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during food processing and storage
CHEMICAL REACTIONS IN FOODS VIII
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Determination Of The Source Of Anthraquinone In Organic Tea Production

Dr. A. Romanotto, Dr. K. Gassert

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Prüfinstitut
Chemische Analytik GmbH

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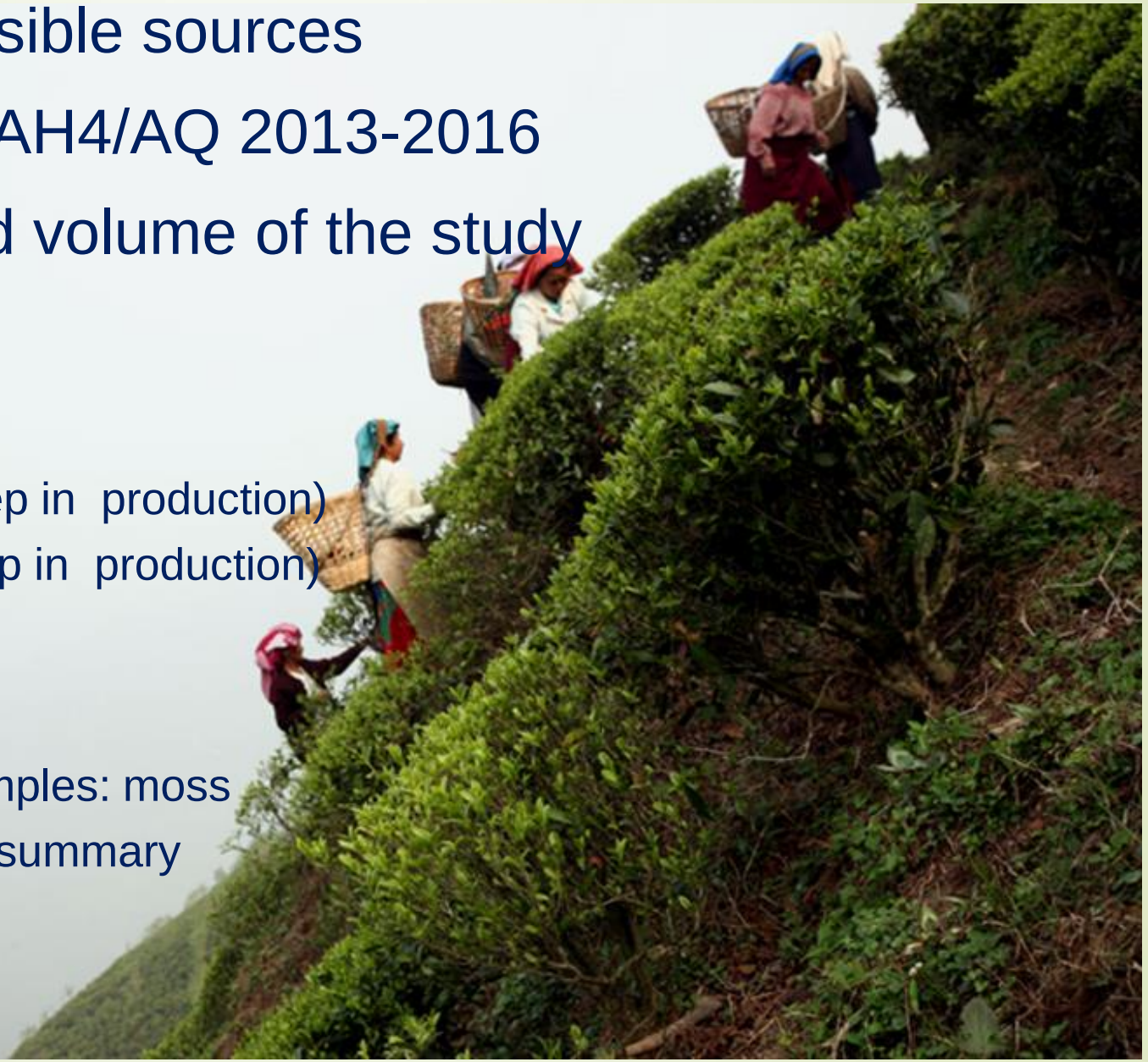
Overview

- Overview of possible sources
- Analysis data: PAH4/AQ 2013-2016
- Composition and volume of the study
- Results
  - Production
    - Tea (each step in production)
    - Air (each step in production)
  - Environment
    - Air
    - Additional samples: moss
    - Soil and dust summary
    - Tea leaves
- Summary



Possible sources for AQ

- ✓ Endogenous formation
- ✓ Exhaust fumes (environment generally)
- ✓ Formation by microorganisms in soil
- ✓ Formation by microorganisms on the tea plant surface
- ✓ Contamination from fertilizers, pesticides and fortifiers

Plantation

- ✓ Developing through fermentation (oxydation)
- ✓ Contamination from using burning of fossil fuels

