International Journal of Ecology and Environmental Sciences
www.ecologyjournal.in
Online ISSN: 2664-7133; Print ISSN: 2664-7125; Impact Factor: RJIF 5.28
Received: 27-01-2021; Accepted: 28-02-2021; Published: 14-03-2021
Volume 3; Issue 1; 2021; Page No. 430-432

Estimation of some toxic metals from different brands of processed tobacco leaves

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Abstract
The aim of present study was to examine the levels of lead, cadmium and chromium in different brands of tobacco. Five different brands of processed tobacco leaves were purchased from local market of Raipur. Each sample was marked properly with a unique marking code, collected in an airtight polythene bag and transferred immediately to the laboratory. Then, samples were kept in the oven at 80 °C for 2 - 4 h. The samples were finely grinded and sieved through 100 (0.1 mm) nylon mesh. After complete drying and sieving, 1 g of each sample in three replicates was weighed with accurate scale; and acid digestion for each sample was done according to the validated procedure by NAFDAC (National Agency for Food and Drug Administration and Control). The digested samples were filtered and the contents were transferred in polythene sample bottles and analyzed for selected elements with Atomic Absorption Spectrometer. The results indicate the values obtained for Pb were ranged from 0.25 mg/kg to 0.34 mg/kg for different brands. Highest value was recorded for sample 1 (0.34mg/Kg) and remaining four samples had almost similar concentration i.e. 0.25 mg/Kg for sample 2, 3, 4 and 0.26 mg/Kg for sample 5. As compared to the permissible values recommended by WHO it is clear that all the samples of tobacco leaves have concentration of lead below the permissible value (0.5 mg/Kg). The values obtained for Cd were ranged from 0.039 to 0.049 mg/Kg. All the brands exceed the permissible values (0.02 mg/Kg) recommended by WHO (Table 2). Maximum value 0.049 mg/kg was recorded for sample 1 followed by 5, 4, 3 is 0.040 mg/kg, 0.040 mg/kg, 0.044 mg/kg respectively and minimum for brand 2 i.e. 0.039 mg/Kg. The overall Cd concentration was more than 0.02 mg/Kg. The values of Cr were ranged from 0.202 mg/Kg to 0.306 mg/Kg which was below the permissible values (1.30 mg/Kg) recommended by WHO. Maximum concentration of Cr was recorded in sample 3 and minimum in sample 2.

Keywords: tobacco leaves, processed, lead, cadmium, chromium

Introduction

India is the world's second largest tobacco growing country (Annual Survey of Industries, 1997-98) [1]. In 1992 it produced 7% of the world's total unmanufactured tobacco and 14% of the world's total manufactured tobacco in the form of cigarettes and biris. The overall contribution of the tobacco industry to India's large agricultural sector is that it employs two-thirds of the country's labour force. Approximately 3.5 million people are employed in tobacco cultivation in India (Jacobs, 2000) [11]. However, manufacturing of tobacco products other than cigarettes like biris and various forms of chewing and smokeless tobacco, largely takes place within the unorganized sector, providing employment for what is estimated to be millions of women and children who work at home (Country case reports, 2001) [5]. With substantial evidences it is confirmed that tobacco contains and delivers a number of harmful substances including cancer causing species into body of its user. Some of these substances are present naturally in the tobacco while a number of them are added externally to it during curing process to make it more delicious and effective.

Both tobacco and tobacco smoke contain a large variety of chemicals. Nearly 3000 chemical constituents have been identified in smokeless tobacco, while close to 4000 are present in tobacco smoke (Lyon, 2007 and J. Fowles *et al.* 2000) [15, 6]. In addition, a wide range of toxic metals including mercury, lead, cadmium, chromium, nickel and other trace elements have been found in Indian tobacco (U. C. Mishra *et al.* 1983 and Shaikh *et al.* 2002) [16, 19].

A hazardous evaluation of tobacco contents indicates that it contains about 200 poisonous and about 63 carcinogenic contents (Lyon *et al.* 1986 and E. Nelson 2001) [14, 17]. Chewing tobacco and snuff contain about 28 carcinogens. Hence both types of tobacco i.e. smoking as well as smokeless tobacco affect the health badly and mainly cause cancer. Former is responsible for lung cancer-adenocarcinoma while later is for mouth, esophageal cancer.

