

Pearson correlation analysis

For correlation analysis, values obtained that were higher than 0.50 were considered to correlate various data points and the results are summarized in Table 5. Values in bold indicated significant correlation between variables at higher confidence interval (** $P < 0.01$). If the values obtained were positive, it showed positive correlation among the variables, whereas, the negative values signified negative correlation. The correlation values near to “zero” indicated poor and nonsignificant positive or negative correlation and on the other hand, values closer to “one” indicated significant correlation among variables. Interestingly, no any pair of elements displayed significant negative correlation among themselves at both the confidence levels ($P = 0.01$ and $P = 0.05$). Rubidium (Rb) showed an interesting behavior by not showing any significant positive or negative correlation with any of the element. Another element, Zinc (Zn), showed positive significant correlation with only one element (Co). Therefore, these two elements were placed in two distinct groups. The remaining elements were placed in three different groups following the interpretation of the correlation matrix. Group I consisted of elements such as Li, As, Sr, Cd, U, Ba, Mn, Be, Se, Co, and Cu. Group II comprised Cs, V, Pb, and Ga whereas, elements such as Ag, Cr, and Tl were placed in group III. Group IV and Group V consisted of Zn and Pb, respectively. This grouping of elements was further analyzed through another multivariate analysis, i.e., principal component analysis.

Principal component analysis

Principal component analysis was performed on the whole data and the results obtained are summarized in Table 6. It shows the rotated component matrix of the principal component analysis. All the variables are grouped in five principal components (PC1 to PC5). Data in bold showed the absolute values >0.50 . Elements of Group I were found to give major contribution to principal component 1. Similarly, Group II elements of the correlation matrix were the major contributor to principal component II. Three major principal components were observed as shown in Figure 3 that depicts the component plot in rotated 3D-space. It shows the exact placement of elements in their principal components according to their absolute values.

DISCUSSION

All the detected elements were classified into (a) trace, (b) toxic, (c) essential, and (d) nonessential elements based on their nature and abundance and their presence in tobacco samples were studied. Various

trace elements present were Li, Zn, Cu, Cr, Co, V, As, Rb, Sr, Mn, and Se, whereas toxic elements such as As, Pb, Cd, Be, Cu, Li, Mn, Ag, Zn, Ba, and Tl were also present. These elements are considered to be toxic when consumed even in smaller quantities for a long period. Arsenic (As) is known to cause cancer and diabetes, whereas, Pb is responsible for anemia, plumbism, and encephalopathy. Cadmium (Cd) causes high blood pressure, lung cancer, and osteomalacia, whereas, Be leads to cardiovascular disorders. Similarly, other elements have various harmful effects on the body when consumed regularly. Essential elements that were present in the tobacco samples were Co, Cu, Cr, Mn, Se, and Zn. These elements were present in trace quantities in all the samples. Furthermore, the presence of nonessential elements was also observed. These elements were V, As, Ba, Be, Cd, Ga, Ag, Pb, Li, Rb, Sr, Cs, Tl, and

Table 6: Component matrix obtained for principal component analysis showing placements of elements in five principal components (PC1-PC5)

| | Rotated component matrix ^a | | | | |
|----|---------------------------------------|--------------|--------------|--------------|---------------|
| | Component | | | | |
| | PC1 | PC2 | PC3 | PC4 | PC5 |
| Li | 0.942 | 0.054 | 0.086 | -0.0106 | 0.110 |
| As | 0.902 | 0.276 | -0.063 | 0.150 | -0.090 |
| Sr | 0.902 | 0.124 | 0.171 | 0.063 | 0.179 |
| Cd | 0.870 | -0.011 | 0.035 | 0.140 | 0.142 |
| U | 0.832 | 0.053 | 0.428 | -0.143 | 0.097 |
| Ba | 0.820 | 0.298 | 0.025 | 0.108 | 0.284 |
| Mn | 0.806 | 0.543 | -0.070 | 0.164 | 0.038 |
| Be | 0.783 | 0.480 | 0.161 | -0.229 | -0.137 |
| Se | 0.778 | -0.207 | 0.397 | 0.336 | 0.105 |
| Co | 0.759 | 0.493 | -0.148 | 0.306 | -0.001 |
| Cu | 0.679 | 0.140 | 0.165 | 0.377 | -0.145 |
| Cs | 0.054 | 0.942 | 0.221 | -0.119 | 0.029 |
| V | 0.270 | 0.898 | -0.081 | 0.240 | 0.035 |
| Pb | 0.077 | 0.845 | -0.054 | 0.423 | 0.013 |
| Ga | 0.623 | 0.748 | 0.089 | 0.123 | 0.006 |
| Ag | 0.066 | -0.029 | 0.982 | 0.025 | 0.060 |
| Cr | 0.241 | 0.073 | 0.901 | 0.031 | -0.008 |
| Tl | -0.074 | 0.423 | 0.549 | 0.474 | 0.355 |
| Zn | 0.214 | 0.284 | 0.067 | 0.870 | -0.108 |
| Rb | -0.227 | -0.025 | -0.087 | 0.082 | -0.920 |

Extraction method: Principal component analysis; Rotation method: Varimax with kaiser normalization. ^aRotation converged in 6 iterations. Values in bold indicates the absolute values >0.50

