ESEM-EDS-BASED APPROACH TO ASSESS NANOPARTICLES FOR FOOD SAFETY AND ENVIRONMENTAL CONTROL

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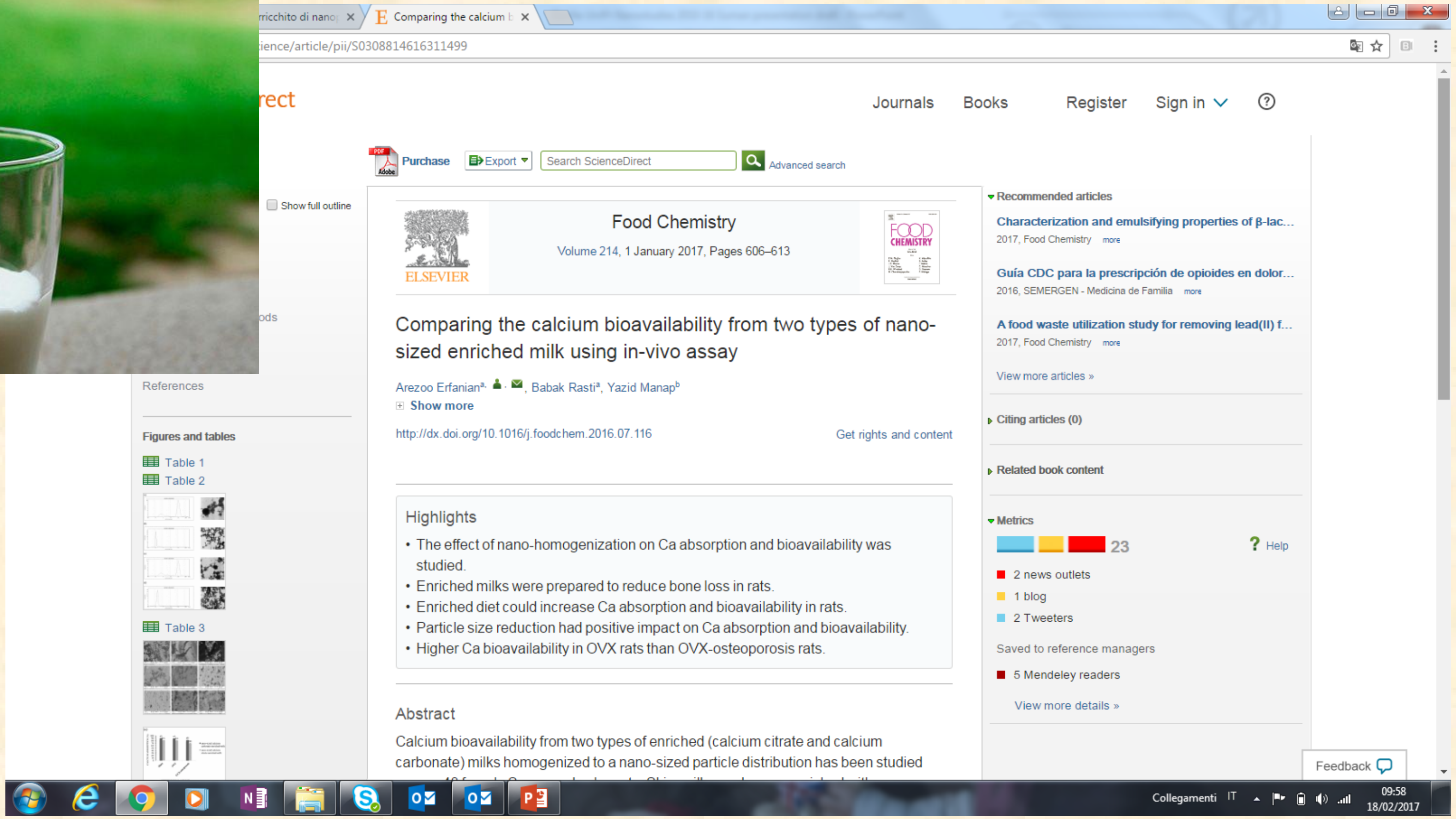
Good Side of NPs

Milk fortified with calcium nanoparticles helps to fight osteoporosis



Dark Side of NPs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Titanium dioxide may interfere in digestive processes: Study

By Will Chu, 17-Feb-2017
Last updated on 17-Feb-2017 at 14:56 GMT

Ingestion of titanium dioxide (TiO2) nanoparticles from products such as agric chemicals, processed food, and nutritional supplements is for the most part unavoidable.©iStock/ClaudioVentrella

Related tags: Titanium, Dioxide, Milk, Additive, Iron, Zinc, Fatty acids, Inflammation, EFSA, Enzyme, Doughnuts, Intestine

Titanium nanoparticles significantly decrease intestinal barrier

Study details

To investigate their short-term effects, researchers from Binghamton University, State University of New York, exposed intestinal cells to a meal's worth of TiO_2 nanoparticles (30 nanometres in size) over four hours as a way to measure acute exposure.