Production

- ✓ Contamination from use of lubrication oil
- ✓ Open questions
- ✓ Conclusion

Anthraquinone: analyses data

- AQ results in the current and last three years

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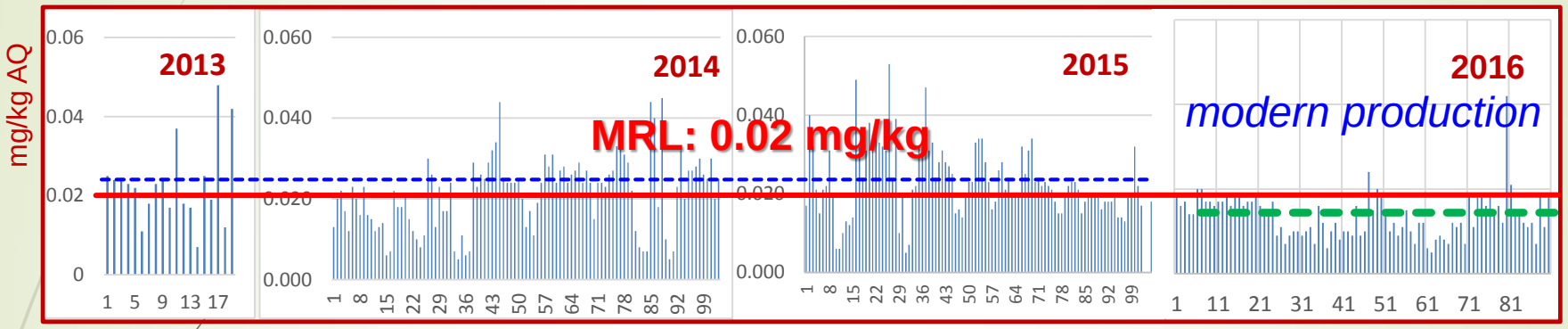
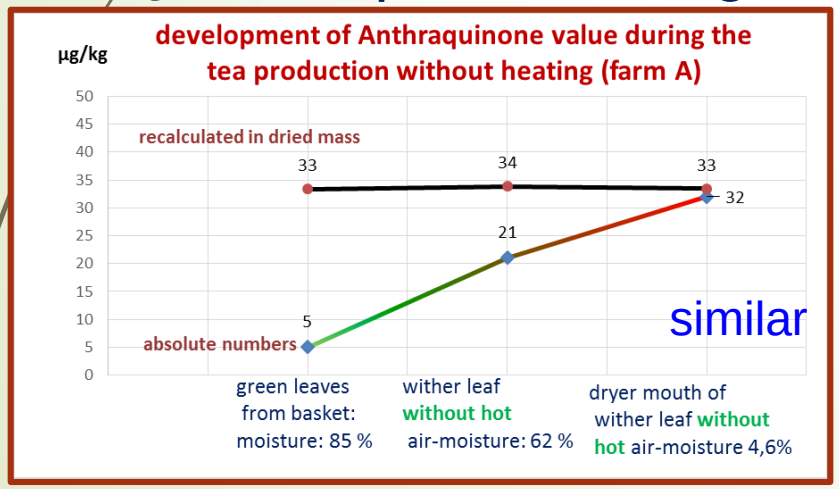
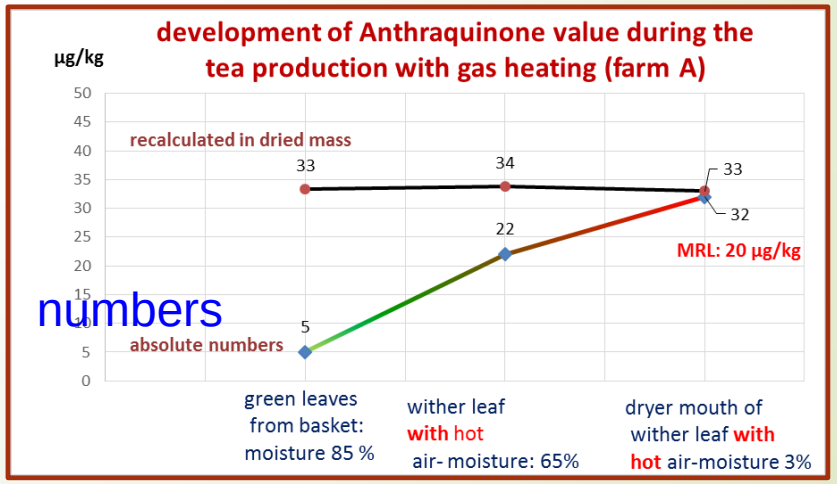


- AQ development during tea production: hot air vs air drying





The origin of Anthraquinone doesn‘t only come from the production - its origin seems to be *earlier*

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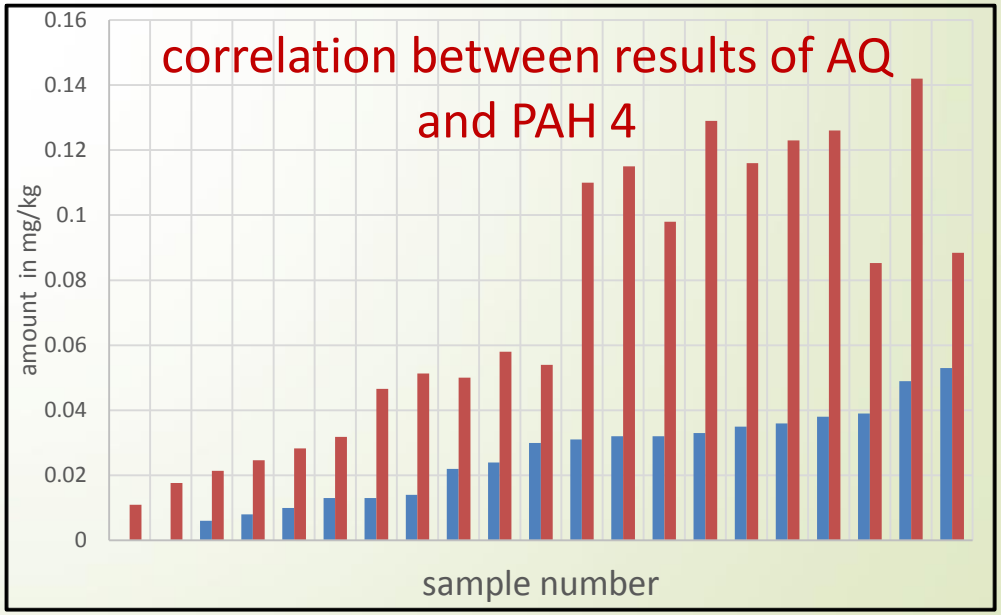
AQ/PAH 4: analyses data

- AQ/PAH 4 relationship is presented with one example of a group tested tea samples in the factory worked with coal burning mode

Nr	AQ, mg/kg	PAH4, mg/kg	F AQ/PAH4	correlation
1	<0.005	0.011	2.8	lowest value PAH, Anthra < LOQ
2	<0.005	0.018	3.5	
3	0.006	0.021	3.6	low level PAH, Anthra≈ LOQ
4	0.008	0.025	3.1	
5	0.010	0.028	2.8	middle PAH level, Anthra OK
6	0.013	0.032	2.4	
7	0.013	0.047	3.6	
8	0.014	0.051	3.7	
9	0.022	0.050	2.3	middle PAH level, Anthra ≈ MRL
10	0.024	0.058	2.4	
11	0.030	0.054	1.8	high PAH Level, Anthra >MRL
12	0.031	0.110	3.5	
13	0.032	0.115	3.6	
14	0.032	0.098	3.1	
15	0.033	0.120	3.6	
16	0.035	0.116	3.3	
17	0.036	0.123	3.4	
18	0.038	0.126	3.3	
19	0.039	0.085	2.2	
20	0.049	0.142	2.9	
21	0.053	0.098	1.8	

