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TOXICITY ASSESSMENT OF TEA LEAVES AND PROCESSED TEA MARKETED IN SONITPUR AND BISWANATH DISTRICTS OF ASSAM, INDIA USING XRF, FAAS AND GC/MS

Dr. Parag Moni Baruah.
Head, Department of Biology, Swadeshi Academy Junior College, Guwahati, Assam, India, Adjunct professor, Mahatma Gandhi University, Ri Bhoi, Meghalaya, India.

ABSTRACT

The aim of this quest was to go for a toxicity assessment of tea leaves and processed tea marketed in Sonitpur and Biswanath districts of Assam in terms of toxic element and residual pesticide contamination. A survey study was carried out to gather information about the pesticides used in some gardens of the concerned region. Samples of tea leaves and processed tea were collected randomly, sundried, treated and analyzed for probable existence of toxic elements and residual pesticides using XRF FAAS and GC/MS. Deltamethrin, Propergite, Glyphosate, Thiomethoxam, 2, 4-D Amine salt and Azadirachtin were found to be the most commonly used pesticides. XRF spectrometric analysis of the samples of tea leaves revealed presence of 16 elements including lead, a toxic metal element. Quantitative estimation of lead employing FAAS, however, shows its concentration below detection limit (0.0001 ppm) of the instrument and much lower than the WHO Permissible standards (10 ppm). GC/MS analysis has shown evidence of 20 compounds in the analyzed processed tea samples. But, none of the pesticides currently in use matched with the detected compounds. The overall results indicated that the samples analyzed are not in a toxic state and considered safe for consumption.

Keywords: XRF, FAAS, GC/MS, Residual pesticides.

INTRODUCTION:

Toxic metal element and Pesticide contamination have been reported in various medicinal plants throughout the globe. The leaves of tea plant, C*amellia sinensis* and tea products best known for their significant medicinal value, have been reported to contain a wide range of toxic elements and residues of pesticides. Amirahmadi et al. detected the existence of 25 pesticides in consumed tea in Tehran Market by GC/MS (1) Cojocariu et al. carried out GC-MS/MS analysis of Pesticide residue in Green Tea by QuEChERS extraction and identified various classes of pesticides such as OC, OP, pyrethroids and so on (2). Schwalfenberg et al. performed analysis of toxic element on thirty different varieties of teas and found all brewed teas contained lead in a concentration considered unsafe for consumption (3). According to World Health Organization (WHO), the maximum permissible limit of the heavy metal, lead in medicinal plants is 10 ppm (4). Zhang et al. carried out the analysis of Agricultural residues on Tea using GC-NCI-MS and UHPLC-MS/MS and quantified 39 pesticides (5).

The present study dealt with the toxicity evaluation of tea leaves and processed tea marketed in Sonitpur and Biswanath districts of Assam, India.

MATERIALS AND METHODS:

Survey study: For toxicity assessment, as a first step, a survey study about pesticide application practices in seventeen tea gardens, ten from Sonitpur and seven from Biswanath district of Assam was carried out through questionnaires and interviews of the Tea garden managers and field officers to gather information about the pesticides used.

Methods for experimental study:

Sample collection: For XRF spectrometric study, fresh mature tea leaves were collected (plucked) from tea plants of some tea gardens of Tezpur (Lat. 26° 36' 34" North, Long. 92° 49' 37" East) and Gohpur (Lat.26° 53' 0" North, Long. 93° 38' 0" East) sub-divisions of Sonitpur and Biswanath districts of Assam about ten days after the application of pesticides. For GC/MS study, processed tea samples available in the markets of Tezpur and Gohpur regions were collected (purchased) randomly.

Experimental procedure:

Pretreatment and sample preparation for XRF analysis:

Before carrying out XRF study, the collected samples were moderately sun dried to ensure complete loss of moisture content. The dried samples were then crushed in a mortar with pestle. After pre-treatment of the samples, the same were pulverized and 1 gm of each sample was mixed with 0.5 gm of boric acid (H_3BO_3). The above mentioned mixture was placed in a sample cup made of aluminium of the size 4cm (outer diameter) and 3.5 cm (inner diameter). The whole thing was then inserted in a hydraulic press and a load of 50 KN was applied for 3 minutes. Samples become solidified at the end of the process.