As is certified from different references, inorganic constituents of tobacco include some metals concluding that smoking constitutes important source of heavy metals input in the user's body (Chiba *et al.* 1992 and Lugon-Moulin *et al.* 2006) [3, 13]. Phosphate fertilizers, which are used in tobacco cultivation, contain high concentrations of metals (Golia *et al.* 2007). The use of phosphate fertilizers in the fertilization of crops is one of the primary factors in the pollution of agricultural soils (Golia *et al.* 2007 and Q.B. He *et al* 1994) [7, 8].

About 76 metals have been detected in cigarette tobacco; of these more than 30 metals have been detected in smoke (Fowles *et al.* 2000) [6]. Lead is a nonessential and toxic metal for human biology and has recently been elevated from a group II B to a group II A carcinogen (International Agency for Research on Cancer, IARC 2004) [10]. High levels of exposure may result in toxic biochemical effects in humans which in turn cause problems in the synthesis of haemoglobin, effects on the kidneys, gastrointestinal tract, joints and reproductive system and acute or chronic damage to the nervous system. Cadmium toxicity especially affects humans because of their longevity and the accumulation of cadmium in their organs by consuming cadmium-contaminated plant products. Tobacco concentrates cadmium, leading to human exposure to this carcinogenic metal through smoking (Clarke and Brennan *et al.*, 1989; Sravrides 2006 and Golia *et al.*, 2007) [4, 22, 7]. Other diseases associated with cadmium exposure are pulmonary emphysema and the Itai-Itai disease (Booth 2005) [2], which results in painful bone demineralization (osteoporosis), because cadmium replaces calcium in the bones. Chromium is an essential trace metal which is required by body for certain physiological processes like glucose metabolism, however at higher concentrations it can cause adverse health effects and serious diseases like cancer, nausea and skin ulcer etc. and ultimately proves lethal to life (Kiilunen 1997) [12].

Material and Methods

Five different brands of tobacco leaves were purchased from local market of Raipur. The brand names of the samples are not written because of legal implications. Approximately 50 gm sample of each type of brand was collected. The collected samples were put in polyethylene bags, and transferred immediately to the laboratory. Then, samples were kept in the oven at 80 °C for 2 - 4 h. The samples were finely grinded and sieved through 100 (0.1 mm) nylon mesh. After complete drying and sieving, 1 g of each sample in three replicates was weighed with accurate scale; and acid digestion for each sample was done according to the validated procedure by NAFDAC (National Agency for Food and Drug Administration and Control). In this method, 10 ml of 69%concentrated nitric acid was added to 20 ml of the sample and the mixture was evaporated on a hot plate in a fume cupboard until the brown fumes disappears leaving white fumes. If brown fumes persist, 5 ml of 69%concentrated nitric acid and 5 ml of 30% H_20_2 was added after cooling the sample. Refluxed the sample at 90ºC until reduces to 2-5 ml. Subsequently, additional distilled water was added to make up the volume to 100 ml which was then filtered with whatman filter paper and ready for analysis using atomic absorption spectrophotometer (AAS).

Stock standard solutions of Merck of 1000 ppm concentrations were used for each metal. A series of standard solutions of Cd, Cr and Pb in the optimum concentration range by appropriate dilution from its stock solution with 0.5% HNO3 was prepared and measured their absorbance. Then absorbance of the test solutions was measured. Before sample analysis, nebulizer was rinsed by aspirating 0.5% HNO3.

Result and Discussion

The level of cadmium, lead and chromium found in different processed tobacco leaves are reported in the table 1. Three samples for each brand were prepared to follow the accuracy of digestion method used. Mean Cd, Pb and Cr concentration for each brand is obtained by analyzing the three samples of that same brand, results obtained for each brand of different tobacco leaves categories are as follows

Table 1: Concentration (mg/Kg) of selected Heavy Metals in some brands of tobacco leaves

SN	Name of Samples (Code)	Concentration of Heavy Metals (mg/Kg)		
		Pb	Cd	Cr
1.	TBL 1	0.34	0.049	0.280
2.	TBL 2	0.25	0.039	0.256
3.	TBL 3	0.25	0.040	0.306
4.	TBL 4	0.25	0.040	0.202
5.	TBL 5	0.26	0.044	0.281

Table 2: Comparison of Heavy Metals in some brands of tobacco leaves with permissible values recommended by WHO

SN	Metals	WHO*/FAO Permissible values (mg/kg)	TBL 1	TBL 2	TBL 3	TBL 4	TBL 5
1	Pb	0.5	0.340	0.250	0.250	0.250	0.260
2	Cd	0.02	0.049	0.039	0.040	0.040	0.044
3	Cr	1.30	0.280	0.256	0.306	0.202	0.281

The results indicate the values obtained for Pb were ranged from 0.25mg/kg to 0.34 mg/kg for different brands. Highest value was recorded for sample 1 (0.34mg/Kg) and remaining four samples had almost similar concentration i.e. 0.25 mg/Kg (for sample 2, 3, 4) and 0.26 mg/Kg for sample 5. All the samples have concentration of lead below the permissible value (0.5 mg/Kg) recommended by WHO (Table 2). Our results are similar to the findings of WHO*/FAO, but the results are far less than the values reported by Verma, (2009) in different tobacco products.

