Characterization of diesel and biodiesel exhaust particles for nanotoxicity studies

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Introduction.

Currently combustion aerosols are gaining an increasing attention because of pronounced impacts on human health. The effects of inhaled aerosols at the cellular level depend on both their size and distribution of toxic components over particles. There are strong indications that biological activity of nanoparticles (NPs) depends on their physicochemical characteristics which are not considered in many toxicity studies (Oberdorster et al., 2005). Since the formation of combustion NPs depends on several factors, e.g. type of fuel, temperature, and combustion conditions, their morphology and composition varies considerably. Fuel additives, oil contaminations, and engine wear produce multicomponent aerosols composed from inorganic compounds (oxides and salts) emitted together with the soot particles. Such complex composition may lead to a significant change of physical, chemical, and therefore toxicological properties of exhaust particles.

Most exhaust characterization studies have aimed to obtain the information on the average chemical characteristics by bulk analytic techniques while characterization of individual particles is significant for health impact assessment, as providing the chemical composition and morphological information at microscopic level. A big advantage is achieved using an electron probe microanalysis performed by scanning electron microscopy (SEM) coupled with energy-dispersive X-ray (EDX) spectroscopy (Kireeva et al., 2010). The range of chemical species in exhaust may be grouped by the clustering technique that reveals the different chemical particle types. With this purpose bulk analytic techniques, such as Fourier Transform Infrared (FTIR) spectroscopy and capillary electrophoresis (CE) are useful to identify the characteristic vibrations of inorganic compounds and mass concentration of ions present in the multicomponent diesel engine exhaust.

Objectives.

This work is devoted to microscopic and chemical characterization of diesel engine exhaust particles produced in EngToxDi project for nanotoxicity studies. The aim is 1) to perform the individual particle analysis for diesel vehicle exhaust, 2) to determine the morphological and composition of NPs in the case of using diesel B0 fuel with various lube oils (low and high SAPS) and biodiesel fuel B20 with high SAPS, 3) to classify the individual particles by separation on different groups of similar composition, 4) to perform the expert analysis of organic/inorganic chemistry and ion content for interpretation of clustering data in terms of physicochemical relevance, and 5) to relate the major characteristics of exhaust NPs to toxicity data.

Experimental.

Opel Astra X20DTL was running on a dynamometer at a constant velocity of 35 km/hr with a force of 66N at the wheel. Diesel B0 fuel of swiss market quality with low and high sulfated ash, phosphorous and sulfur (SAPS) lube oil or biodiesel fuel B20 with high SAPS were used. Sampling of exhaust particles were performed from CVS system on PallFlex filters during various operation conditions. The individual particle analysis was performed for sampled particles by SEM/EDX microscopy. Combination of hierarchical (HCA) and non-hierarchical cluster analysis (NHCA), k-means and g-means, was applied to separate individual particles in definable groups of similar morphology and chemical composition using software Deductor. The bulk chemical structure and ion content was analyzed by Fourier Transform Infrared (FTIR) spectroscopy and by capillary electrophoresis (CE), respectively.

Exhaust was diluted then folds with heated filtered ambient air to expose cell cultures at the air-liquid interface. Before entering the cell exposure chambers, it was brought to temperature of 37°C, relative humidity of 85% and CO_2 partial pressure of 5%, which reflects physiological conditions in the human lung. A complex three dimensional cellular model of the human lung epithelium was used to present the lung cells (Rothen-Rutishauser et al., 2008). In parallel to exhaust exposures, identical cell cultures were exposed to filtered ambient air, which by subtracting the biological response to filtered air exposure from the biological responses to exhaust exposures, allows to differentiate between exposure system and cell culture handling related effects from purely exhaust related effects. The cell cultures were exposed for 2 or 6 hrs to exhaust samples or filtered air followed by a 6 hrs post-incubation. A preliminary toxicity assessment was performed by toxicity tests of exposed cells, i.e. cytotoxicity, oxidative stress, inflammation, and genotoxicity. Pro-inflammatory responses were assessed by quantification of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-8 by enzyme linked immune-sorbent assay (ELISA).

Results.

Chemical structure of diesel and biodiesel exhaust particles are dominated by aliphatic C-H groups of alkanes and alkens with small amount of carbonyl C=O groups of carboxylic acids, ketones, and aldehydes. Relative concentration of oxygen–containing groups is small in comparison with aliphatic groups, higher on biodiesel than on diesel particles. This finding indicates a hydrophobic character of nonoxidized surface of exhaust particles. Total ion mass concentration in all exhausts does not exceed 3%. Sodium, ammonium, and calcium ions compose the major part of total ionic species for particles produced by using high SAPS lube oil. There are sulfates and phosphates only in diesel exhaust and biodiesel exhaust, high SAPS.

Diesel exhaust is found to compose from polluted soot agglomerates internally or externally mixed with inorganic salts and metal oxides. The chemical characteristics and shape of fine particles (0.1 - 0.5 μm) are different from those of larger-size particles (>0.5 μm), that can be explained by different mechanism of formation for fine soot particles and larger inorganic particles. The most abundant group (around 50%) in each exhaust is polluted soot. Representative micrographs for five groups of B0, low SAPS are shown in Fig.1 in order of group abundance. Individual particles of major Group 1 (42%) show typical features

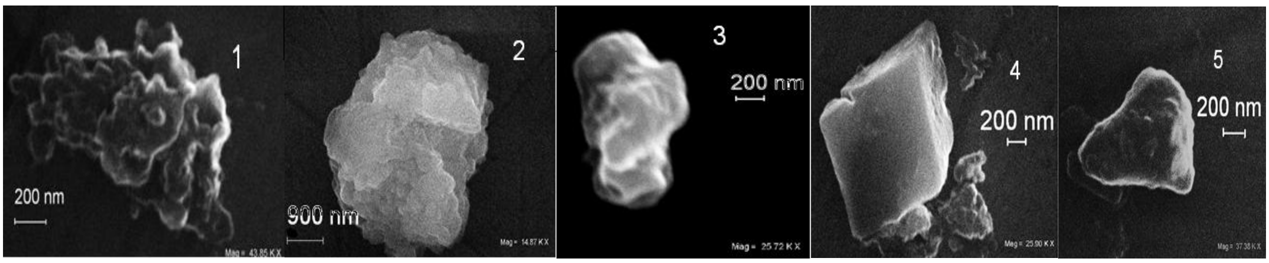

Fig.1. SEM images of five groups of diesel B0 exhaust particles, low SAPS.

of soot agglomerates with primary particles of diameters from 50 to 200 nm which are well-fused each other. The other three particle groups are composed of irregular shape particles in a size range >500 nm. Their average composition is characterized by silicon, iron, calcium, and aluminium as dominant elements besides carbon and oxygen. The particles of Group 2 are identified as Si-rich with variable amounts of O and other elements (Al, Fe) typical for fuel and lube oil contaminations, classified as silicates. The particles of Group 3 are dominated by Fe and O associated with Ni, Ca, and Si, their structure corresponds to iron oxide. The less abundant Group 4 (8,7%) and Group5 (6,6%) are presented by Ca- and Al-rich particles which are composed of calcium sulfates and aluminum oxides, respectively.

Using high SAPS with larger ash content leads to abundance of groups of Al oxide and Al fused with Fe. The most prominent impact of high SAPS relates with presence of additional two groups of Nb oxides and W particles. Biodiesel exhaust particles, high SAPS also contain groups of soot, Ca, Al-rich, Fe-rich, and Zn-rich, and of W particles. The major difference from diesel particles is the appearance of separate groups created mostly by Na, Cl and Si, Cl, K, the elements which are commonly recognized as biomarkers of biofuel combustion.