The experiment was repeated with the cells being exposed to three meal's worth of nanoparticles over five days as a way to test the effects of chronic exposure.

Exposure to these nanoparticles significantly decreased intestinal barrier function following chronic exposure.

Harmful reactive chemicals containing oxygen increased in number as did proinflammatory signalling and intestinal alkaline phosphatase enzyme activity, which all showed increases in response to nano-TiO_2.

The team also found iron, zinc and fatty acid transport were significantly decreased in activity following exposure to TiO_2 nanoparticles.

Food Safety & Authenticity

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ANALYTICAL FOOD SCIENCE

Living with nanoparticles

64 nanotoday FEB-APR 2008 | VOLUME 3 | NUMBER 1-2

The Confused side of NPs…

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Dai tessuti agli alimenti: il pericolo delle nano

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Luisa De Montis - Dom, 19/

Un nuovo pericolo nanos
pitture agli alimenti. Son

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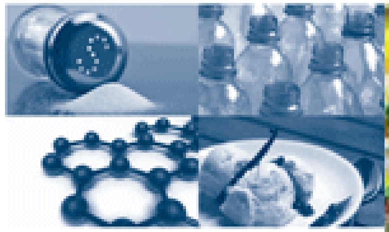
NANOTECHNOLOGY IN FOOD

9 and 10 September 2015
in Cologne/Germany

HIGHLIGHTS:



THE EXPERTS:

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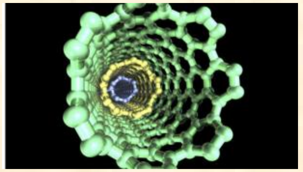
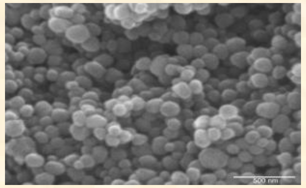
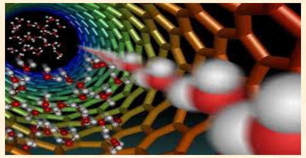
NANOPARTICLES – HEALTH IMPLICATIONS



Nanomaterials: very large surface area to mass ratio. This determining their high reactivity which is known to interfere with normal cellular processes and induce inflammatory reactions/oxidative damages



Large surface area, reactivity/electrical charge can also generate 'particle aggregation' (physical forces) or 'particle agglomeration' (chemical forces)

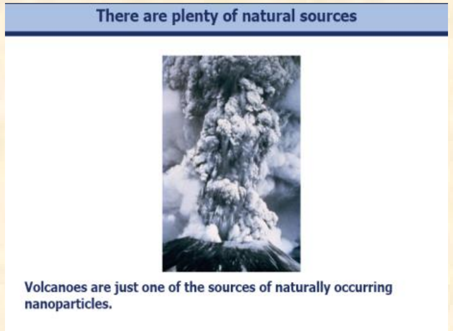


NPs can override cellular barriers, passing through the epithelium, reaching the gut/lymphatic vessels and entering directly in the bloodstream

Inorganic nanoparticles such as metals and metal oxides are considered to be the most hazardous being non-biodegradable, and potentially they may be retained for a long time leading to an accumulation in tissues

There are some indirect indications about connections between NPs and human diseases but no direct evidence of instances where ingested NPs harmed human health has been reported in scientific literature yet.

NANOPARTICLES - ORIGIN



WHAT THEY ARE:

- Particles commonly regarded with at least one dimension ≤ 0.1 micron

WHAT IS THEIR ORIGIN:

- Combustions processes (natural and anthropomorphic), unintentionally sources (e.g. diesel exhaust), abrasion processes, can be intentionally engineered
- Presence in food could derive from the environment (fall out on raw materials,..), production processes (pneumatic transport, nanoencapsulated aromas and so on..)
- Food constituents (such as proteins, starches, fats) undergo structural changes at the nano-micrometer scale during food processing (milling, homogenization,…)

Many food products are naturally structured at the nanoscale.

All foods, whether natural or processed, contain nano-particles. Examples: milk contains caseins at the nanoscale level, meat is made up of protein «nano»filaments (these affects the texture, etc…)

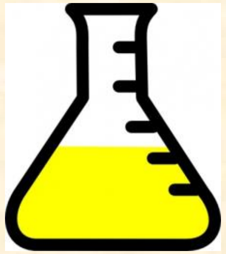
Moreover, during digestion food is reduced to progressively smaller components, e.g. sugars and amino acids, which are at the nanoscale.

