they exist in non-native body locations. In this study, 5 brands of most popular tobacco products of India (Khaini, Zarda and three different Paan masala) were quantified by Inductively Coupled Plasma Optical Emission Spectroscopy for the presence of these heavy metals As, Cd, Cu, Ni, Fe, Pb, Zn and Tl. The variations in metal concentration were observed in all tobacco samples. These heavy metals Cd, Cu, Fe, Zn and Tl were higher in Khaini, and followed by rest samples, but not beyond the toxic level. This could be due to the preferential metal enrichment during both chemical and physical processing in finished product. The concentrations of all heavy metals in all samples were below the permissible limits issued by *Joint FAO/WHO Expert committee on Food Additives*. But, there is a regular need of proper processing and monitoring for the presence of heavy metals, so that limited and controlled value of heavy metals may remain in them.

**KEYWORDS**: Paan masala, Toxic Heavy metals, Micronuclei, Smokeless tobacco, Khaini, Zarda.

## INTRODUCTION

Heavy metals are mandatory parts of biological structures, but at high concentration it can have an inhibitory and toxic biological function.[1] Tobacco products (Table 1) are rich source of heavy metals.[2,3] Their consumption by non-smoking and smoking methods is not only affecting the health of smokers directly, but also affects the health of non-smokers passively.[4] As for example if Selenium (Se) is present in trace amount i.e. 0.1 ppm then it is good for health but its higher concentration i.e. 10 ppm causes Selenosis.[5] Any element, with specific density value greater than 5g/cm³, is considered as heavy element.[6] *International Agency for Research on Cancer* (IARC) has identified and classified about 4000 chemicals in different groups according to its health index.[7] Certain heavy metals like Arsenic (As), Cadmium (Cd), Beryllium (Be), Iron (Fe), Cobalt (Co), Lead (Pb), Nickel (Ni) are declared as human carcinogens by IARC.[7] International bodies such as *World Health Organization*,[8] *Agency for Toxic Substances and Disease Registry*[9]and *Scientific Committee on Emerging and Newly Identified Health Risk*[10] have extensively studied and reviewed regularly the effects of these heavy metals on human health.[11]

Evidence of chromosomal damage in oral cancer patients due to tobacco products, leading to increased micronuclei formation in oral epithelial cells has also been reported.[12] Symptoms like oral sub mucosal fibrosis, leukoplakia, erythroplakia and the stiffening of the oral mucosa are the early indicators of cancer risk.[13,14] One of the major causes for fibrosis in mouth cavities is found to be Copper (Cu), a heavy metal in gutkha, a type of tobacco product.[12] Five million deaths per annum worldwide are attributed to tobacco use. In India, according to WHO,[15] the predicted death due to tobacco consumption may exceed 1.5 million per year by 2020. A large amount of variation in the concentration of these heavy metals in tobacco products has been observed, which are country and product specific.[16]

The aim of this study is to give the updated information about the presence of different heavy metals like Arsenic (As), Lead (Pb), Cadmium (Cd), Iron (Fe), Nickel (Ni), Zinc (Zn), Copper (Cu) and Thallium (Tl) in different Indian tobacco products and paan masala, and compare the occurrence frequency of micronuclei in buccal cells of smokeless tobacco consumers with that of non-consumers.

## MATERIALS AND METHODS

Five different brands of tobacco products were selected from the local market of West Bengal- Khaini (1), Zarda (1) and Paan masala (3), on the basis of popularity amongst the people. In the present study, brand names of the samples are not written because of legal implications. Each sample was marked properly with a unique marking code, collected in an airtight polythene bag and stored in refrigerator. All glasswares were washed and autoclaved properly with deionized water before use. The information for sample preparation was adopted from the most up-to-date published literature.[3]

## SAMPLE PREPARATION

Heavy metals in different tobacco product samples were analyzed through two step process: Dry ashing method followed by Acid digestion method.[17] In Dry ashing method, each raw sample was carefully processed and grinded, separately. Two grams of grinded sample were taken in a silica crucible, and placed in the preheated muffle furnace at a temperature of 500° C. After four hours of proper heating, ash form of sample was obtained. For acid digestion second step, 1ml of concentrated HCl was used to dissolve the obtained ash and was left on a hot plate. When the solution became dry, small dissolving amount of 1% $HNO_3$ was added with appropriate dilution (the samples being diluted to 25 ml with deionized water). The dissolved samples were filtered using Whatman filter paper, in another 25 ml volumetric flask and used for the analysis on *Inductively Coupled Plasma Optical Emission Spectrometry* (ICP–OES), model number iCAP 6300 DUO made by Thermo Fisher Scientific. Standard solution was prepared from 2.5 ml of 100 µg/ g multi standard solution obtained from *National Institute of Standard and Technology* (NIST) in the laboratory for the calibration of ICP-OES.