1.8 < F AQ/PAH4 <3.6:



! Not only the general environment: also PRODUCTION

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Study composition

Environment (main focus)

➢ altitude factor

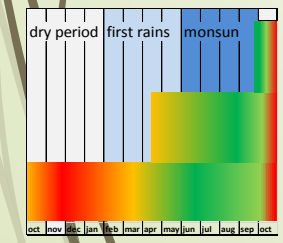
correlation: altitude/AQ amount

➢ ecological factor

distance to urban area
roads/railways/factories
with/without coal burning

➢ foreign tissue

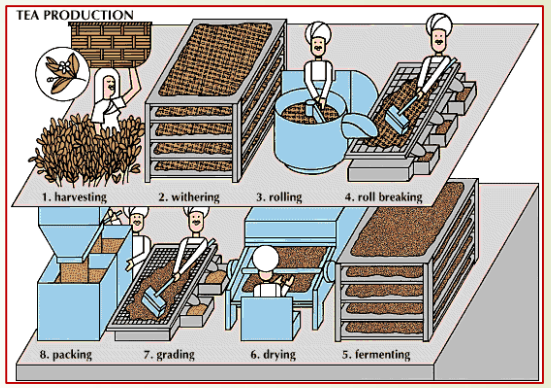
comparability of the AQ findings test model moss:



➢ seasonal factor

correlation: rainy season/
dry season

Production

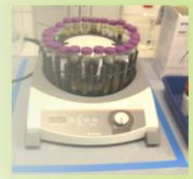


➢ AQ developments in different kinds of heat

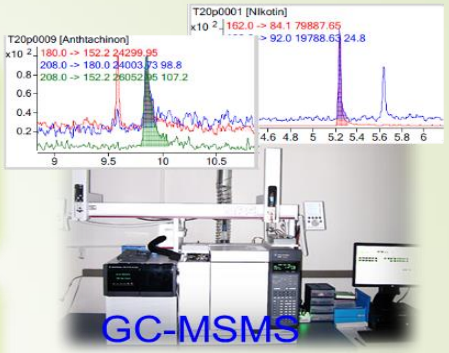
gas burning vs coal burning

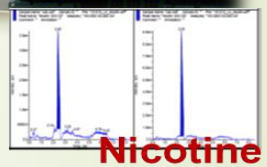
is AQ built from anthra-quinon glycosides during fermentation?

Sample analyses



PAHs/ AQ





2015 118 plant material samples (ENV and Prod),
50 dust samples (ENV and Prod)
30 soil samples (ENV)
24 samples of roots and seedlings

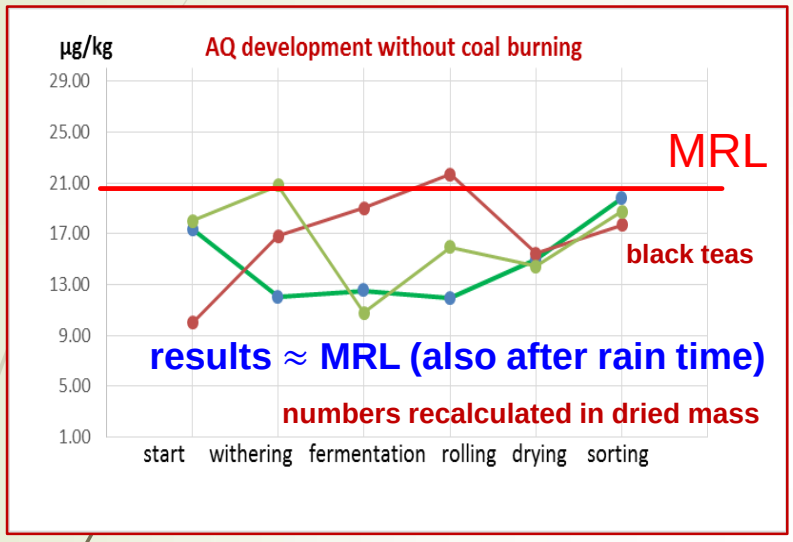
2016 20 air and leaves samples: January/April