The toxicological investigation is focused on pro-inflammatory responses and necrotic (cytotoxicity induced) cell death. Independently on exhaust or exposure duration, no significant cytotoxic effects are detectable when compared to filtered air exposure. Relative to filtered air exposure, 2 hrs exposure to B0,high SAPS and B20,high SAPS exhausts resulted in increased secretion of TNF-α, the effect being stronger for B0,high SAPS (1.6 fold compared to filtered air). In contrast, 2 hrs exposure to B0,low SAPS resulted in decreased TNF-α secretion (0.5 fold). The effects of 6 hrs exposures are comparable for all three exhausts, in the range of 1.2 to 1.4 fold. Increased IL-8 secretion was only observed upon 2 and 6 hrs exposure to B0,high SAPS and 2 hrs B20,high SAPS exhausts (in the range of 1.2-1.3 fold, compared to filtered air). 6 hrs exposure to B20,high SAPS and B0,low SAPS did not affect IL-8 secretion. In accordance with the decreased levels of TNF-α secretion, IL-8 secretion was also decreased upon 2 hrs exposure to B0,low SAPS exhaust (0.8 fold compared to filtered air).

Conclusions.

The comprehensive analysis of morphology and composition of multicomponent exhaust particles elucidates how combustion aerosols are changed in dependence on fuels and lube oils used. Approach developed for clustering individual particle characterization supported by bulk expert analysis reveals a powerful tool for nanotoxicity studies. It indicates multicomponent composition of diesel exhaust particles emitted in various groups of soot, metal oxides, and salts while using of high ash lube oil increases a number of metal oxide groups. Using biodiesel fuel brings additional groups with biofuel impurities. The results obtained for cell pro-inflammatory responses indicate weak effects of the differences in exhaust composition. The higher differences are related to both diesel and biodiesel exhaust, high SAPS due to more metal oxide particle types. Organic chemistry does not pay any role in differences in pro-inflammatory responses.

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References.

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Kireeva E., Popovicheva O. et al. J. Atmos. Chem., 64,129-147, (2009)
Rothen-Rutishauser, B. et. al. In vitro models of the human epithelial airway barrier to study the toxic potential of particulate matter. Expert Opin Drug Metab Toxicol, 4,1075-108, 2008

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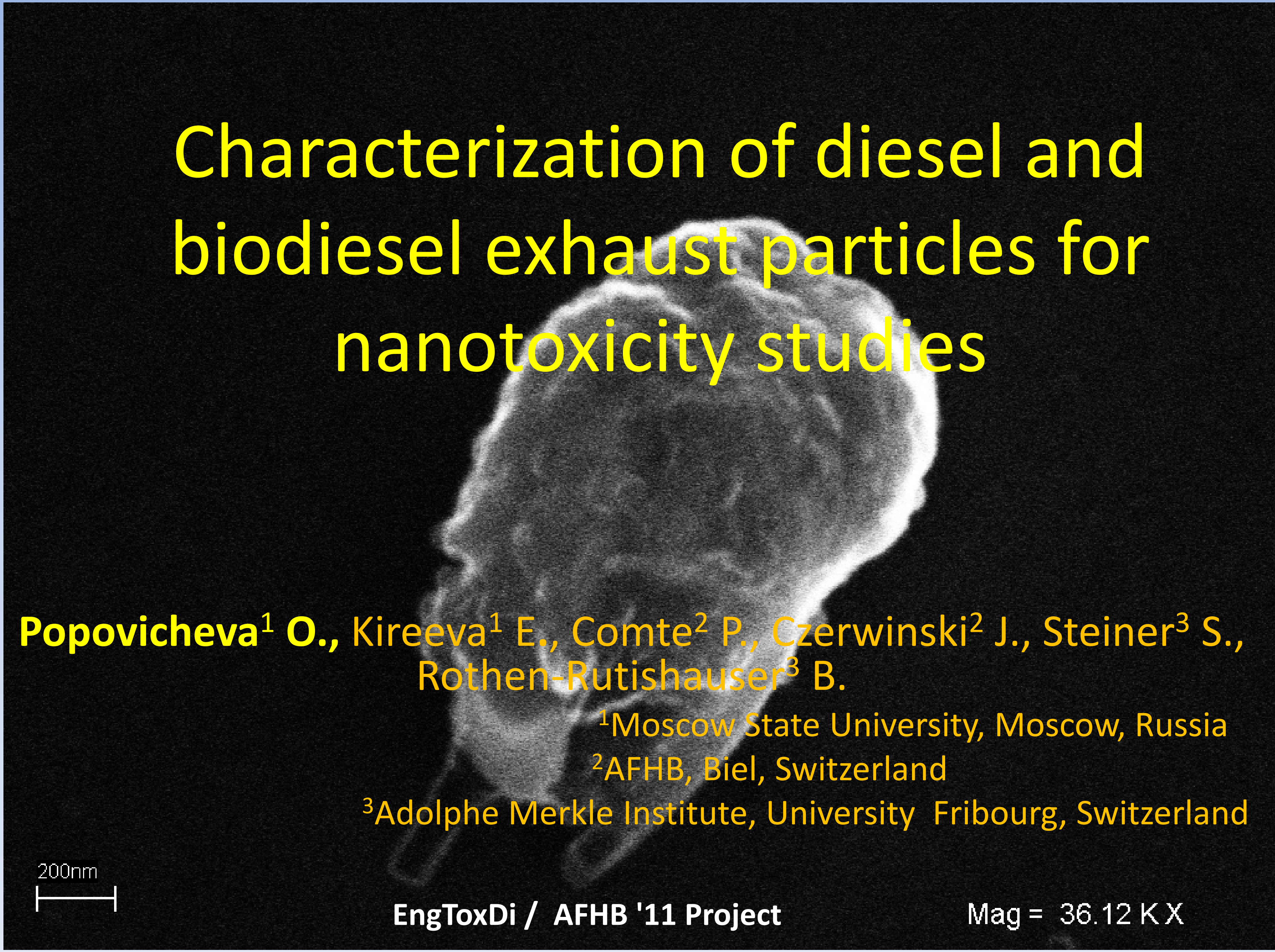
EngToxDi / AFHB '11 Project



Motivation:

Effects of aerosols at the cellular level depend on both size and **toxic components of single particles.**

There is strong indication that biological activity of nanoparticles (NPs) depends on physicochemical characteristics which are not considered in many toxicity studies (*Oberdorster et al., PFT, 2005*).

Individual particle characterization of combustion exhaust is most relevant for nanotoxicity assessment as providing chemical composition and morphological information at microscopic level.

Problems:

Formation of combustion NPs depends on several factors, e.g. fuel, engine, and combustion conditions, their composition ***varies considerably.***

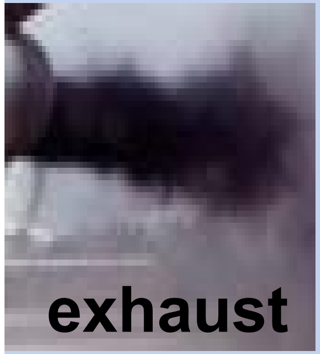
Whatever there is an engine, fuel, or operational conditions, its emission is ***multicomponent (!).***

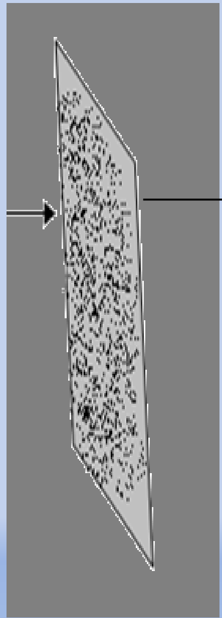
Many studies use bulk analytic techniques providing just average characteristics.