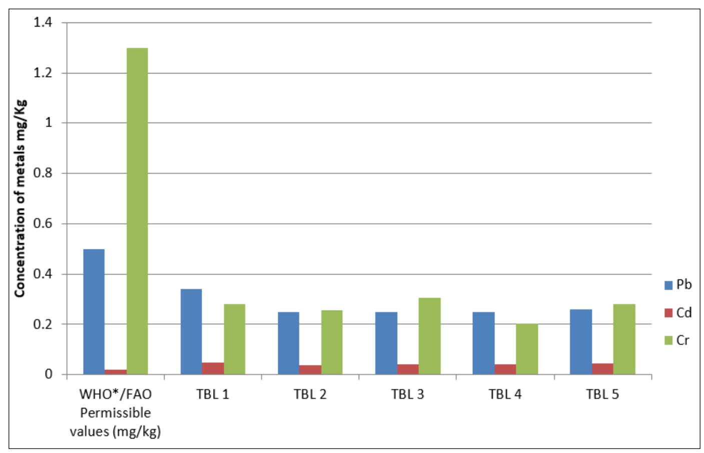

Fig 1: Graphical presentation of comparison of Heavy Metals in some brands of tobacco leaves with permissible values recommended by WHO

The values obtained for Cd were presented Table 1, which ranged from 0.039 to 0.049 mg/Kg. All the brands exceed the permissible values (0.02 mg/Kg) recommended by WHO (Table 2). Maximum value 0.049 mg/kg was recorded for sample 1 followed by 5, 4, 3 is 0.040 mg/kg, 0.040 mg/kg, 0.044 mg/kg and minimum for brand 2 i.e. 0.039 mg/Kg. The overall Cd concentration was more than 0.02 mg/Kg. The overlook of result for cadmium indicates a very small variation among different brands. The tobacco leaves are processed almost in the same way but added additives may differ, which results in small variation among metal concentration for different brands. Tobacco plants have a specific ability to absorb cadmium from soil and to accumulate it in unusually high concentrations in leaves (Canberra, Australia 2000 and Country case reports 22 june 2001) [5] and (Smith, et.al 2000 Sonnenfeld 1985) [20, 21]. During the comparison of our study with other reported Indian literature our results are in good agreement with the study made by Shaikh *et al*. 2002., less than the results of (Mishra *et al* 1983) [16] but more similar to the findings of WHO*/FAO. All these studies clearly indicate that tobacco smoking/use creates an additional cadmium load in the body.

The values of Cr ranged from 0.202 mg/Kg to 0.306 mg/Kg which was below the permissible values (1.30 mg/Kg) recommended by WHO, presented in Table 2. Maximum concentration of Cd was recorded in sample 3 and minimum in sample 2. Idejhe *et al* 2013 determined the level of Cr, Cd and Pb in selected cigarettes & tobacco leaves and were found below the WALOH standards for human consumption and plant uptake. Regassa and Chandravanshi 2016 found that concentrations of Cr and Ni in tobacco leaves from Billate were below the detection limits. Their study showed that the metal contents of tobacco leaves varied with the geographical origin in which the tobacco plant grows.

Conclusion

The investigation has indicated the presence of heavy metals (Pb, Cd, Cr), provided baseline data for comparison, give good awareness for general people (smokers and tobacco chewers), Indian Tobacco Enterprises and Health Organizations. The concentrations of most of the metals detected are found to be within the range of literature values, except cadmium, which was found to be slightly higher than literature values. Fertilizer and processing method could be the main contributor for the higher concentration of Cd in these processed tobacco leaves. The Cd content that determined in the samples is not only the content of processed leaves itself but also the metal originated from contamination of raw leaves during the process, starting from harvesting to manufacturing. Therefore, to control further contamination of tobacco leaves with toxic metals, well treatments in handling, transportation and storage of tobacco leaves are recommended.

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