Digestion procedure using Microwave digest for FAAS study:

1g of sample was mixed with 5 ml. of HNO_3 (65%) and 2.5 ml. of H_2O_2 (30%). The sample mixture was then introduced into microwave digest and kept there for five minutes. After completion of the digestion process, the solution obtained was diluted by adding 10 ml de-ionized water.

Sample preparation for GC/MS analysis following QuEChERS protocol:

2g of dried processed tea was mixed with 10 ml distilled water in a 50 ml plastic centrifuge tube, shaked for 30 seconds and then allowed to leave for another 30 minutes for matrix hydration. 10 ml of acetonitrile was added to the above solution and agitated vigorously for 1 Minute.10 ml of acetonitrile was added to the above solution and agitated vigorously for 1 minute. 4 g $MgSO_4$ and 1 g NaCl was added to the solution and agitated for 1 minute. 5 ml of 0.1 M solution of Triphenyl phosphste was then added as an internal standard and agitated vigorously for 1min. Sample tube was then centrifuged for 5 minutes at 10000 r.p.m. 1 ml of acetonitrile layer (upper) was taken in a 15 ml plastic centrifuge tube, added 1 ml n-hexane and 5 ml 20% w/w aqueous NaCl solution. The solution was agitated for 1 min and then centrifuged at 10000 r.p.m. for 1min. Finally, the upper n-hexane layer (the final tea extract) was taken in a 2 ml auto sampler vial.

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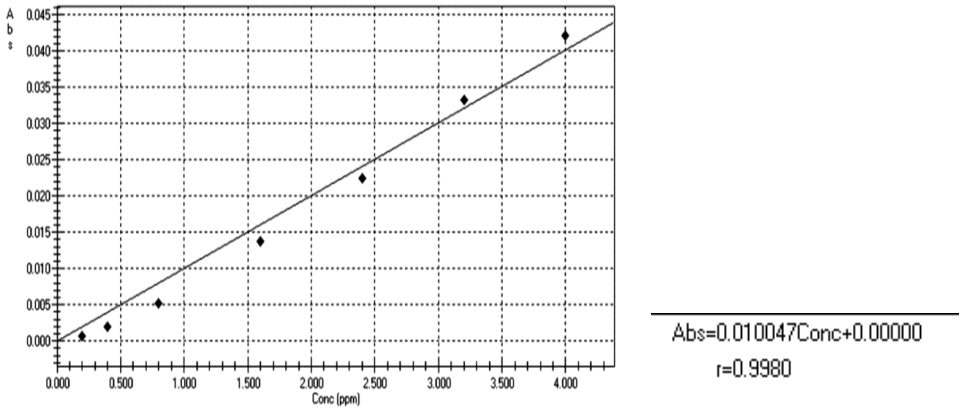
TOXICITY STUDY:

For elemental analysis of the samples of mature tea leaves, solidified samples were subjected to XRF spectrometer (PANalytical Axios, Model: DY 824). For quantitative estimation of lead in the sample employing Flame Atomic Absorption Spectrometry (FAAS Model: Shimadzu Series AA-7000)), 100 ppm of lead stock solution was used.Standard solutions of the concentration 0.2, 0.4, 0.8.1.6, 2.4, 3.2 and 4 ppm were prepared and the standard calibration curve (Fig.1) obtained was used to measure the concentration of lead in the sample twice to obtain a precise result. For GC/MS analysis, 2 ml. of the upper n-hexane layer (the final tea extract) of each sample was injected into GC/MS system**.** Compounds in the samples were identified on the basis of the pattern of TICs, Mass Spectra and considering highest Hit (Hit1) and matching percentage (Qual: 95-100) reflected in the library search reports for all samples generated by inbuilt Library Software (Turbo Mass NIST 2008 &NIST05a.L).Overall results were compared with the available literature for toxic elements in plants including tea and with the data relating to pesticides used in the surveyed gardens to draw conclusion regarding toxicity level of the collected samples.



Result: Pb (ppm) in tea sample Tea T4 -2.0703

-2.14

Fig.1. Calibration graph for quantitative estimation of lead showing the values of Absorbance (Abs), concentration (Conc.), r (correlation coefficient) along with the result of quantitative estimation of lead (in ppm) in the tea sample T4.

