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Identification of nicotine sources in Indian tea

A. Hofmann*, A. Romanotto* *(corresp. autor)*, K. Speer**, J. Scholl**, K. Gassert***
** PiCA GmbH, Berlin Germany*
*** Technical University Dresden*
*in Cooperation with Teekampagne Potsdam****

TEEKAMPAGNE

Investigations of the German Federal Institute for Risk Assessment (BfR) in 2009 demonstrated that various plant-based foods including tea exceed the residue limit of 0.01 ppm for nicotine (NIC) according to regulation (EC) No 396/2005 [1]. Even the European Food Safety Authority (EFSA) raised the limit temporary to 0.6 ppm for nicotine, because the nicotine sources are unknown [2]. The only plant with a significant nicotine content is tobacco (1 % in dry mass). Because India is simultaneously one of the five biggest producers of tea and tobacco in the world, we **suspected** a correlation between **the tobacco cultivation** and the nicotine findings in Indian tea.

The second possibility is the Environmental Tobacco Smoke (**ETS**), which simplified includes all kinds of burned tobacco products from smoking to the burning of any residues from tobacco production.

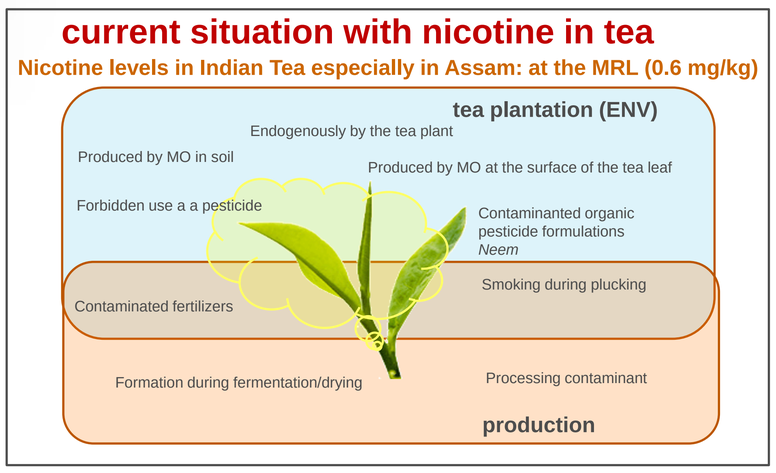

figure 1. theoretical proposals for NIC sources in tea
at the plantation (env. contaminant or pesticide) and during the production (process. contaminant)

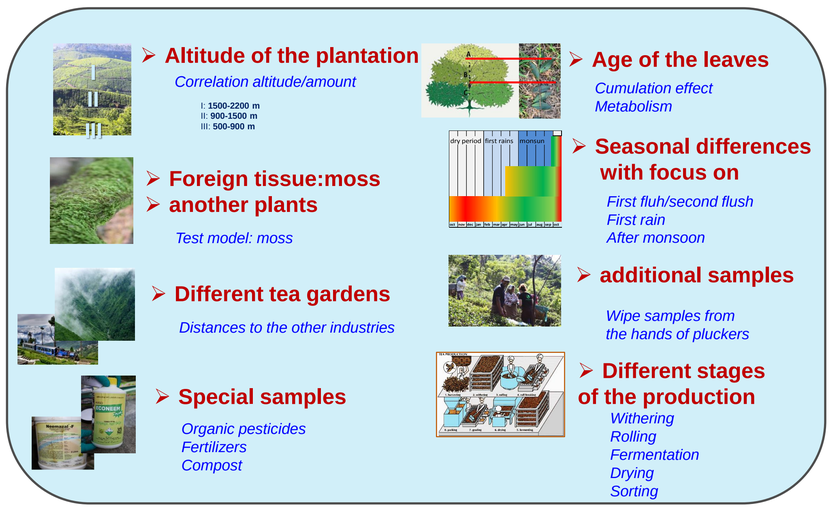

figure 2. composition of the study/ sampling volume

short summary of the first results

AIR
- Principle values from 50 ng/m³ to 800 ng/m³ (1.000 liter)
- No significant difference 2016/2015 > no related dependencies on the surrounding circumstances

tea leaves
- Amounts between 20 µg/kg and 50 µg/kg
- In higher altitudes (<1500 m) higher NIC amounts (max. 20 % difference)
- Rainy/dry seasons play no significant role > NIC source is not only fine dust

moss
- NIC enrichment takes place, values up to 100 µg/kg > impact source: AIR

plants in the surrounding
- All positive

tea production
- Enrichment through drying, no additional entry during production

For the identification of tobacco and tobacco smoke as the source of nicotine, specific markers were established [3]. Various plant materials from the tea-growing area Darjeeling were examined with UHPLC-MS/MS for nicotine and further tobacco alkaloids nornicotine and anabasine and cotinine (COT), as an oxidation product of nicotine, with a LOQ of 0.01 mg/kg of each. The nicotine and the tobacco alkaloids analyses were carried out according to the QuEChERS method [4].