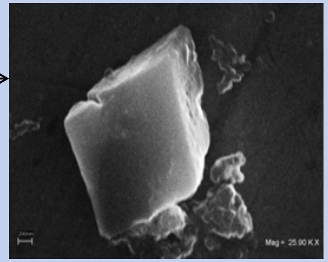
Approach for characterization of multicomponent exhaust

SEM/EDX

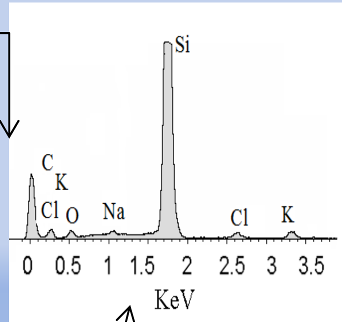
sampling







100 nm < particle size< 3 µm



morphology and elemental composition of ~300-500 individual particles

Expert analysis

Data matrix

Cluster analysis

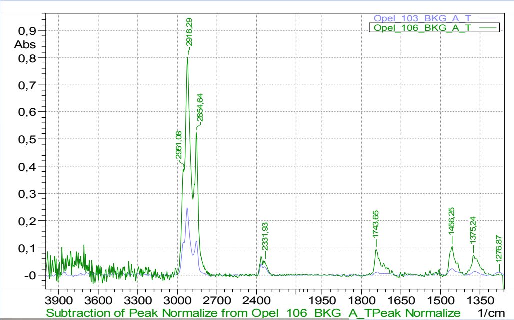
C	O	F	Na	Al	Si	K	Ca	Ba
24,75	16,67	48,05	2,72	1,05	6,76	0	0	0
15,58	35,88	23,5	3,55	2,19	17,71	1,05	0,53	0
7,54	33,92	21,08	4,91	2,44	23,16	2,08	0,98	3,89
27,96	8	56,83	1,6	0,5	4,68	0,43	0	0
13,72	30,17	32,3	3,9	1,87	16,29	1,05	0,7	0
3,64	45,07	8,03	3,9	3,23	29,18	1,63	1,06	4,26
18,84	21,82	25,49	3,59	2,08	20,64	2,14	1,2	4,19

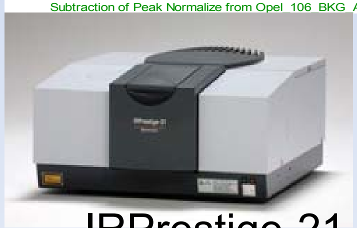
Principals of Cluster Analysis

- combination of hierarchical (HCA) and non-Hierarchical cluster analysis(NHCA), to reveal data structure, software Deductor, k-means and g-means methods,
- separation into distinct groups of similar composition,
- group identification according to best separation of different chemical particle types ,
- **expert analysis** for choosing the final number of clusters and interpretation of choice in terms of physicochemical relevance.

BULK ANALYSIS

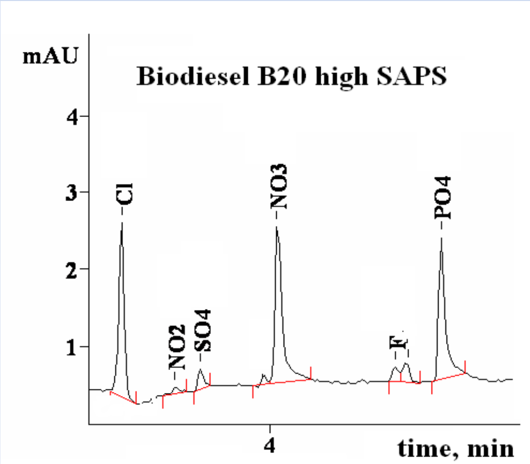
FTIR characterization of organic/inorganic compounds