## CALCULATION

The calculation for daily amount consumption of tobacco products by the consumers was done with the following formula and adopted by Dhaware *et al*.[3]

Daily Intake of Metal (DIM) = Metal concentration in Gutkha sample taken for analysis × Weight of Gutkha sample taken for analysis (pouch weight) × Daily intake of pouch.

Here, the above calculation was done on this assumption that 10 pouches were taken by each person, daily. The obtained data of DIM is given in Table 3.

## BUCCAL CELL COLLECTIONS AND MICROSCOPIC OBSERVATIONS

All persons gave the written assent for the participation. They were guided to clean their mouth properly with sterile water before the collection of buccal fluid containing epithelial cells from their mouth. The collected fluid from the mouth was smeared onto the autoclaved microscope glass slides. Four pre-treated slides with 0.9% NaCl solution per person were prepared and quickly stained with 1 drop of methylene blue aqueous solution (1%). And 400 cells per slide were observed under the microscope with high magnification (1000 X).

## RESULTS AND DISCUSSION

India has one of the largest growing markets for chewing tobacco products and ranks first in it.[18] In the present investigation the calculated micronuclei frequency is given in Table 5 where the mean and standard deviation (SD) for non-consumers are found in the range of 0.33±0.48 (MEAN±SD) while for consumers are 4.92±2.55. The long time use of non-smoking tobacco (Paan masala/ Gutkha/ Chewing tobacco) increases the risk of micronuclei formation and the different oral consequences, too. In Fig. 1 we can see the presence of micronuclei along with the normal nucleus. Literature surveys have given the experimental evidences for the occurrence of carcinogenic effect of non-smoking tobacco and its derivatives. The quantitative analysis for the presence of heavy metals is discussed in the Table 2. Average concentration (AC) and standard deviation (SD) of 8 different heavy metals from 5 different tobacco samples are presented in Table 2 in µg/ g units. The concentration of As and Cd is present Below Detectable Limit (BDL) in some samples (Table 2). On the contrary, other mentioned heavy metals like Cu, Fe, Pb, Zn, and Ni are present more frequently and in more concentrated amount but within the Recommended Permissible Daily Intake Concentration of Heavy Metals (RPDIM) given by JECFA[20] (Table 4). It is found that concentration of Fe followed by Zn is present in higher amount as compared to other heavy metals in all the samples. The overall order of concentration of metals determined in the present study in all product types is Fe > Zn > Cu >Tl > Pb > Ni > Cd, which is similar to the order of metal concentrations found in all types of soil.[19] The absorption of elements by plant is function of pH and different related factors of soil.[21] It is accepted that tobacco plants specifically absorb few heavy metals from soil and accumulate them in leaves[21] but, an interesting result is obtained for As of Khaini sample. This Khaini is produced in West Bengal: a state known for having high As concentration in its ground water[22,23] but its concentration in the khaini sample is below detection limit. Among the different types of paan masala (PM) of present study, PM2 and PM3 show higher metal concentration whereas the PM1 shows comparatively lower metal concentration. This shows that processing of tobacco products enriches the metal content.[24-26] It becomes mandatory to note that these different tobacco products are processed preferentially from the raw tobacco leaves and priced accordingly; in the present case of tobacco products, PM2 and PM3 are the cheapest paan masala compared to PM1 paan masala. However, it is important to mention here that these metals become toxic if it is present in higher concentrations than required for the human health. The regular analytical improvement to this line of research has been made available by various literatures from time to time. Therefore, it becomes mandatory to give a comparative data of this literature with earlier published data (Table 3). In the present study, the mean concentration of As (0.1µg/ g) is comparable as Serbian tobacco products (0.15µg/ g),[27] but on the contrary, the average concentrations of remaining heavy metals are lower than the data provided by the different countries (Table 3).

Although the limits of permissible concentration for different heavy metals have been defined for almost all food materials[28] but very petite information is available on tobacco products, hence there is an urgent need to pay attention to the standardization of limits for heavy metals in these tobacco products.