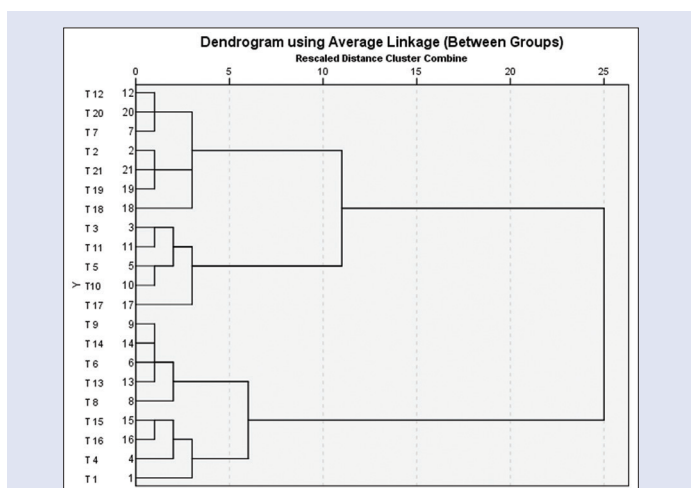


Figure 2: Hierarchical cluster analysis – dendrogram for 21 samples

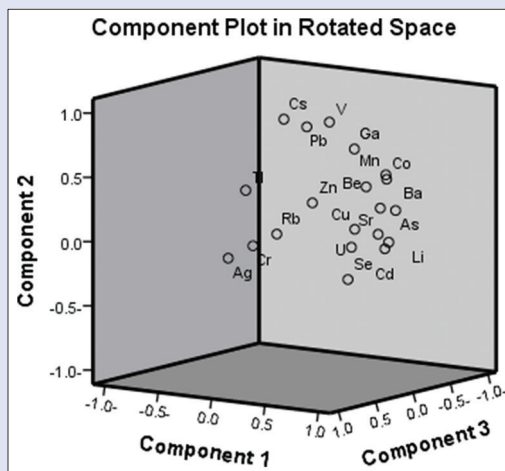


Figure 3: Component plot obtained after Principal component analysis showing the placement of elements in three major principal components

U which were present in low-to-high concentrations.

Metals, such as Co, Cu, Cr, Mn, Se, and Zn, are referred to as essential nutrients because these are required for various physiological and biochemical functions of the body.^[15] The insufficiency of the supply of these elements results in various diseases or syndromes. These elements have important roles as constituents of several enzymes and take part in several redox reactions in biological systems.^[15] For example, Cu is an essential cofactor for many oxidative stress-related enzymes, such as peroxidase, cytochrome c oxidases, catalase, monoamine oxidase, etc.^[16-18] Moreover, Cu has toxic properties too as it can exhibit transitions between Cu (II) and Cu (I), which generate reactive oxygen species (ROS), including superoxide and hydroxyl radicals.^[16-19] Excessive Cu intake has been reported to cause cellular damage, which leads to Wilson disease in humans.^[18,19] Similar to Cu, other essential elements that are present in tobacco are required for biological functioning. However, excessive amounts of these metals produce damage to tissues and cells, resulting in various harmful effects and diseases in humans. Cu along with Cr has a very narrow range of concentrations between beneficial and toxic effects.^[19,20]

Other elements, such as As, Ba, Be, Cd, Ga, Pb, Li, Ag, Sr, Tl, V, and Uranium (U), which are present in all tobacco samples in high to medium concentrations, do not have any established roles in biological functions and are thus considered non-essential elements.^[20] Strontium (Sr) was found to be present in highest concentrations in all the samples that is similar in properties and uses to calcium (Ca), but its use is restricted nowadays because of its harmful effects on the body including various cardiovascular disorders.^[21] After Sr, the other highly abundant metal found in tobacco samples was Mn, which comes under the essential metal ions category. Mn has an important role in maintaining human health and is essential for the development and metabolism in humans. However, its excessive intake causes a set of disorders called manganism, which is a neurodegenerative disorder that affects the dopaminergic nerves and leads to a condition similar to Parkinsonism.^[22-23]

The presence of heavy metal ions in tobacco samples was also investigated, and their concentrations were compared with the prescribed limits. The heavy metals present in these tobacco samples were As, Cu, Cr, Co, Zn, Ag, Cd, Pb, Se, and Tl. Although, except Zn, the presence of other heavy metal ions was comparatively less but cannot be ignored. Heavy metals in biological systems are known to have pronounced effects on cellular components and various enzymes involved in metabolism and repair.^[24] They interact with the cellular components and cause conformational changes that damage these components and lead to cancer and apoptosis.^[24,25] Heavy metals, such as As,^[26-28] Cd,^[29] Cr,^[30,31] and Pb,^[32,33] are known to produce carcinogenic effects due to their capability to produce ROS in biological systems. These elements are among those that pose a significant hazard to human health. These elements are all systemically toxic and cause damage to many organs, even at low concentrations. Various experimental and epidemiological studies have reported that these elements are placed under the category “known” carcinogens by the U. S. EPA and the International Agency for Research on Cancer.

CONCLUSION

The concentrations of various elements in all 21 tobacco samples were measured successfully by ICP-MS. The tobacco samples were of different varieties, color, and origin, and most of the elements in these samples showed some variation in concentration. Elements were found in either significantly high or relatively low concentrations. Different types of heavy and toxic metals, which could lead to various health hazards like cancer and cardiovascular diseases, were found in all tobacco samples. Further studies should be conducted to discover other toxic substances that are present in these tobacco samples to justify their potential in causing diseases.

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Conflicts of interest

There are no conflicts of interest.

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