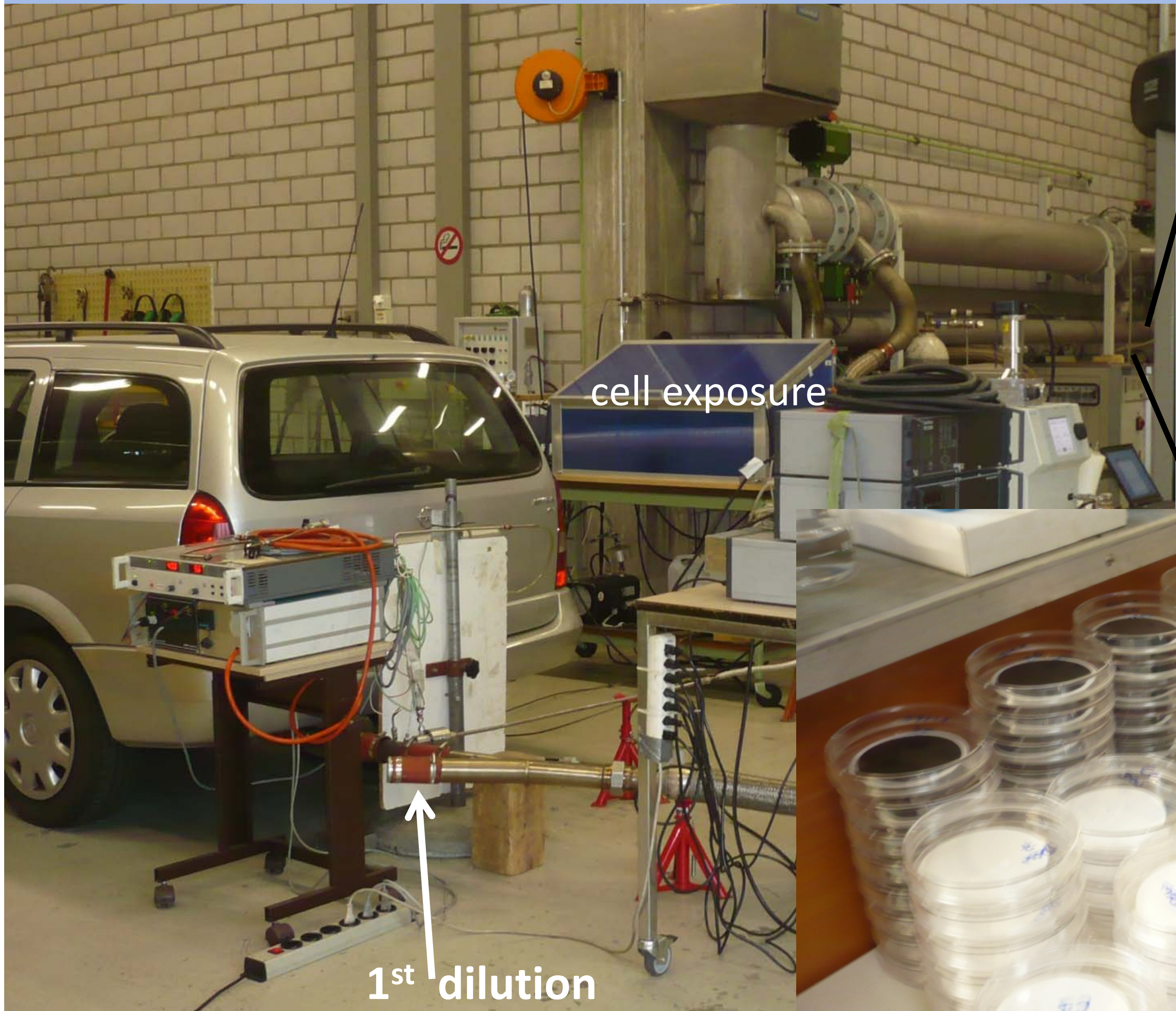
IRPrestige-21

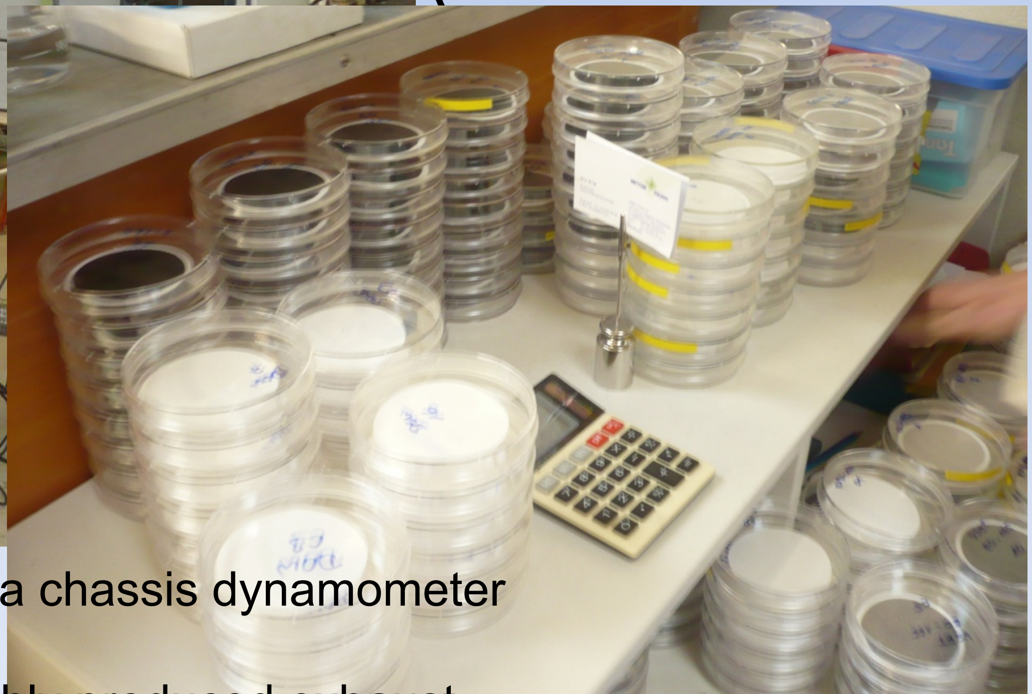
Ion content by capillary electrophoresis



Capel-103PT

Overview of sampling and exposure system in BFH/AFHB





- Opel Astra at velocity of 35 km/h on a chassis dynamometer
- exposure of lung cell cultures to freshly produced exhaust

Filter list and operation conditions

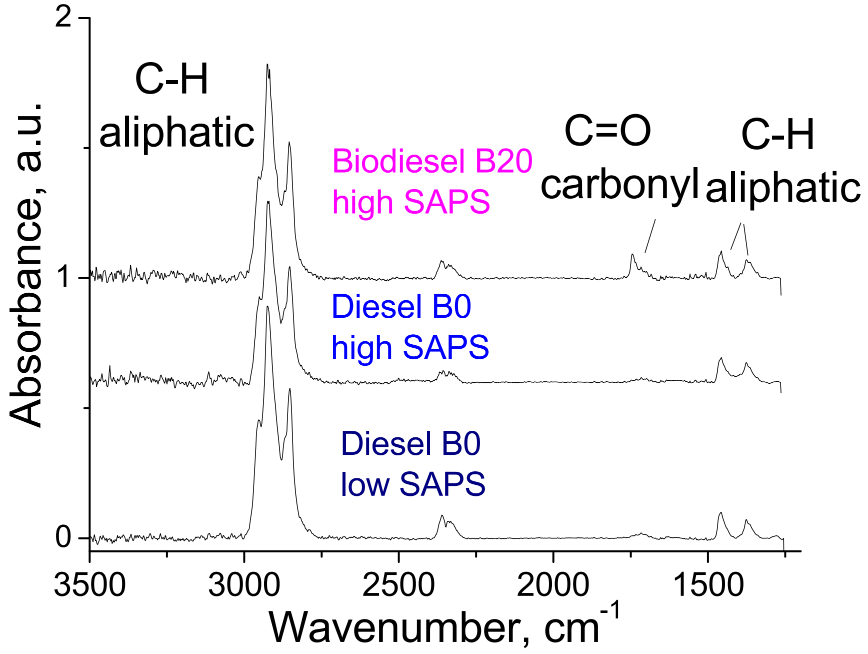
Filter n°	Weight [mg]	Fuel	Lube oil
312	3.165	Diesel B0	High SAPS
123	0.727	Diesel B0	High SAPS
105	2.987	Diesel B0	Low SAPS
27	0.697	Diesel B0	Low SAPS
210	4.097	B20	High SAPS
133	0.982	B20	High SAPS
in 2 hours 106	4.076	B20	High SAPS
103	1.04	B20	High SAPS

Fuel:
Diesel BO, max 10 ppm of S
Biodiesel B20, 20% biofuel

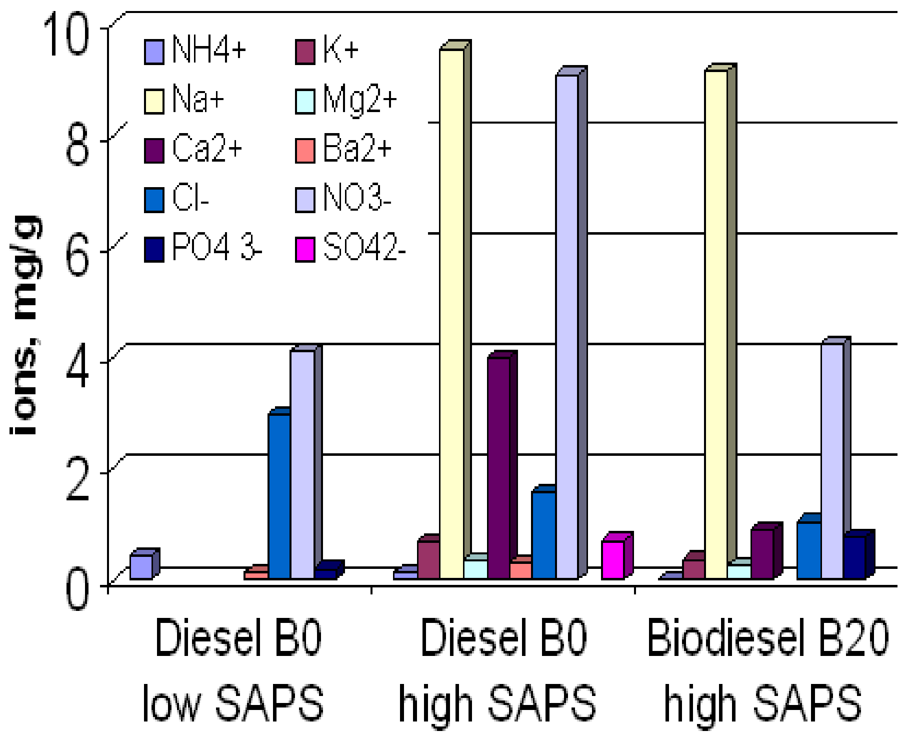
Lube oil: SAPS Sulfated ash, phosphorous and sulfur
high SAPS, 1.5% ash
low SAPS, ≤ 0.5% ash