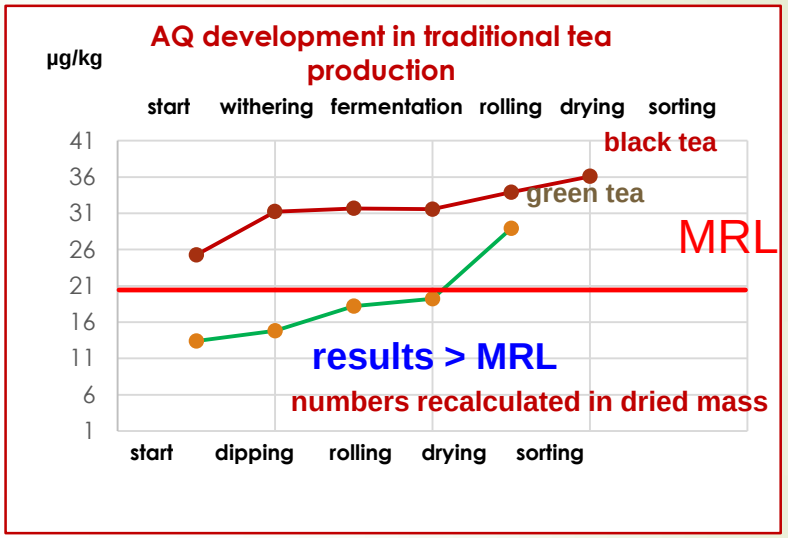
appr. 300 samples

AQ development: production

tea

AQ developments with air heating through gas burning vs coal burning





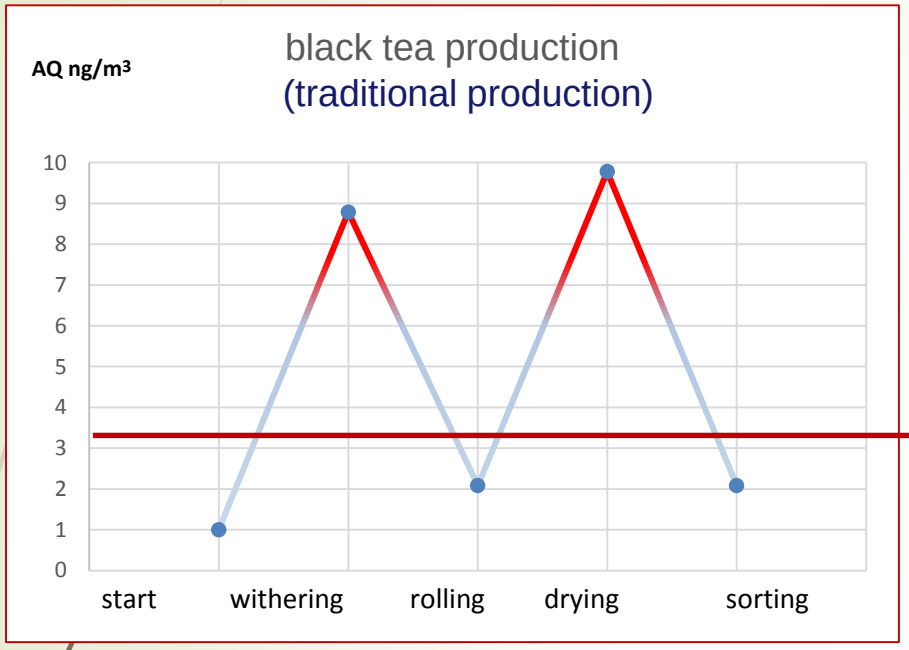
AQ source in production is clearly the contact with the hot air: coal burning

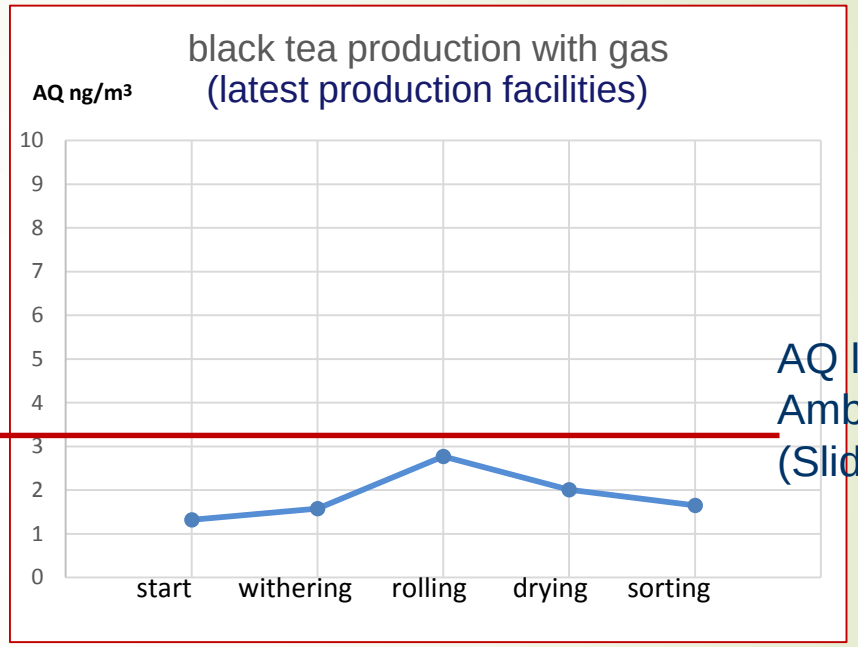
- AQ results from the initial findings and contamination during coal combustion: *withering* (black tea) and *drying* (both kinds of tea)
- No AQ increase before and after the fermentation-> AQ-Gly are not a source
- AQ increases also between drying and sorting after drying the water content falls <3% *strongly hygroscopic*->

AQ development: production

air

AQ development in the air: all production stages in the factory





AQ level in Ambient air (Slide 13)

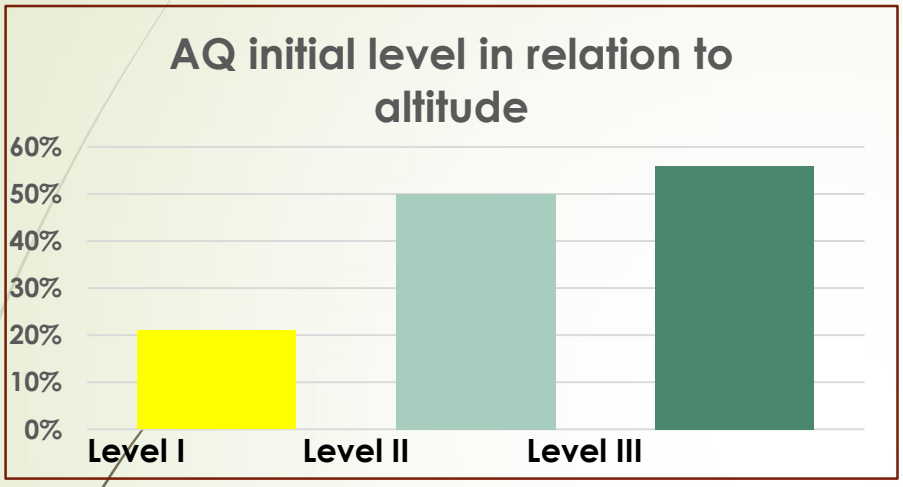
- In the factories with traditional heating by coal combustion, withering and drying are the steps resulting in the highest AQ levels in the air
- In the factories (latest production facilities) with heating by gas burning no increase of AQ in the ambient air was detected

- **burning of fossil fuels/use of direct heat is the source of AQ in air**
- **It corresponds with the AQ findings in tea**

AQ development: plantation

tea leaves

Altitude factor



altitude levels:

I: 1500-2200 m

II: 900-1500 m

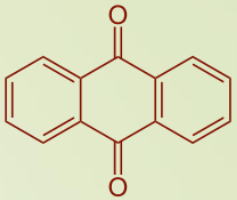
III: 500-900 m

60 tea leaves samples

7 different tea gardens

- Among the different altitudes (I, II and III),the lowest AQ levels were detected on Level I(1500m-2200 m) → 21% **> LOQ**
- At altitudes II (900 mm-1500 m) and III (500 m-900 m) 50%-56% of the samples show an initial level of > 0.001 mg/kg
- all samples with **AQ** also show **PAHs** findings