RESULTS AND DISCUSSION:

XRF spectrometric analysis:

XRF spectrometric study of the samples of tea leaves has shown the presence of 16 elements viz., K, Ca, S, P, O, Mn, Fe, Mg, Si, Rb, Al, Cl, Ni, Zn, Na and Pb in the tea samples (Table 1).Certain elements such as Al and Ni that may become potentially toxic in high intensities were detected in low intensities in the samples of tea leaves of both regions. Detection of essential elements such as Mg, Ca, K, P and the toxic element lead in the tea

Table 1. Elements present in samples of mature tea leaves analyzed and their intensities (in kcps).

ELEMENTS:	SAMPLES WITH MAXIMUM INTENSITIES (kcps) SHOWN BY DIFFERENT ELEMENTS:									
	T1	**T2**	**T3**	**T4**	**T5**	**G1**	**G2**	**G3**	**G4**	**G5**
1. Potassium.	64	64	74.5	64	43	64	50	64	64	53.7
2. Calcium.	16	16	17	16	10.9	16	16	16	16	16
3. Phosphorus	16	16.2	12.5	-	8.8	16	22	-	13.4	-
4. Oxygen	8	4.5	2.2	3.2	1.7	8.3	4.3	3.4	3.9	4.3
5. Sulphur	6.5	18	16	15	7.8	17	18	14	13	11.2
6. Manganese	5.2	4.5	5	5.2	3.5	2.8	4.2	3.5	4	2.6
7. Rubidium	5.5	8	-	-	-	-	-	5.5	-	-
8. Chlorine	3.9	3.1	3	3.3	2.4	5	5	2.5	3.0	2.8
9. Iron	2	2.2	3.5	1.9	2	2.5	1.4	2.7	1.5	2.1
10. Aluminium	1	-	-	2.4	-	2.6	-	-	-	-
11.Magnesium	2.4	5	3.6	3.8	2	5.9	4.0	3.6	3.5	3
12. Silicon	-	4	3.9	3.1	3.5	4	-	-	2	4
13. Nickel	-	-	18	-	-	-	-	3.8	-	-
14. Zinc	-	-	0.9	-	-	-	-	-	-	-
15. Sodium	-	-	-	-	-	0.3	-	0.5	-	-
16.Lead	-	-	-	4.6	-	-	-	-	-	-

samples was in agreement with the result obtained by Schwalfenberg et al. (2013). The significant experimental finding was the detection of heavy metal lead in one tea sample with an intensity of 4.6 kcps (Fig.1).Conversely, the result of quantitative estimation of lead using FAAS shows its conc. below detection limit of the instrument (0.0001 ppm). Moreover, the concentration of lead was also found to be much lower than the World Health Organization (WHO) Permissible standards (10 ppm).The pesticides widely used in the gardens were: Deltamethrin, Propergite, Glyphosate 41% SL, Thiomethoxam 25% WG, 2, 4-D Amine salt 58% SL and Azadirachtin 5% EC. The overall GC/MS analysis has shown the presence of twenty different compounds; Triphenyl phosphate (TPP),Tetradecane, Hexadecane and Cresyl diphenyl phosphate being the most abundantly occurring compounds (Table 2).The survey study for pesticide application in the gardens and the experimental study (GC/MS) for residual pesticides in the processed tea samples have shown that none of the pesticides used in the gardens matched with the compounds detected in final tea extracts of the samples. Moreover, none of the compounds is included in the current list of registered pesticides.

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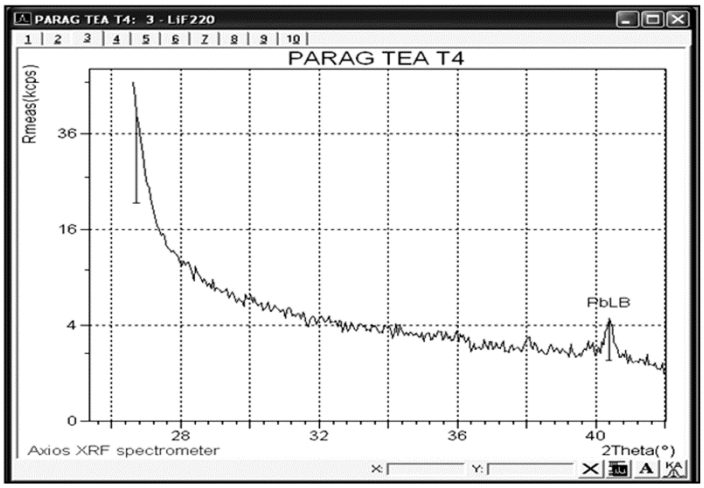

Fig. 2. Pb in tea sample Tea T4.