It can be very difficult to distinguish between particles of engineered origin and particles from a natural or other source

OUR SAMPLE PREP APPROACH:

- ✓ Different raw materials and food products, like wheat, durum wheat, wheat flour, semolina, cookies and pasta were considered

- ✓ Protocol aimed at avoiding artefact formation (i.e. particle aggregation/agglomeration): precooking/defatting steps combined with enzymatic treatments and membrane filtration



samples (0.1 g) placed in flask adding TRIS buffer

Incubation in agitating bath (90° C 1h)

30mg amylase added (40° C overnight, agitating bath)

high fat

10 mL hexane – shaking and phase separation

low fat

filter weighing/pre-treatment (5 mL EtOH). Wheat seeds, grain cuticles discarded by a plastic sieve during filtration

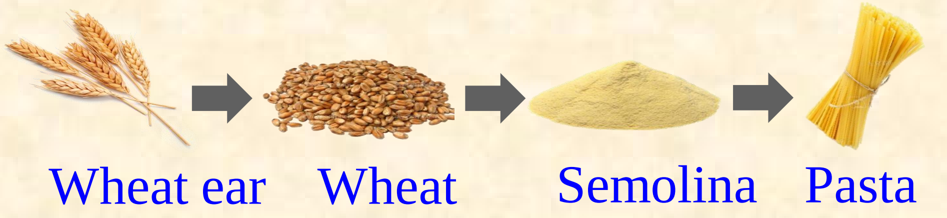
Filters placed in Petri with absorbent paper, dried at room temperature

Isopore ™ polycarbonate filters for ICP-MS and SEM, whereas ESEM samples using Durapore™ PVDF filters

all operations carried out in a laminar flow hood.

A more gentle treatment to reduce artefacts risks especially starting from wheat ear samples

Pasta production chain from industrials pilot plants



Processing steps, Extraction and Analysis Methodology

→ Pasta production and parallel analysis obtained by mixing the 4 wheat ears varieties provided in equal ratio.

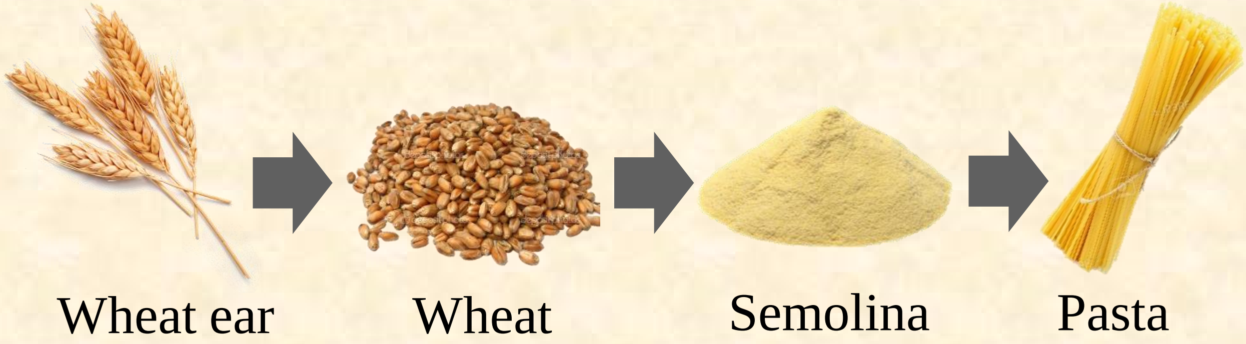
→ Grain samples and semolina and pasta submitted to the same extraction procedure used for the samples of wheat ears (washing in Milli-Q water in ultrasound for 1 h, filtration and subsequent ESEM with EDX microanalysis and to spectroscopic analysis ICP-MS.).

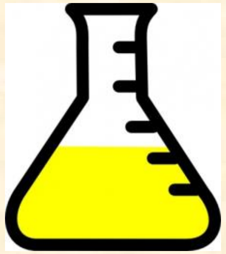
→ This procedure put in place in order to "remove" the outer part of the contamination without at the same time introduce the in situ generation of aggregates artifacts at the nanoscopic level, given the great complexity and level of impurities characterizing in particular the wheat ears.

OUR SAMPLE PREP APPROACH: focus & improvements on pasta chain

Pasta production chain from both industrial/pilot plants

The particles were collected on polycarbonate 0.1 µm filters after immersion in milli-Q water for 1 h.





samples (0.1 g) immersion in milli-Q water (with/without sonication)

low fat

filter weighing/pre-treatment (5 mL EtOH). Wheat seeds, grain cuticles discarded by a plastic sieve during filtration

Filters placed in Petri with absorbent paper, dried at room temperature

Isopore ™ polycarbonate filters for ICP-MS and SEM, whereas ESEM samples using Durapore™ PVDF filters

all operations carried out in a laminar flow hood.