AQ development: plantation



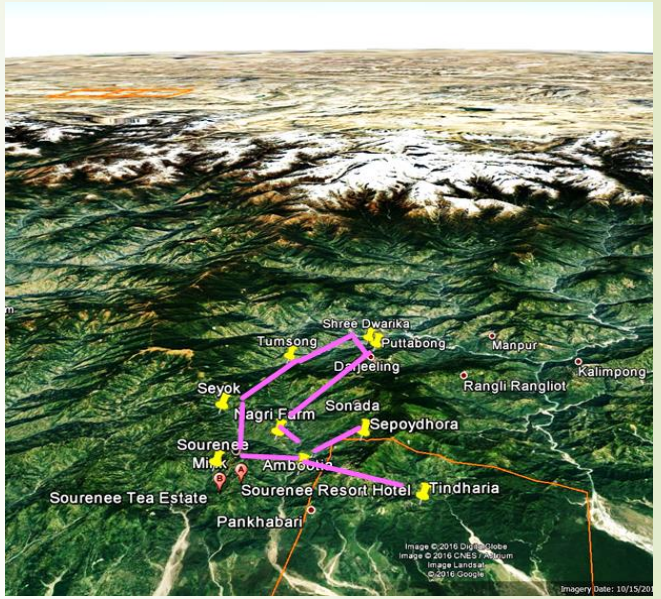
tea leaves

Distance factor

distance to urban area, railways and factories*

Tea garden	Factory*	City	Road	Number of factors
A. Nagri Farm	-	-	-	0
B. Sourenee	+	-	-	1
C. Tindharia	+	-	-	1
D. Ambootia	-	+	+	2
E. Tumsong	-	+	+	2
F. Puttabong	+	+	+	3
G. Seeyok	+	+	+	3

** with coal burning mode*



PAH/AQ RESULTS in µg/kg in tea gardens Nagri Farm and Seeyok	Sample	Anthraquinone	Benzanthracene Results	Chrysene Results	Benzo[a]pyrene Results	Benzo[b]fluoranthene Results
Env_LEAF / NAGRI FARM-I_A	T20-135	<1 ppb	<1 ppb	<1 ppb	<1ppb	<1 ppb
Env_LEAF / NAGRI FARM-I_B	T20-136	<1 ppb	<1 ppb	<1 ppb	<1 ppb	<1 ppb
Env_LEAF / NAGRI FARM-I_C	T20-137	<1 ppb	<1 ppb	<1 ppb	<1 ppb	<1 ppb
Env_LEAF / Seeyok_factory_2	T20-79	1.8	< 1 ppb	1.7	1.8	1.5
Env_LEAF / Seeyok_factory_3	T20-80	1.7	< 1 ppb	2.5	2.4	1.5
Env_LEAF / Seeyok_factory_4	T20-81	1.9	< 1 ppb	2.1	1.9	1.6

- **60 tea leaves samples**
- **7 different tea gardens**

→ **Nargi Farm ENV factor: 0**

→ **Seeyok ENV factor: 3**

- **Environmental factors** such as the distance to cities, roads, railways: location plays an important role
- The lower and more inhabited regions (<1500 m) show higher numbers (**Initial findings**)

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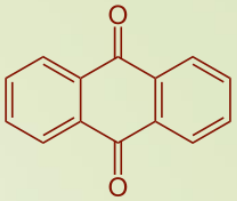
AQ development: plantation



moss

Endogenous formation: moss

- Why moss?

- exactly the same environment → same influencing factors
- foreign tissue → no plant; no similar synthetis pathways
- no collective metabolism → not a parasite
- no contact with the ground → no assimilation from the ground
- known as fine dust absorber of air *
- air: the only common medium

* Professor Dr. Jan-Peter Frahm
Nees-Institute for Biodiversity of plants,
University in Bonn

Chlotolaria spp. *Albizza Lebek*

Ambient plants ≈ 1 µg/kg AQ

Fern spp.

Weeping love grass

moss

moss

„big surface effect“

Sampel label	AQ [µg/kg]
Env_LEAF / _IIB	0.95
Env_LEAF / _IIA	0.92
Env_LEAF /_IIC	0.86
Env_Moos II	7.8
Env_LEAF / _IIIC	1.3
Env_LEAF / _IIIB	1.6
Env_LEAF / _IIIA	1.2
Env_Moos III	3.8

at the altitude 900-1500 m
tea: appr. 1 µg/kg
moss: appr. 8 µg/kg

at the altitude 500-900 m
tea: appr. 1,5 µg/kg
moss: appr. 4 µg/kg

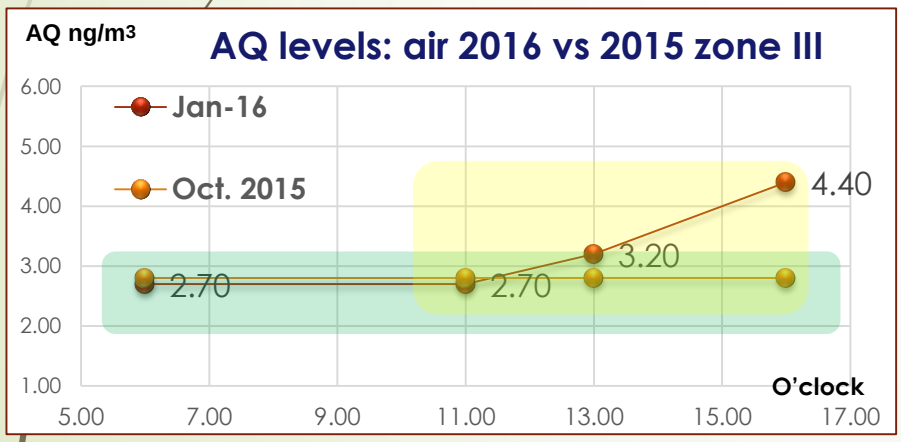
- **AQ is unlikely to be formed endogenously in the tea plant**
- **Air seems to be the only common source of contamination**