**Arsenic-** According to ATSDR and IARC, As is Group 1 element for its carcinogenic nature.[28,29] The JECFA has set the As level to 1 µg/kg bw/day.[20] In the present study, only in one tobacco sample, khaini, As is found (0.1 µg/ g). This level is lower than the RPDIM value. [20]

**Cadmium-** According to IARC monograph (2012), Cd is Group 1 element. In the present study (Table 2), Cd is found below the permissible level in all samples (Table 4). According to JECFA,[20] the RPDIM value of Cd is 0.5 µg/kg bw/day. This gives the information that at higher uptake of Cd (greater than 0.5 µg/kg bw/day), adverse symptoms on brain and kidney are observed.[30] Due to having half-life period of almost 10 years, its accumulation is observed in kidney. This causes many diseases like Tubular Cell Necrosis.[31] In the present study, its DIM value (Table 2) is not greater than the RPDIM value (Table 4).

**Copper-** Cu is an essential trace element in human metabolism. According to FAO/WHO (2011), the RPDIM value is 140 µg/ kg b.w. /day. As per previous studies, Cu is responsible for the formation of free radical, via Fenton or Haber-Weiss reaction. This gives the information that at higher uptake of Cu (greater than 140 µg/kg b.w./day), adverse effect on DNA and mitochondrial membrane is observed.[32] Gutkha containing Cu has significant role in the stimulation of Sub Mucous Fibrosis.[12,13] In the present study, its DIM value (Table 2) is not greater than the RPDIM value (Table 4).

**Iron-** IARC Monographs (2012) has declared Fe as Group 1 element. According to FAO/WHO (2011), the RPDIM value is 800 µg/kg b.w./day. As per previous studies, at higher uptake (greater than 800 µg/kg b.w./day) Fe is responsible for the shock, failure of liver, coma, metabolic acidosis and death.[33] In the present study**,** its concentration is highest among all samples (Table 2), but within the permissible limits (Table 4).

**Lead-** The FAO/WHO has confirmed a RPDIM value for Pb as 3.6µg/kg b.w./day. IARC (2012) has declared Pb under Group 2B. According to the JECFA,[20] inhalation (50%), dietary source (40%) and food and drinking water (10%) are the contributors for the accumulation of Pb in body. This gives the information that higher uptake of Pb (greater than 3.6 µg/kg b.w./day), supports the retention of Pb in blood, thereby impacting the cardiovascular, immune and hematic system.[27,30,34] It is found that in present tobacco samples, the Pb level is within the permissible range, as shown in Table 4.

**Zinc –** Zn is an essential trace metal for human. According to IARC Monographs (2012), Zn is not classified in any carcinogenic group. According to FAO/WHO (2011), the RPDIM value is 500 µg/kg b.w./day. This gives the information that at higher uptake of Zn (greater than 500 µg/ kg b.w./day), adverse effect like ataxia, hemolytic anemia and kidney damage is observed.[33] In the present study, DIM value (Table 2) is not greater than the RPDIM value (Table 4).

**Nickel –** IARC Monographs (2012) has classified Ni as Group 2B element. According to FAO/WHO (2011), the RPDIM value is 50 µg/kg b.w./day. This gives the information that at higher uptake (greater than 50 µg/kg b.w./day), Ni compounds produce carcinogenic effect and initiate genetic defects like breakage in DNA strands, cross linking between DNA-Protein, micronuclei formation, alteration in concentration of nucleic acid etc. On the contrary, there are some experimental evidences that support immunomodulatory and immunotoxic nature of Ni.[35] In this study, the calculated DIM value of Ni (Table 2) is not greater than the RPDIM value (Table 4).

**Thallium –** Tl is another element which is found in all samples, in variable concentrations. Its permissible daily intake value is not standardized by IARC and JECFA but is retrieved from some publications. Data reports that its lethal dose is from 10 to 15 mg/kg.[36]

Because Tl has equal charge and ionic radius as similar as Potassium (K) ion, so it interferes easily in K-dependent metabolic pathway. Probable toxic metabolic interactions of Tl cause disturbance in homeostasis of calcium, stoppage of cellular respiration due to interaction with riboflavin cofactor.[37] Its charge similarity with potassium ion also helps in easy absorption through digestive system and respiratory system.[38]

**Table 1: Types of tobacco products and their common ingredients.**

| Products | Source |
|---|---|
| **Khaini** | Tobacco leaves, Slaked lime paste. |
| **Zarda** | Dry or moist form of tobacco leaves having spices for various tastes. |
| **Gutkha and Paan Masala** | Mixture of Betel Nut, Tobacco flavoring agents and Catechu. |