The results from the table 1 indicate a correlation between tobacco and nicotine contamination. The analyzed cotinine to nicotine ratios in the various sample types (1 - 30 %) were similar to the particle phase in tobacco smoke (5 - 15 % [6]) but not to the ratio in tobacco plants (≤ 0.01 - 0.3 % [5]). Therefore a **coherence** between **tobacco smoke** and **tobacco nicotine** in Indian tea could be assumed.

1. Nicotine was **not** determined in the investigated **fertilizers** and **tea roots**. This result **excluded** the use of nicotine **as a pesticide**.
2. In addition to tea plants, nicotine was detected in the air, dust and other plant materials from Darjeeling. The *anabasine* and *nornicotine* to nicotine ratios ranged between 0.2 and 18 % (tobacco plants: 1 - 10 % [5]).
3. In **isolated** small **tea plants** (seedlings) **no NIC** was detected

table 1. results table for COT to NIC ratios in tea, tea leaves and air

kind of sample		cotinine to nicotine ratio %	number of the analysed samples
Literature	Tobacco [7]	<1	x
	ETS [6]	5-15	x
analyzed	air	2-300	45
	tea leaves	5 to 20	31
	tea	5 to 30	48

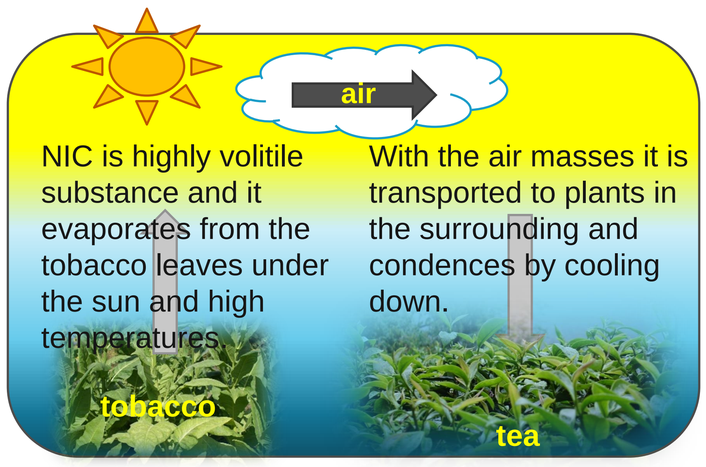

figure 3. NIC transport with the AIR from tobacco plantation to tea plantation [8]

Additional studies of **sage plants in Albania** with similar results **confirmed our thesis** about the correlation between **tobacco** plantations and **nicotine in sage** products. Moreover the closer a tobacco plantation was to other plants the higher was the nicotine contamination.

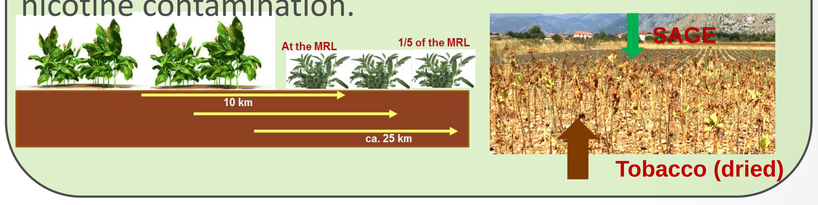

figure 4. correlation between NIC levels in sage and distance to tobacco plantation (*Koplik i Sipërm*, Albania)

By analyzing various specific tobacco alkaloids in addition to nicotine we confirmed that the nicotine contamination in Indian tea is due to tobacco cultivation instead of nicotine as a pesticide. The tobacco alkaloids in plants dissolve in the surrounding moisture, volatilize under solar exposure and enter the environment. The high amounts of cotinine in different sample types can be explained by the formation by UV light of nicotine in higher air layers. Because of positive results for nicotine in various plant materials and negative results in isolated cultivated tea plants (seedlings) an endogenous biosynthesis of nicotine in tea plants can be excluded. A similar way of contamination, observed in mushrooms through fungi mycorrhiza from soil, is another possibility. Furthermore, India like China and Indonesia are three of top five world biggest tea and tobacco producers of the world.

In India, Bihar is one of the most important tobacco producing state, which is only 20 km from Darjeeling region. Assam is famous for growing/ producing of both, tea and tobacco.

Nicotine direct from tobacco and as ETS is clearly the source of NIC levels in Indian Tea.

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[4] §64 LFBG: determination of pesticide residues in fruit and vegetables using GC-MS and/or LC-MS/MS after acetonitrile-extraction/distribution and cleaning with dispersive SPE (QuEChERS) (acc. to DIN EN 15662); L 00.00-115
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[7] Yong Li, Tao Pang, Junli Shi, „Simultaneous Determination of Alkaloids and Their Related Tobacco-Specific Nitrosamines in Tobacco Leaves Using LC–MS-MS," *Journal of Chromatographic Science,* pp. 1730 - 1736, 26 06 2015
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E-Mail: Anna.Romanotto@pica-berlin.de