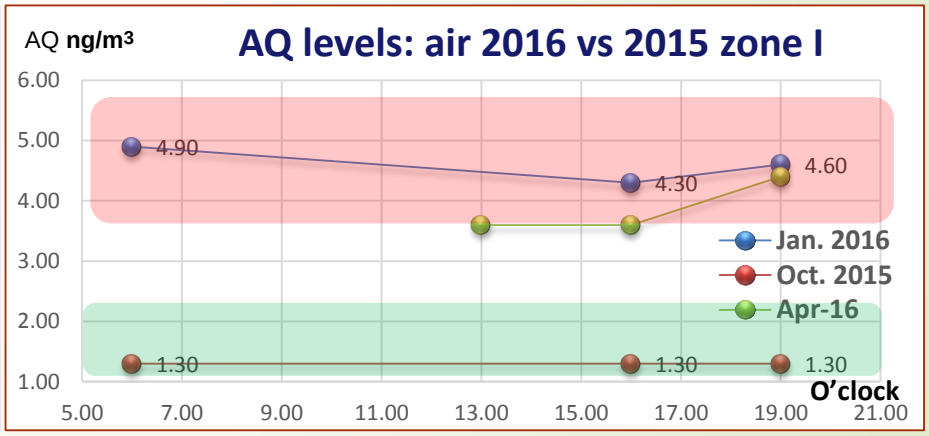
AQ development: plantation

air

Air: 45 samples taken during different seasons, times at the same place

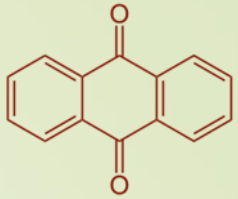
Anthraqinone, ng/m3 2016	start time	Jan 2016 zone III	Jan 2016 zone I	Apr. 2016 zone I
morning	6.00	2.70	4.90	x
early afternoon	11.00	2.70		x
late afternoon	1:00 PM	3.20		3.60
evening 1	4:00 PM	4.40	4.30	3.60
evening 2	7:00 PM		4.60	4.40
ENV_AIR 2015	x	2.80	1.30	x





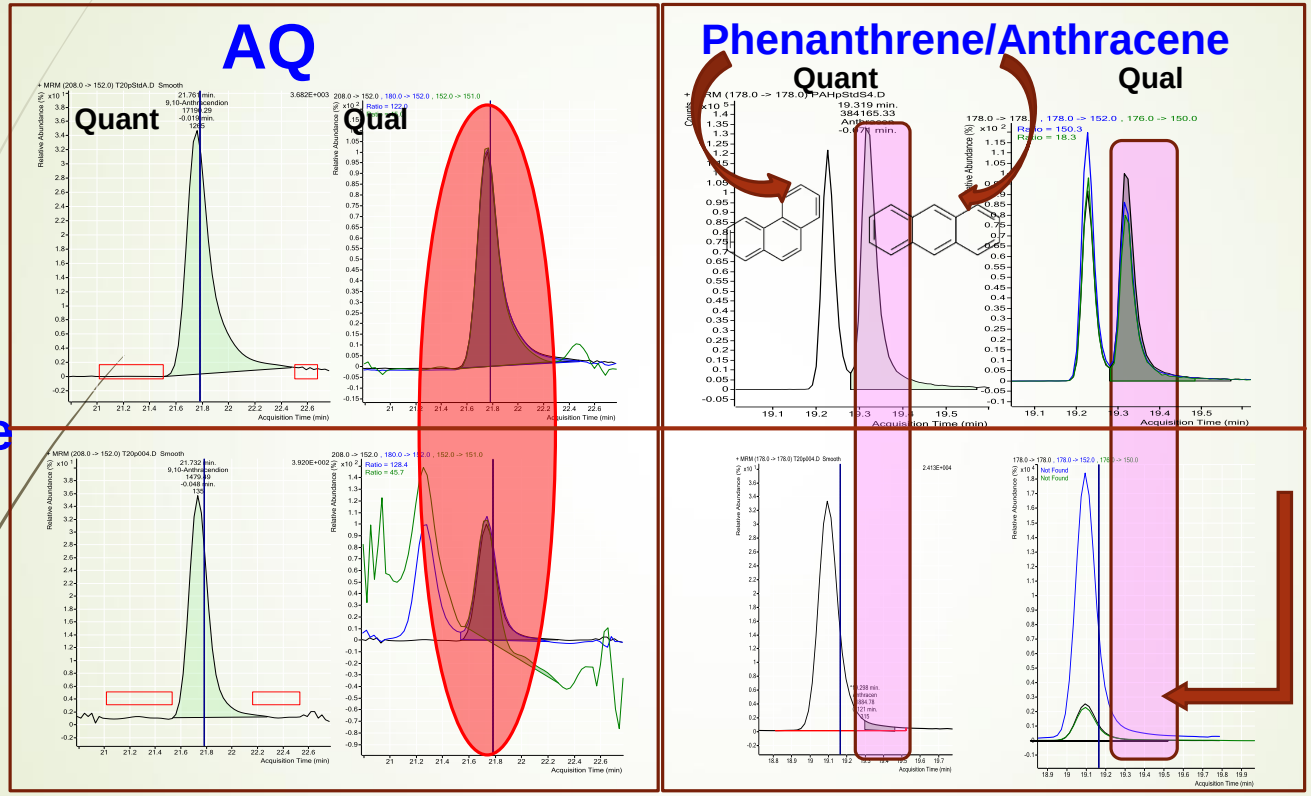
- Air in the rain free season is more loaded with AQ than after the monsoon
- First flush tea, harvested after the rain free time shows higher AQ levels than second flush tea

Summary Air: plantation



air

- PAHs and OxyPAHs are present in all air samples



extracted air samples

no anthracene/ phenanthrene only

no phenanthrene-diones/ AQ only

- The studied samples were taken immediately after monsoon -> nonethess all detectable
- **All PAHs except for ANTHRACENE detectable!**

is ***Anthracene-> precusor for AQ? Phenanthrendions present?***

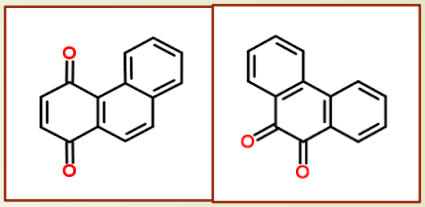
open question

- Is this peak AQ only?