Characterization of organic/inorganic compounds

ORGANICS



Water soluble IONS



similar organic chemistry,

aliphatic C-H groups dominate,

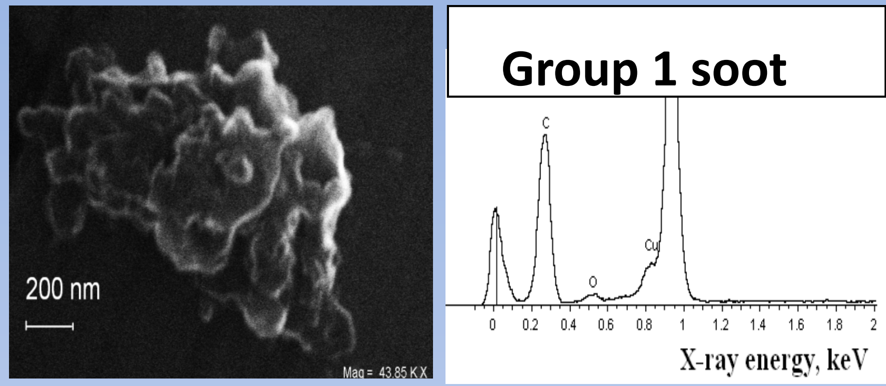
more oxidized surface of biodiesel particles

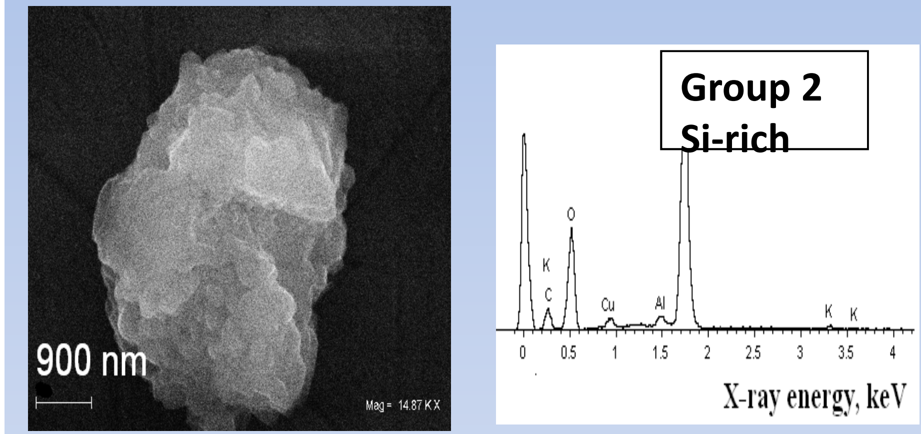
Ions mass ~ 3%PM,
high SAPS determine ion content,
Na^+, NH_4^+, Ca^{2+} dominate,
SO_4^{2-} is specific for diesel B0

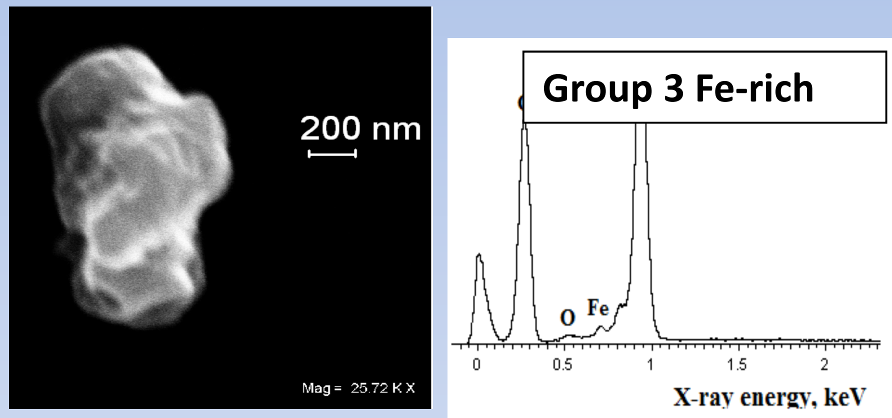
Individual particle analysis of diesel B0, low SAPS exhaust

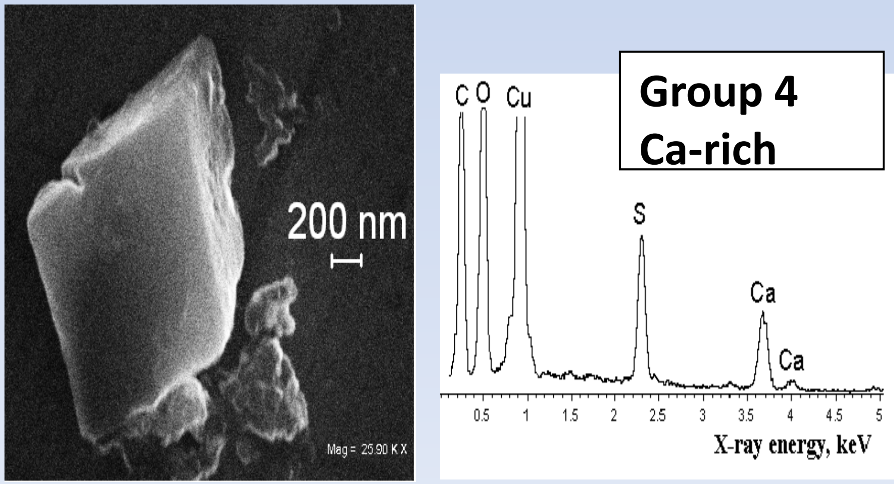
	C	N	O	F	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Mn	Fe	Ni	Nb	Pd	Pt
Group 1 soot 42.1 %																				
max	100	32.6	55.8	20.2	10.5	0.6	15.7	12.2	0	4.7	4.3	4.4	8.0	14.5	0	12.0	0	59.6	16.1	80.5
mean	**79.9**	0.3	**13.5**	0.2	0.4	0.0	0.7	1.1	0	0.2	0.2	0.1	0.3	0.1	0	0.3	0	0.6	0.2	1.8
Group 2 Si–rich 21.5 %																				
max	51.7	0	100	1.0	4.6	3.6	14.1	42.3	1.2	2.1	0	100	3.7	4.9	6.5	30.2	0	0	0	0
mean	15.0	0	**50.4**	0	0.4	0.4	1.1	**20.9**	0	0.1	0	4.4	0.5	0.1	0.1	6.5	0	0	0	0
Group 3 Fe-rich 21.1%																				
max	38.3	0	55.2	0	2.1	0	0	11.9	0	0.7	0	0	3.4	0	0	75.8	26.1	0	0	0
mean	14.2	0	**27.7**	0	0.1	0	0	0.9	0	0	0	0	0.3	0	0	**56.3**	0.5	0	0	0
Group 4 Ca-rich 8.7 %																				
max	57.0	0	57.1	0	0.4	4.7	0.1	1.2	2.8	14.6	0.6	0.5	39.4	0	0	11.8	0	0	0	0
mean	33.7	0	39.1	0	0	0.3	0	0.1	0.2	1.3	0	0	**24.7**	0	0	0.6	0	0	0	0
Group 5 Al-rich 6.6 %																				
max	41.8	0	48.8	0	0	0	50.9	0	0	0	0	0	0	1	0	0	0	0	0	0
mean	10.7	0	42.7	0	0	0	**46.3**	0	0	0	0	0	0	0	0	0	0	0	0	0