**Table 2: Analyzed concentration of heavy metals with standard deviation and daily intake value of heavy metals (DIM) from smokeless tobacco and its products.**

| HEAVY METALS | | SAMPLES | | | | |
|---|---|---|---|---|---|---|
| | | **KHAINI** | **ZARDA** | **PM 1** | **PM 2** | **PM 3** |
| **As** | **AC±SD** | BDL | BDL | BDL | BDL | 0.10±0.0012 |
| | **DIM** | NA | NA | NA | NA | 1.75 |
| **Cd** | AC±SD | 0.34±0.0003 | 0.10±0.0001 | BDL | BDL | BDL |
| | **DIM** | 6.85 | 1.00 | NA | NA | NA |
| **Cu** | AC±SD | 8.58±0.0029 | 6.61±0.0056 | 8.00±0.0024 | 5.31±0.0035 | 5.20±0.0009 |
| | **DIM** | 170.35 | 66.13 | 80.05 | 119.47 | 95.67 |
| **Fe** | AC±SD | 288.20±0.0985 | 233.12±0.1696 | 77.01±0.0150 | 74.87±0.037 | 137.62±0.0740 |
| | **DIM** | 5765.00 | 2331.25 | 770.12 | 1684.57 | 2546.06 |
| **Pb** | AC±SD | 0.87±0.0023 | 0.90±0.0019 | 0.83±0.0005 | 0.78±0.0006 | 0.60±0.0009 |
| | **DIM** | 17.50 | 9.00 | 8.33 | 17.60 | 10.93 |
| **Zn** | AC±SD | 38.51±0.0199 | 8.92±0.0029 | 6.42±0.0027 | 6.13±0.0014 | 7.50±0.0016 |
| | **DIM** | 770.25 | 89.22 | 64.20 | 138.00 | 138.50 |
| **Ni** | AC±SD | 0.71±0.0005 | 0.91±0.0003 | 0.54±0.0004 | 0.64±0.0002 | 0.61±0.0001 |
| | **DIM** | 14.3 | 9.175 | 5.47 | 14.48 | 11.39 |
| **Tl** | AC±SD | 1.76±0.0272 | 0.85±0.0357 | 0.30±0.0512 | 0.25±0.0164 | 0.35±0.0262 |
| | **DIM** | 35.00 | 8.55 | 3.02 | 5.76 | 6.40 |
| **AC** = Average Concentration (µg/ g), **SD** = Standard Deviation, **DIM** = Daily Intake of Metal (µg/day), **BDL**= Below Detectable Limit, **NA=** Not Available. (p = 0.05). | | | | | | |

**Table 3: Concentration (µg/ g) of Heavy Metals in different samples of tobacco products obtained from different countries.**

| As | Cd | Cu | Fe | Pb | Zn | Ni | Tl | COUNTRY | REFERENCE |
|---|---|---|---|---|---|---|---|---|---|
| - | - | - | 1703 | - | 12.25 | - | - | India | [39] |
| - | - | - | 1565 | - | 19 | - | - | India | [40] |
| - | - | 11 | 1050 | 4.5 | 20 | - | - | India | [41] |
| - | 0.8 | 12 | 1100 | 4.5 | 28 | 1.5 | - | India | [42] |
| - | 0.38 | 42 | 853 | 8.38 | 56 | 2.37 | - | India | [2] |
| 0.15 | - | - | - | 0.93 | - | - | - | Serbia | [27] |
| - | - | 16.05 | 520.59 | 1.52 | 27.42 | 3.75 | - | Pakistan | [16] |
| - | 0.472 | 4.17 | 684.53 | 3.30 | 25.41 | - | - | India | [35] |
| **0.1** | **0.22** | **6.47** | **162.16** | **0.79** | **13.49** | **0.69** | **0.702** | **Present Data analysis** | |

**Table 4: Recommended Permissible Daily Intake concentration of heavy Metals (RPDIM).**

| Heavy Metals | Acceptable Daily Intake | For Adult (60 kg) | Source |
|---|---|---|---|
| **As** | 1 µg/ kg b.w. / day | 60 µg/day | JECFA |
| **Cd** | 0.5 µg/ kg b.w. / day | 30 µg/day | JECFA |
| **Cu** | 140 µg/ kg b.w. / day | 8400 µg/day | JECFA |
| **Fe** | 800 µg/ kg b.w. / day | 48000 µg/day | JECFA |
| **Pb** | 3.6 µg/ kg b.w. / day | 216 µg/day | JECFA |
| **Zn** | 500 µg/ kg b.w. / day | 30000 µg/day | JECFA |
| **Ni** | 50 µg/ kg b.w. / day | 3000 µg/day | JECFA |
| **b.w.** = Body weight, **JECFA** = Joint FAO/WHO Expert Committee on Food Additives. | | | |