OUR ANALYTICAL APPROACHES: ICP-MS

- Inductively Coupled Plasma-Mass Spectrometry (ICP–MS) multi-detection capabilities, to quantify different metal NPs in food, water, biota and soil at trace and ultra-trace levels

- Sample preparation for analysis–microwave-assisted mineralization: one half of each sample filter mineralized by using a MLS-1200 MEGA microwave apparatus in presence of nitric acid 65%, then cooling and dilution with Milli-Q water

- Experimental measurements were performed by ICP-MS X7 series II ICP-MS (ThermoFisher, Bremen, D) equipped with an AS-500 autosampler (CETAC, Omaha, NE, USA)

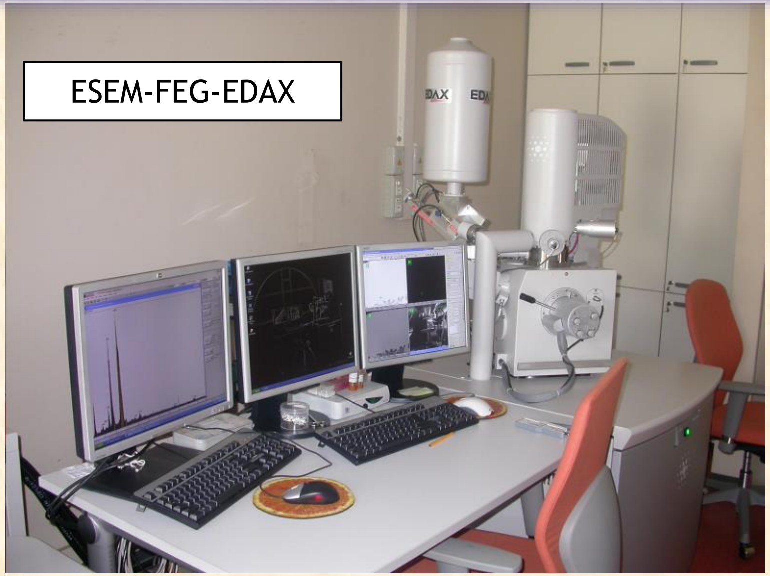
- Method validation for ICP-MS analysis was carried out according to EURACHEM guidelines in terms of
  - linearity (0.8-80 µg/g and 0.09-9 µg/g for Fe and Ti respectively),
  - quantification limits (0.73 µg/g for Fe and 0.09 µg/g for Ti),
  - repeatability (RSD%: 10% for Fe and 20% for Ti - wheat matrix)
  - extraction recoveries (93±2% - 101±2%)
  - uncertainty equal to 20%