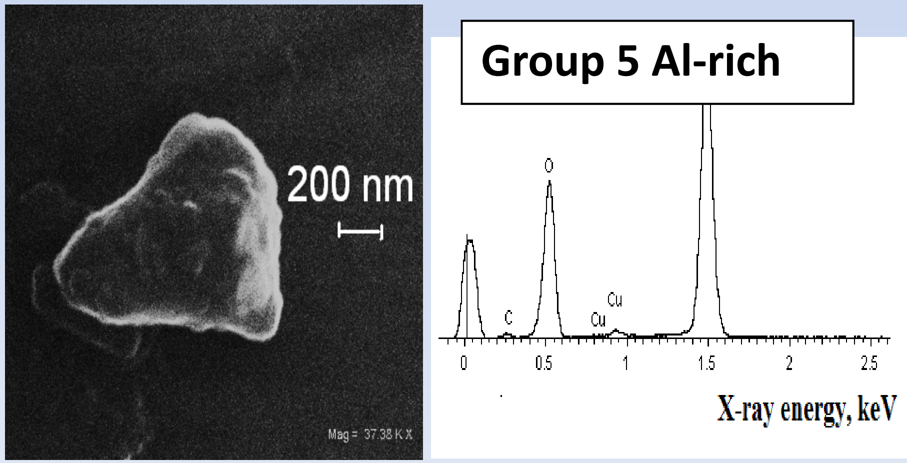
Individual particle groups in diesel B0, low SAPS exhaust







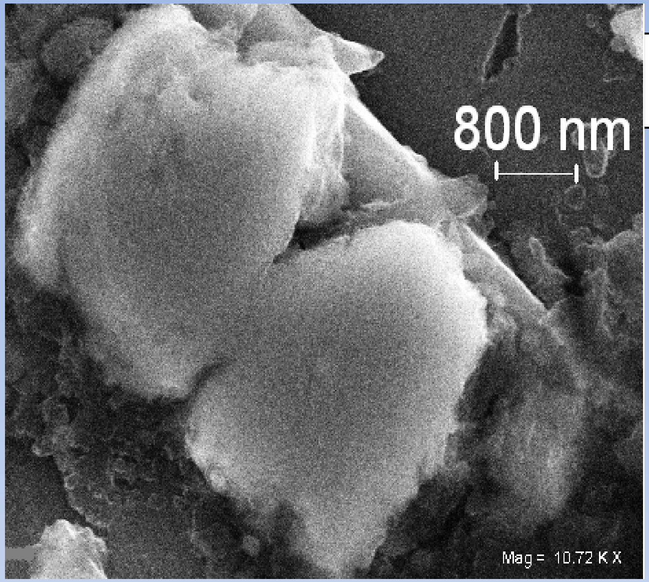




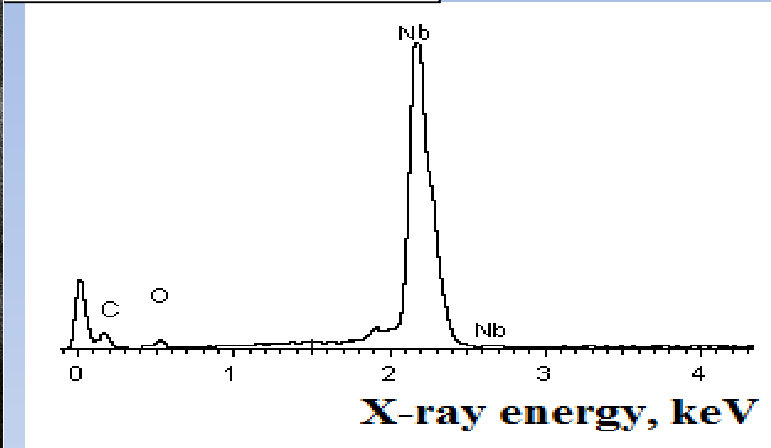
Individual particle analysis of diesel B0, high SAPS exhaust

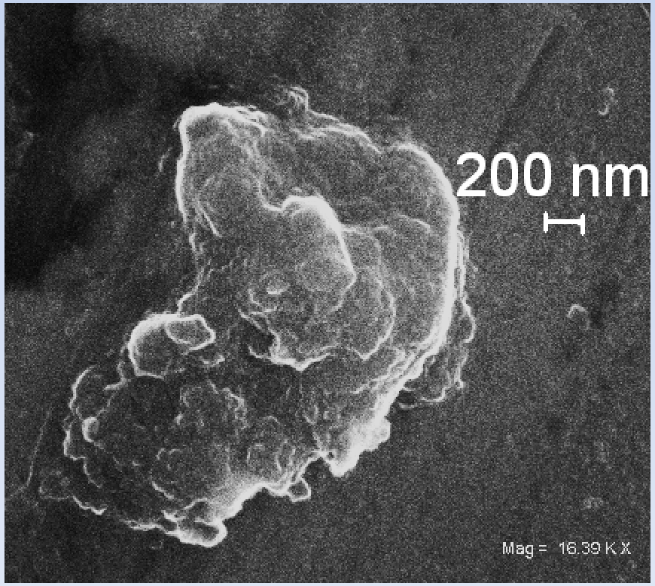
	C	O	F	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	V	Fe	Ni	Nb	W
Group 1 soot 34.6 %																		
max	100	36.8	0.2	6.2	6.7	18.6	5.4	0.6	3.9	3.4	1.6	3.5	0	23.1	2.3	0	0	0
mean	90.7	7.3	0	0.2	0.2	0.7	0.1	0.1	0.2	0.0	0.1	0.1	0	0.3	0.1	0	0	0
Group 2 Al-rich 34.6 %																		
max	31.9	55.1	0	0	0	54.3	28.1	0	0.3	0.0	1.9	1.5	1.1	0	0	0	0	0
mean	9.6	44.1	0	0	0	45.4	0.4	0	0	0	0	0	0.3	0	0	0	0	0
Group 3 Si,Ca 10.4 %																		
max	55.4	61.8	24.1	9.7	28.3	9.7	38.2	2.7	36.1	1.0	16.8	27.5	5.4	1.2	38.2	0	0	0
mean	27.8	41.9	1.1	0.7	2.1	1.0	10.2	0.2	2.1	0.1	1.0	6.6	0.2	0.1	5.0	0	0	0
Group 4 Al,Fe 6.9 %																		
max	31.9	91.0	0	1.7	3.8	17.0	5.6	0	1.8	0.0	0.5	3.6	0	0	20.9	1.6	2.8	0
mean	8.2	79.8	0	0.2	0.9	4.2	1.1	0	0.2	0	0.1	0.6	0	0	4.1	0.1	0.6	0
Group 5 Fe-rich 5.6 %																		
max	19.5	39.8	0	0	0	0	0	3.6	0	0	0	0	0	0	100	24.2	0	0
mean	6.5	15.3	0	0	0	0	0	0.3	0	0	0	0	0	0	63.3	14.6	0	0
Group 6 Nb-rich 4.3 %																		
max	23.5	46.8	0	0	1.1	1.5	1.2	0	0	0	0	0.5	1	0	1.0	0	90.4	0
mean	12.3	27.3	0	0	0.4	0.6	0.2	0	0	0	0	0.1	0.5	0	0.2	0	58.8	0
Group 7 W-rich 3.5 %																		
max	14.3	1.0	0	0	0	0	0	0	0.0	0	0	0	0	0	0	0	0	94.3