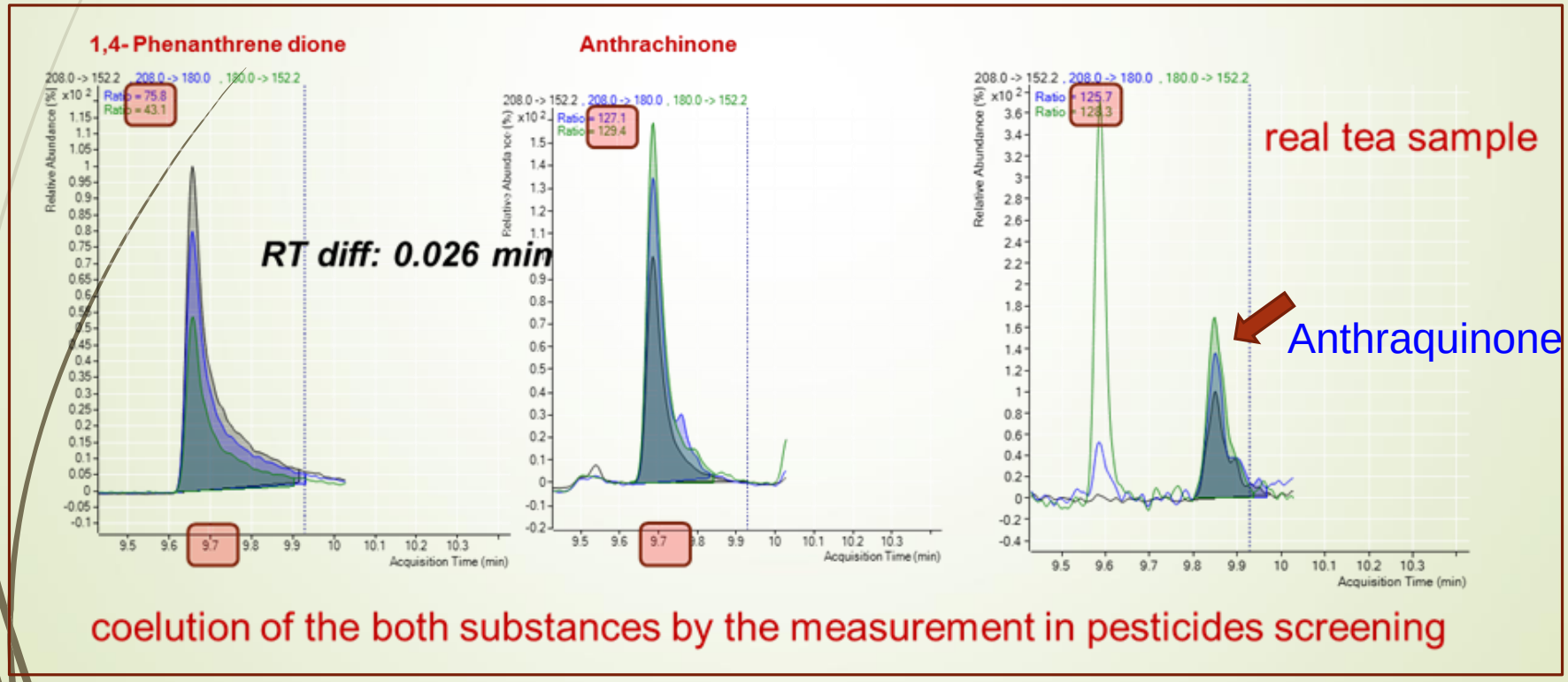
Summary Air: plantation

- PAHs and OxyPAHs are present in all air samples

- Anthracene/Phenanthrene: isobaric compounds
- What are we measuring AQ only or/and phenanthrenedion(s) ?



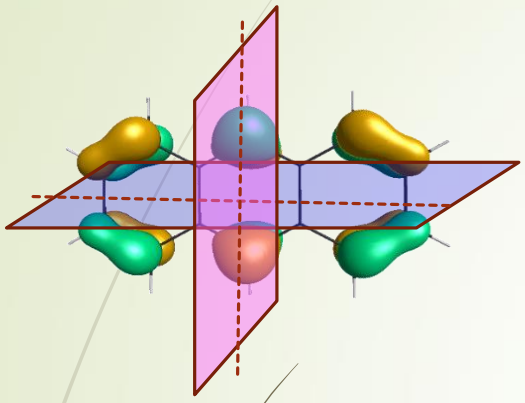
Structures of 1,4- and 7,8-phenanthrendiones



- Similar transitions, similar RTs, but different ion proportions
- In tea **AQ only** (formation of phenanthrendiones less possible -> next slide)

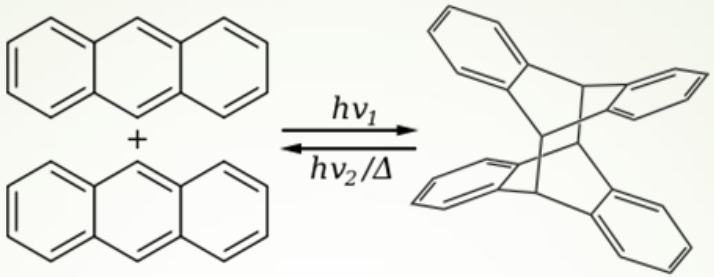
open question
- where is anthracene?-

Conversion of Anthracene in AQ

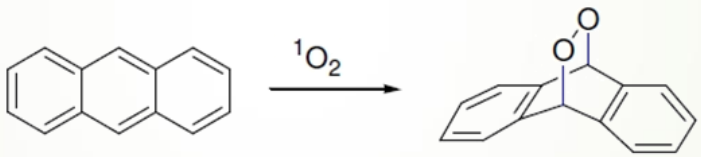


anthracene

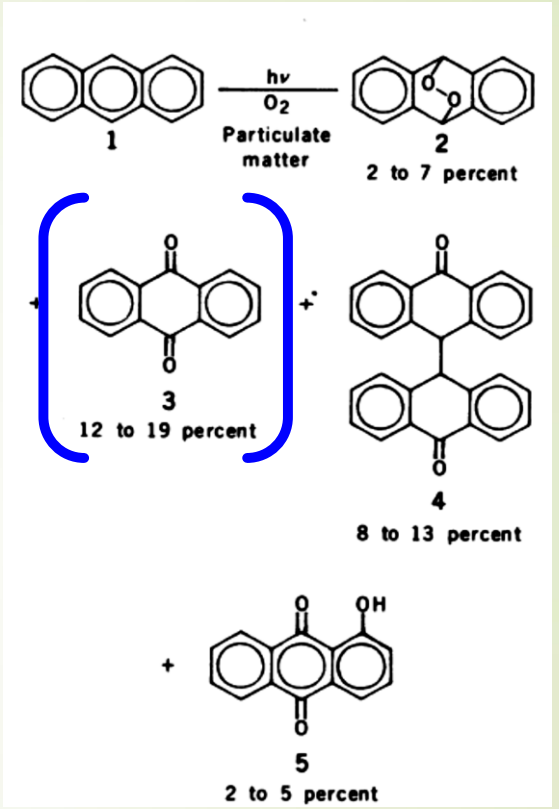
delocalized π-electrons
in central ring pos. 9 and 10



reversible dimerization



peroxydation



photooxidation of anthracene in PM*

Abstract*: Anthracene that has been dispersed into atmospheric particulate matter is photooxidized to an array of products... Significant disappearance of anthracene also occurs by pathways not involving oxygenation. * **SCIENCE, VOL. 205, 10 AUGUST 1979***