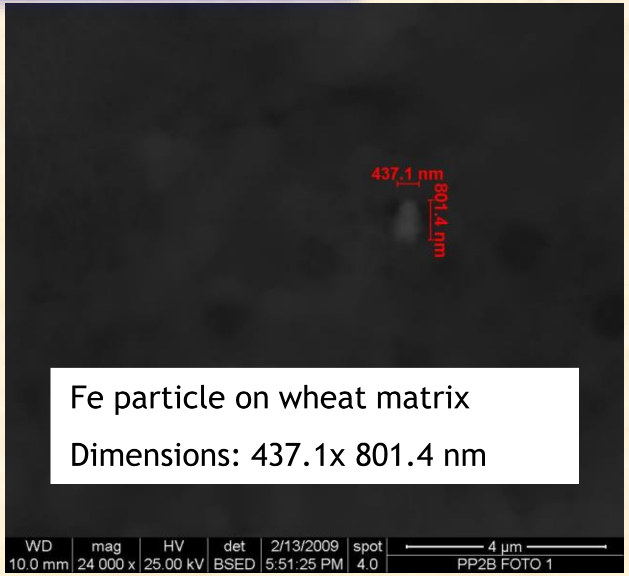
OUR ANALYTICAL APPROACHES: ESEM-FEG/EDAX

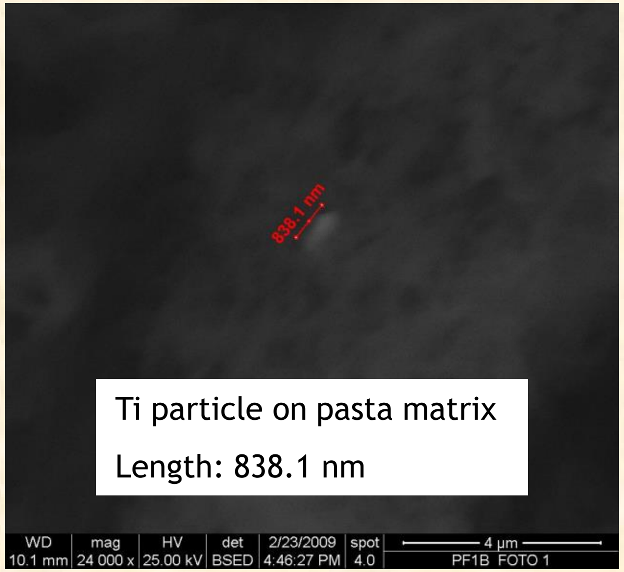
- Environmental scanning electron microscopy (ESEM) investigation performed on a FEI-QUANTA-250 ESEM-FEG equipped with an EDAX EDS apparatus with Si/Li detector
- ESEM-FEG allows imaging samples under ambient conditions at lower relative humidity, to some extent, circumvent artefacts
- Preliminarily, the sample-filters were analyzed with BSI spectra analysis characterization: (0.2 mm^2 area - 100 images of neighboring 50 × 45-μm2 zones along a straight line)
- Then, an EDS spectrum was collected for each bright spot/shape in the image together with data on particle size and shape. The particles having composition corresponding to the metals under investigation were counted and catalogued (three sample-filters for each kind of sample)
- As in the case of SEM-EDS mapping analysis, the localization of bright zones in BSI was performed manually
- Particle counting and identification were also performed on automatically acquired images, by Feature Analysis Esprit 1.9 software, over a proper representative filter area.

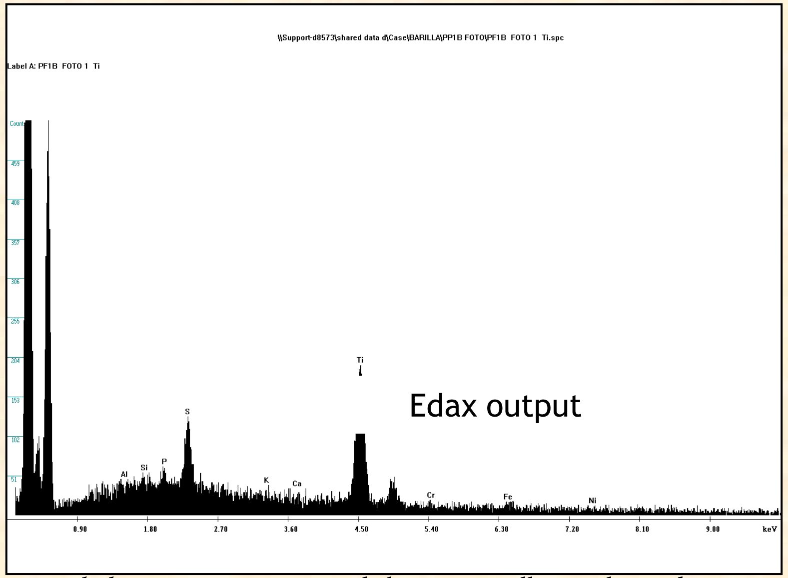
ANALYTICAL OUTCOMES: ESEM-FEG/EDAX

Generally, the largest number of particles was found in the case of Fe, whereas particles of Ti, Cu and Zn were occasionally found