Individual particle groups of diesel B0, high SAPS exhaust

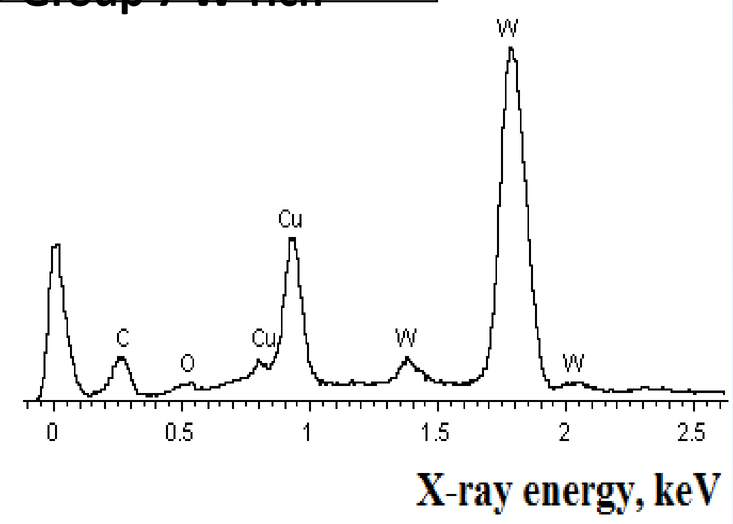


Group 6 Nb-rich





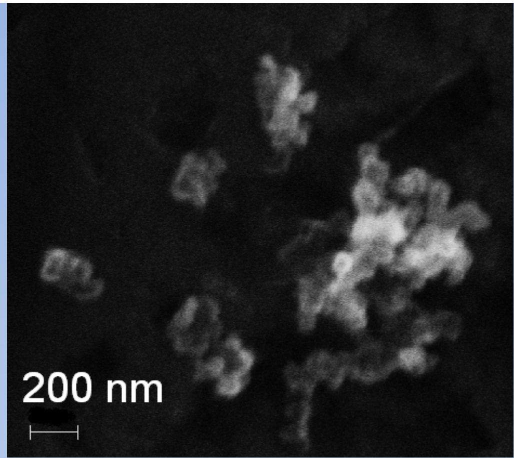
Group 7 W-rich

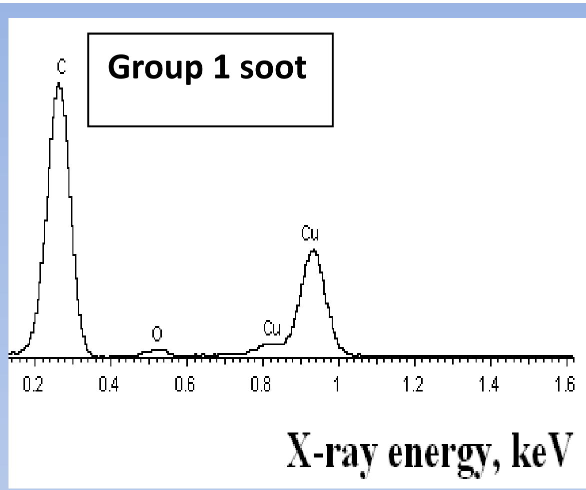


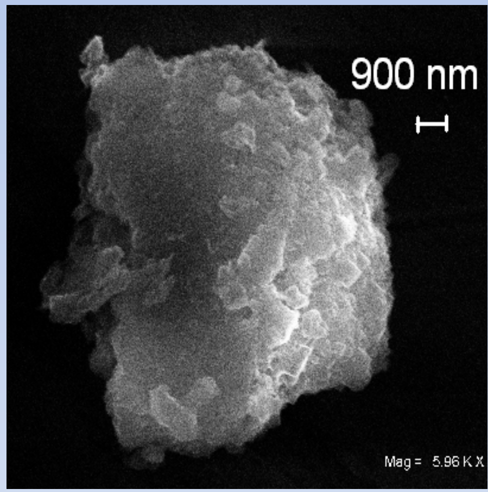
Individual particle analysis of biodiesel B20 high SAPS exhaust

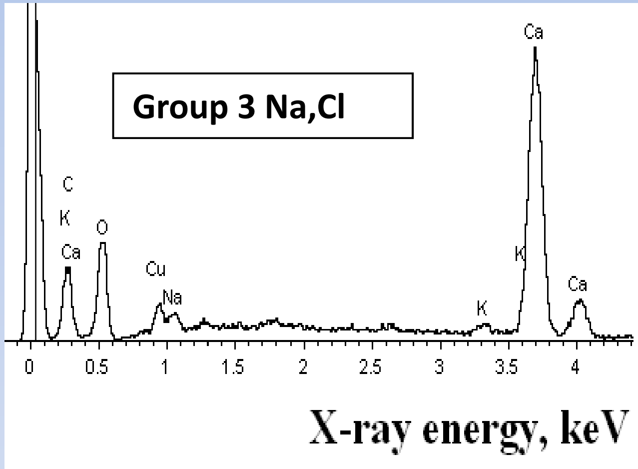
	C	O	N	Na	Mg	Al	Si	S	Cl	K	Ca	Ti	Fe	Zn	W
Group 1- soot 43.0%															
max	100	8.9	0	0	0	0.4	2.2	0.4	0.6	0.6	2.3	0	0	0	0
mean	99.0	0.9	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0	0
Group 2 Ca ,Al – rich 17.6%															
max	74.7	59.0	0	5.3	10.9	44.9	10.0	17.8	5.1	4.3	32.4	3.8	11.6	0	0
mean	43.4	33.0	0	0.2	0.5	7.4	2.0	0.8	0.6	0.2	11.3	0.3	0.3	0	0
Group 3 Na ,Cl 11.4%															
max	81.2	28.3	19.3	65.0	1.1	9.2	3.0	1.1	19.1	10.7	7.4	0	0	0	0
mean	61.5	13.6	1.6	13.3	0.1	0.7	0.5	0.4	5.0	2.1	1.1	0	0	0	0
Group 4 - Fe– rich 9.3%															
max	36.7	27.7	0	0	0	0.8	0.9	0	6.4	0	0	0	75.7	0	0
mean	10.0	23.6	0	0	0	0.0	0.1	0	4.0	0	0	0	62.4	0	0
Group 5 W-rich 9.3%															
max	14.5	20.6	0	0	0	0	0	0	0	0	0	0	0	0	89.2
mean	3.7	15.5	0	0	0	0	0	0	0	0	0	0	0	0	80.8
Group 6 Zn-rich 6.2%															
max	23.2	18.8	0	12.8	0	0	2.7	2.3	1.2	0.9	2.7	0	0	79.8	0
mean	11.8	14.8	0	1.1	0	0	0.8	0.2	0.3	0.1	0.2	0	0	70.7	0
Group 7 Si. Cl. K 3.1%															
max	52.7	48.9	0	7.8	0	2.7	24.2	0	43.8	42.6	0.9	0	0	0	0
mean	27.5	29.1	0	1.3	0	1.1	13.8	0	12.8	14.2	0.1	0	0	0	0