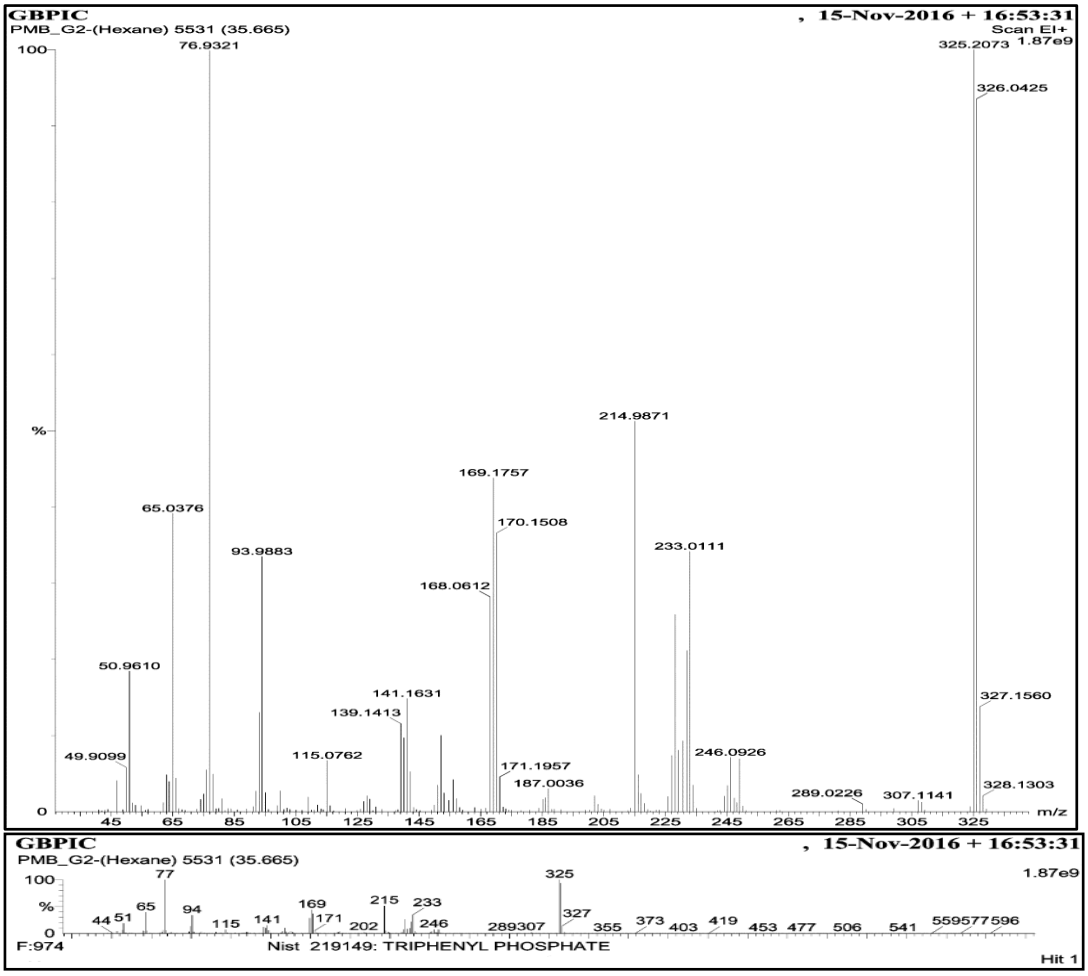

Fig.3.Mass Spectrum of the peak at RT 35.665 (Sample G2) with associated library search (in part). Compound name: TRIPHENYL PHOSPHATE - vide reference code: Nist 219149.

Table 2. Results of GC/MS analysis of processed tea samples.