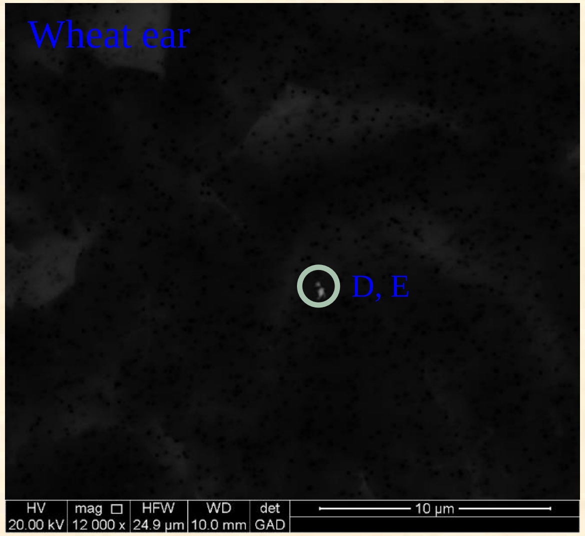


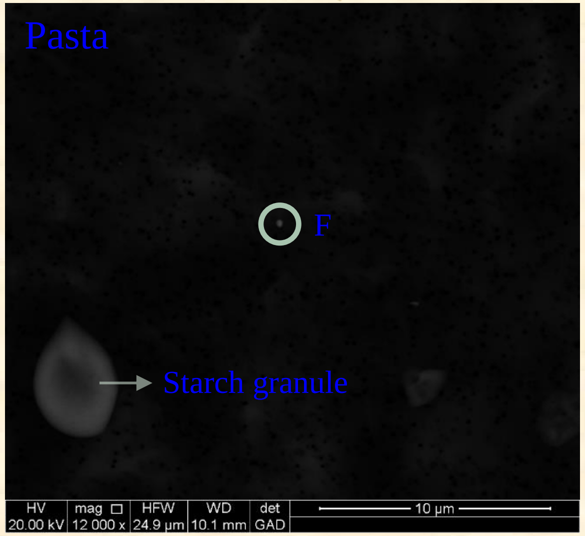




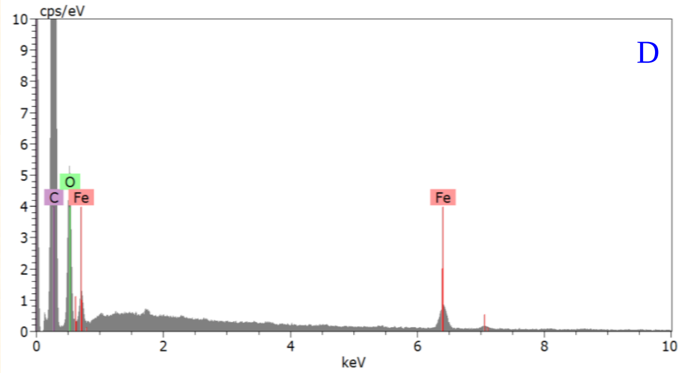


ANALYTICAL OUTCOMES: ESEM-FEG/EDAX

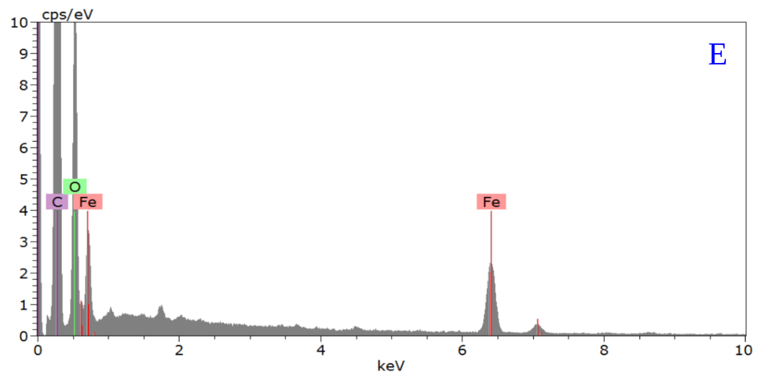


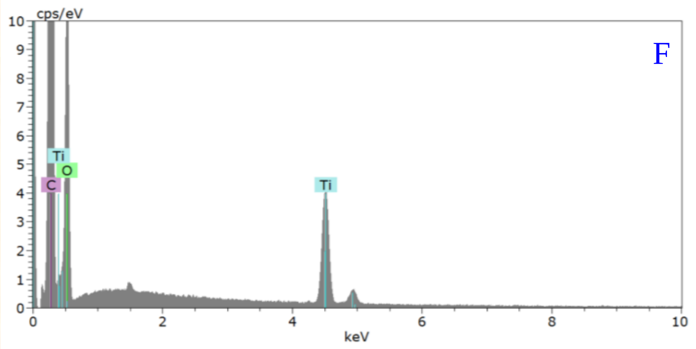


ESEM micrographs (BSE)