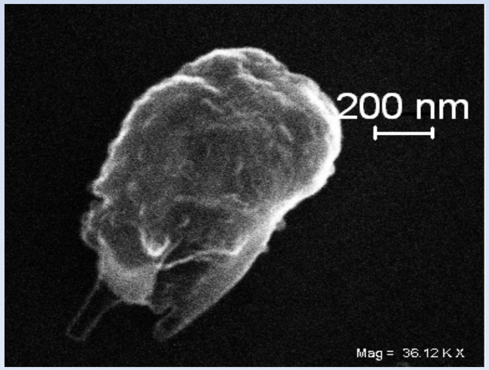
Individual particle groups of biodiesel B20 high SAPS exhaust

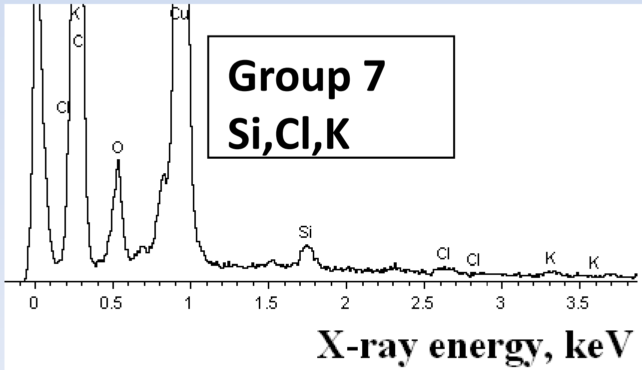






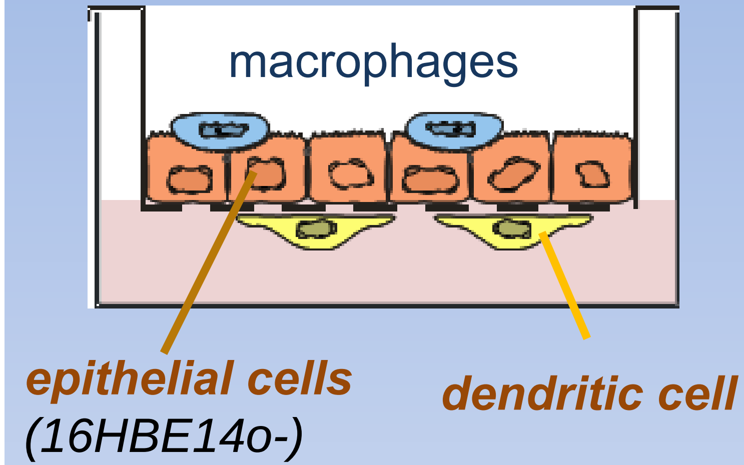






Cell exposure studies

Lung cell cultures



co-culture system simulating the human epithelial airway barrier

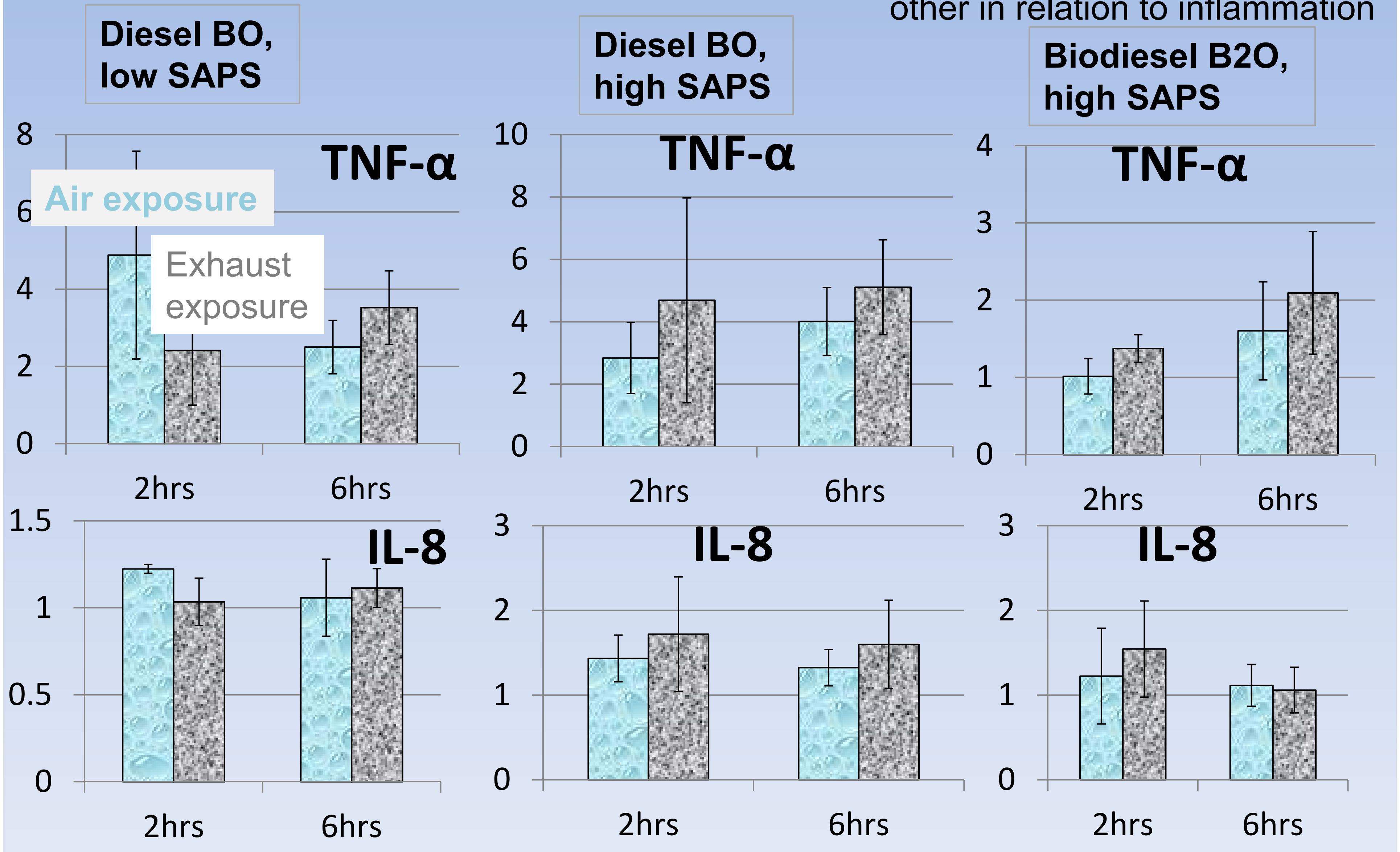
In order to simulate the situation in the lung *in vivo*, the cell cultures were exposed at the air-liquid interface

Toxicity tests of exposed cells , i.e. cytotoxicity, oxidative stress, inflammation, and genotoxicity

- ***LDH test of cell viability: no cytotoxicity, independently on the exhaust type and exposure duration.***

Inflammatory responses exposed vs unexposed

Determination of TNF- α and IL – 8 cytokines, two signal molecules used for communication of cells with each other in relation to inflammation



Concluding remarks:

Approach developed for exhaust particle characterization is a powerful tool for nanotoxicity studies.

Characterization at microscopic level reveals multicomponent composition of exhaust particles:

- ***for diesel particles : groups of soot, metal oxides ,and salts, for high SAPS - increased amount of metal oxide groups***
- ***for biodiesel particles: groups of soot, metal oxides, salts, and group with biofuel impurities.***

Differences in inflammatory cell responses are prominent for both diesel and biodiesel exhaust, high SAPS, they are due to different types of exhaust particles, not to organic chemistry.

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