- **Anthracene is highly reactive especially in positions 9 and 10 It oxidizes lightly to AQ**

Summary: dust and soil

- Dust from the environment (fine dust) and production (production dust) are similarly contaminated -> PAHs, AQ
- Special affinity of tea leaves because of the special nature of tea hairs of young tea leaves. The younger the leaves, the more the dust is retained
- Young leaves have a particularly active metabolism / - stomata number they absorb dust better than the old leaves
- Dust in the factories mostly consists of the tea dust / tea hairs and dust are additional sources of contamination, when badly ventilated

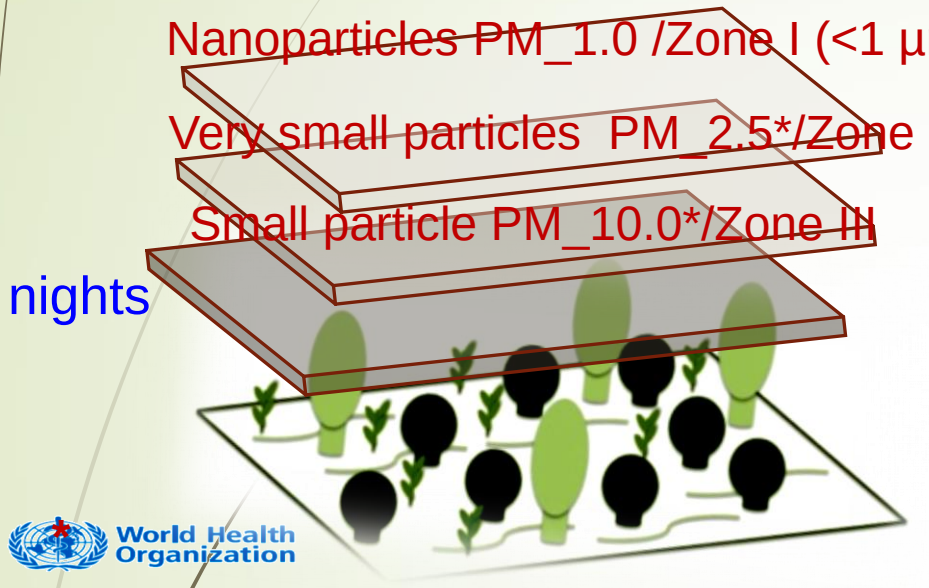
!

9-Fluorenone ? ✓✓		Paper III Eriksson *et al.* 2000 [66] Meyer & Steinhart 2001 [137] Wischmann *et al.* 1997 [138]	Contaminated soil [64,66,69] Diesel and gasoline exhaust [62,87,9] Fly ash [61,91] Air particulate matter [65,70,90,91] Sediment [67,89] Sewage sludge [63]
1,2-Acenaphthenedione ?		Meyer *et al.* 2001 [137]	
4-Hydroxy-9-fluorenone and isomers ?		Paper III and VI Eriksson *et al.* 2000 [66] Meyer & Steinhart 2001 [137]	Contaminated soil [66] Air particulate matter [70]
Anthracene-9,10-dione		Paper VI Andersson *et al.* 1996 [41] Saponaro *et al.* 2002 [88] Wischmann *et al.* 1997 [138] Lee *et al.* 1998 [135]	Contaminated soil [66] Diesel and gasoline exhaust [87] Fly ash [61] Air particulate matter [65,70] Sediment [67] Sewage sludge [63]

- Soil: AQ positive only in factories with coal operation / roads nearby

Summary Environment: Air

Particulate Matter (PM): fine sized particles/pollutants in air



World Health Organization

WHO's Urban Ambient Air Pollution database - Update 2016
version 0.2

Environment: divided into three subgroups:

- **global environment**: contamination caused by worldwide anthropogenous influences e.g. accumulation and current release of global contaminants due to the melting of glaciers as a result of global warming ZONE I
- **distant environment:** influence of cities and factories or coal mines from Tibet, cigarette factories, etc. within a circle of five thousand kilometers ZONES II/(III)
- **immidiate environment:** influence of local traffic and cities in the region ZONES III/(II)

Summary: two main sources of AQ

Environment:

- One AQ source is the particulate matter (contamination) from the air;
- Tea leaves have a **special affinity** for absorption due to
  - **big surface** of tea leaves
  - very active mtabolism / high amounts of stomata of young leaves
  - **hook-shaped hairs** of young tea leaves
- **The hypothesis that AQ is endogenously produced has been refuted**

Production:

- Heat creation by **burning coal/wood** is the most important source of AQ in production
- The **tea dust** itself, in the so-called „sorting area“ of the factory, is also a cause of contamination
- **The hypothesis that AQ develops during fermentation has been disproven**

Anthraquinon as Multicontaminant in tea cultivation

- contaminant in production (fossil fuels and wood)
- contaminant in the environment (present in fine dust, air)
- contaminant in paper industry

§

- not a pesticide in tea cultivation (bird repellent, for tea irrelevant-> no use, counterproductively)

- proven, that developed from the PAH (Anthracene)
- other oxyPAH present, which are not declarated as a pesticide

next step: Dec. 2016 meeting by the Commission (EFSA) and European Tea and Herbal Infusion Association (THIE) and presenting of this data

to be conitued...

Acknowledgments

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Thanks to

- Organizing committee of the CRF2017

International conference on new knowledge on chemical reactions during food processing and storage
CHEMICAL REACTIONS IN FOODS VIII
February 15 - 17, 2017 ● Prague, Czech Republic
CRF 2017

Thanks to

- Teekampagne for asking me to visit their plantations and for trusting me to do such important study in the region Darjeeling