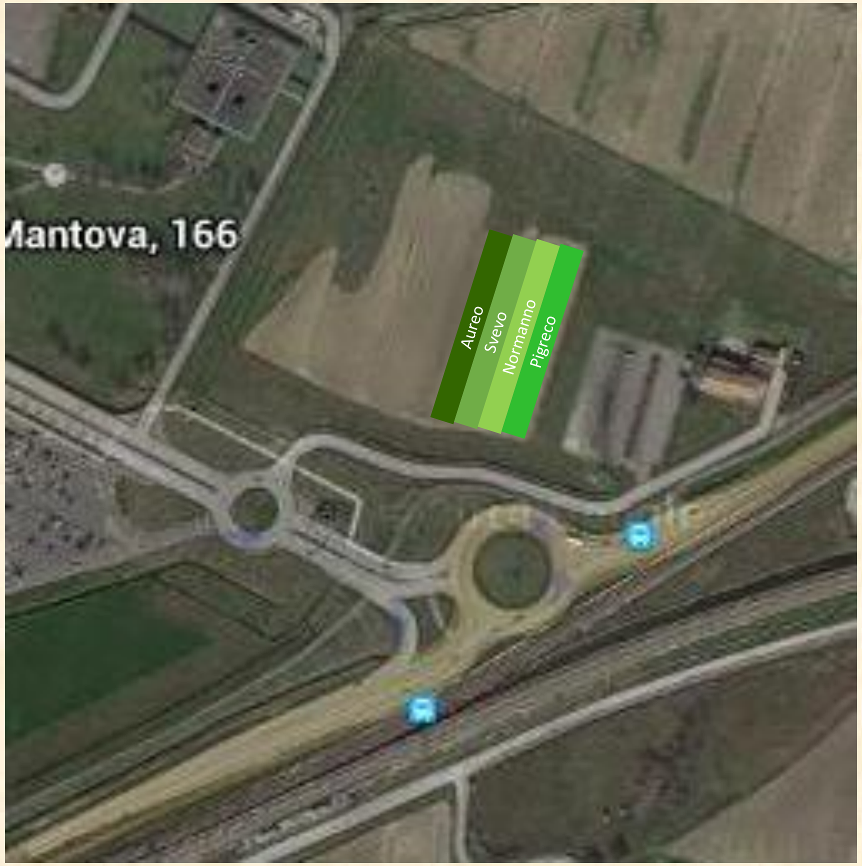


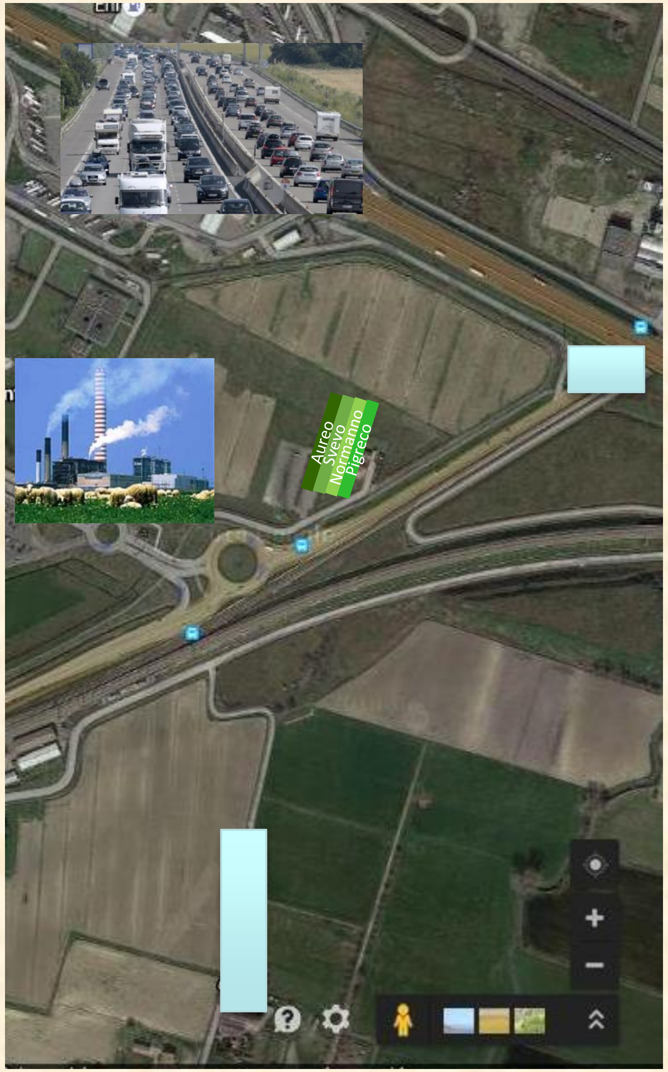
EDS spectra of selected particles





Focus more on fields contamination…





Staying on the field…what happen to wheat ears…

→ ESEM-FEG measurements indicated that number of metal nanoparticles present on wheat ears were dependent on the involved wheat varieties, coupling a similar trend when looking to Svevo/Aureo e Pigreco/Normanno variety combinations.