**Table 5: Comparative analysis for the distribution of micronuclei between Non-Consumers and Consumers of smokeless tobacco products.**

| **Non-Consumers** | | | | | **Consumers** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Individual** | **Micronucleus** | | | | **Individual** | **Micronucleus** | | | |
| | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 |
| **1** | 0 | NA | NA | NA | **1** | 5 | NA | NA | NA |
| **2** | 1 | NA | NA | NA | **2** | 3 | NA | NA | NA |
| **3** | 0 | NA | NA | NA | **3** | 7 | NA | NA | NA |
| **4** | 0 | NA | NA | NA | **4** | 8 | NA | NA | NA |
| **5** | 0 | NA | NA | NA | **5** | 4 | NA | NA | NA |
| **6** | 1 | NA | NA | NA | **6** | 1 | NA | NA | NA |
| **7** | 1 | NA | NA | NA | **7** | 4 | NA | NA | NA |
| **8** | 0 | NA | NA | NA | **8** | 1 | NA | NA | NA |
| **9** | 0 | NA | NA | NA | **9** | 1 | NA | NA | NA |
| **10** | 1 | NA | NA | NA | **10** | 2 | NA | NA | NA |
| **11** | 0 | NA | NA | NA | **11** | 4 | NA | NA | NA |
| **12** | 0 | NA | NA | NA | **12** | 9 | NA | NA | NA |
| **13** | 0 | NA | NA | NA | **13** | 5 | NA | NA | NA |
| **14** | 1 | NA | NA | NA | **14** | 7 | NA | NA | NA |
| **15** | 0 | NA | NA | NA | **15** | 1 | NA | NA | NA |
| **TOTAL** | 5 | NA | NA | NA | **TOTAL** | 62 | NA | NA | NA |
| **NA=** Not Available. | | | | | | | | | |

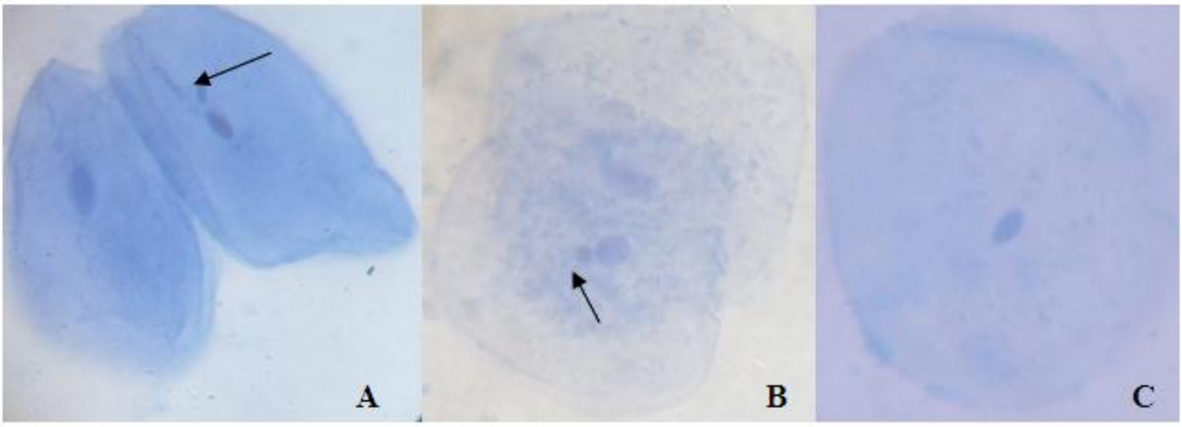


**Fig 1: Buccal Cells. (A) and (B) Micronuclei Containing Buccal Cells, (C) is Normal cell. (1000 X).**

## CONCLUSION

In conclusion, this quantitative investigation is giving the overall information about the present concentration level of heavy metals in taken tobacco samples, which is not beyond the toxic level. But, there is a regular need of proper processing and monitoring for the presence of heavy metals, so that limited and controlled value of heavy metals may remain in them. Here on the basis of comparative analysis it is found that the concentration of these heavy metals Cd, Cu, Fe, Zn and Tl is higher in Khaini, but not beyond the toxic level. Along with the most studied metals we should also monitor the presence and entry of other elements, because their presence can adversely affect consumer's health. We have to give regular information to all consumers about the atrocious effects of smokeless tobacco consumption. This noble and strenuous effort may decrease the dreadful effects of future oral carcinogenic and cellular abnormalities.

## CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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