Sl.No.	Compounds detected as per library search considering Highest Hit (Hit 1), MS peak / match percentage (Qual: 95 – 100)	RT of peaks	MS peak	Match %	Reference	M.W.
1.	Triphenyl phosphate	45.35	76,326		NIST 219131/219149	326
2.	Isopropyl Myristate.	21.28``		99	NIST05a.L 105615	270.457
3.	9, 15-Octadecadienoic acid, methyl ester,	24.59		99	NIST05a.L 121114	294.472
4.	9-Octadecenoicacid, methyl ester.	24.67		99	NIST05a.L 122326	296.487
5.	Tetradecane.	15.08		98	NIST05a.L 55975	198.394
6.	Octadecane.	20.97		98	NIST05a.L 94931	254.502
7.	Hexadecane.	18.12		98	NIST05a.L7609	226.448
8.	Hexadecanoic acid, methyl ester	22.57 & 26.91	74,270		NIST05a.L105639/ Nist 200488:	270.450
9.	Eicosane	23.49		98	NIST05a.L113492	282.556
10.	9-octadecenamide	41.04	59, 97,126		Nist 198010	281
11.	Cyclononasiloxane, octadecamethyl.	23.57	73,147,221		Nist 37893	666
12.	Cyclodecasiloxane, eicosamethyl.	26.48	73,147,281		Nist 37485	740
13.	7, 9-Di-tert-butyl-1-oxaspiro[4, 5] deca-6, 9-diene-2,8-dione.	26.62	57,205,232		Nist196731	276
14.	Cresyl Diphenyl phosphate.	36.78	77,165,340		Nist 183776	340
15.	Methyl 3-methyl-3-(Methoxy-Tert-Butyloxy) Amino-Butanoate.	23.59	57, 73, 86, 94		Nist 35222	233
16.	3- Butoxy-1, 1, 1, 7, 7, 7-hexamethyl-3,5,tris(trimethylsiloxy) Tetrasiloxan	29.14	57, 147,221,281		Nist 36448.	590
17.	Silane,trichlorodecyl.	26.64	57, 175,205,217		Nist 21938	274
18.	Pentane, 2, 4-dimethyl.	26.65	57, 73, 86, 94		Nist 7125.	100
19.	3-Butoxy-1, 1, 1,5,5,5 hexamethyl					

CONCLUSION:

In comparison with WHO Permissible standards (10 ppm) for lead in medicinal plants, the observed finding was negligible. The tea extracts used for GC/MS analysis were not the pure extracts of tea and that various reagents and solvents were used in between different steps of sample preparation prior to GC/MS analysis. Presence of certain compounds in the samples, may therefore, be attributed to the fact that they might have arisen during different steps of sample processing such as sample preparation using different solvents, clean up regime till the preparation of the final tea extract. Considering the result of quantitative estimation of lead in the sample of tea leaves and GC/MS analysis of the processed tea samples for residual pesticides, it is assumed that the samples analyzed are not in a toxic state and considered safe for consumption.

REFERENCES:

(1) Amirahmadi, M., S. Shoeibi,M. Abdollahi, H. Rastegar, R. Khosrokhavar and M.P Hamedani: Monitoring of some pesticides residue in consumed tea in Tehran market. *J. Environ. Health Sci. and Engg.* 10:9, 1-6 (2013).

(2) Cojocariu, C., P. Morgan, P. Silcock, and M. Godula: GC-MS/MS Analysis of Pesticide Residue in Green Tea Extracted by QuEChERS with Acetonitrile as Final Solvent. *Thermo Fisher Scientific*, EP22099 (2014).

(3) Schwalfenberg, G., S.J. Genuis and I. Rodushkin: The Benefits and Risks of Consuming Brewed Tea: Beware of Toxic Element Contamination. *J. Toxico.* Volume 2013, Article ID 370460, 1-8 (2013).
(4) World Health Organization: Quality control methods for medicinal plant materials, WHO, Geneva (1998).
(5) Zhang, X., N. Mobley, J. Zhang, , X. Zheng, , L. Lu, O. Ragin, and C.J. Smith: Analysis of Agricultural Residues on Tea using d-SPE sample preparation with GC-NCI-MS and UHPLC-MS/MS. *J. Agric Food Chem*., 58, 11553-60 (2010).