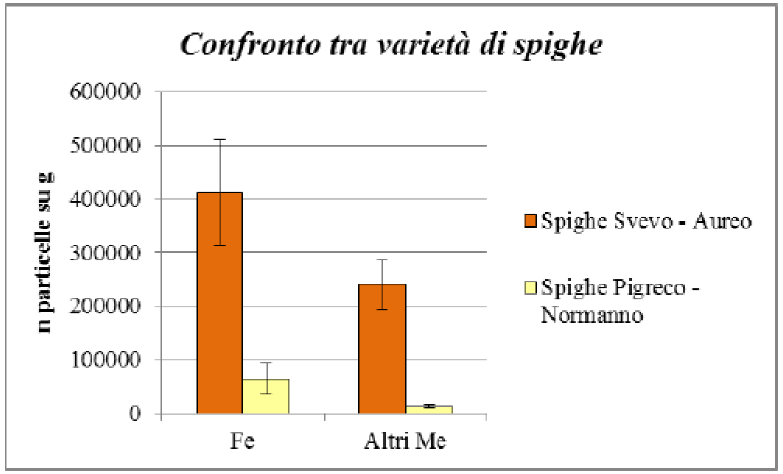
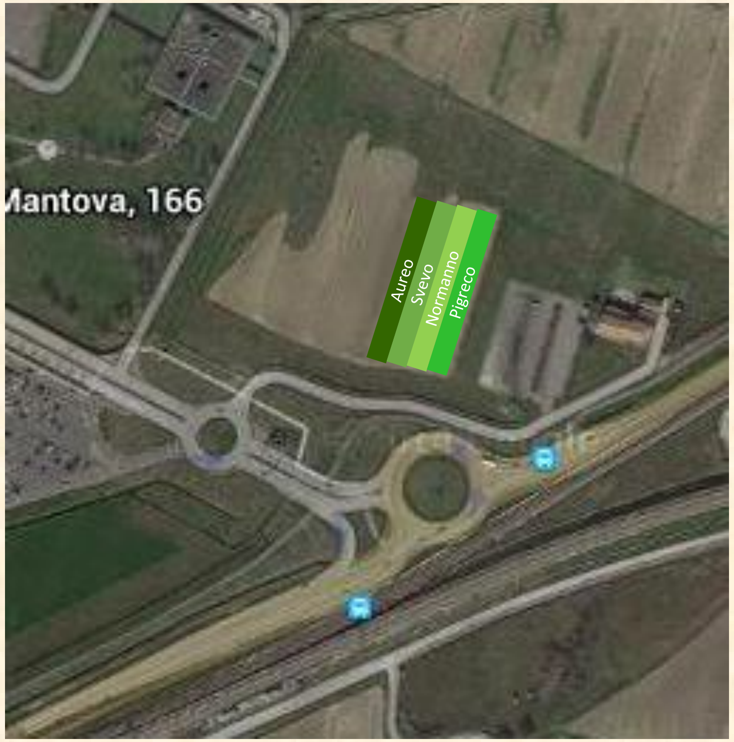


Fig. 7. Confronto degli andamenti del numero di particelle di ferro e di altri metalli con dimensioni <0.80 μm per unità di massa nei campioni di spiga



Staying on the field…what happen to wheat ears…

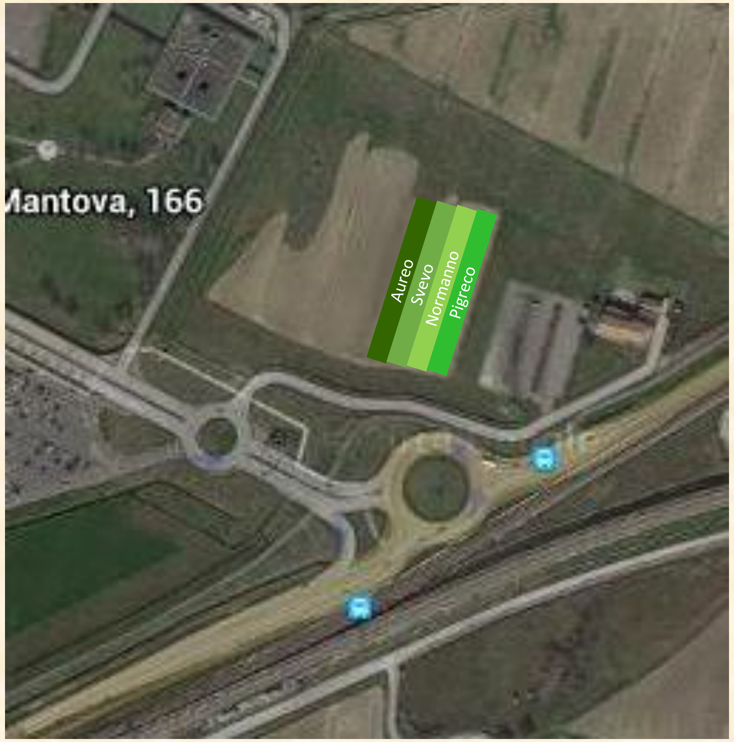
SOME INFERENCES FOR EXPLAINING THESE RESULTS (COUPLIG OF VARIETIES IN TERMS OF «ABSORPTION» OF NPs INTO EARS)

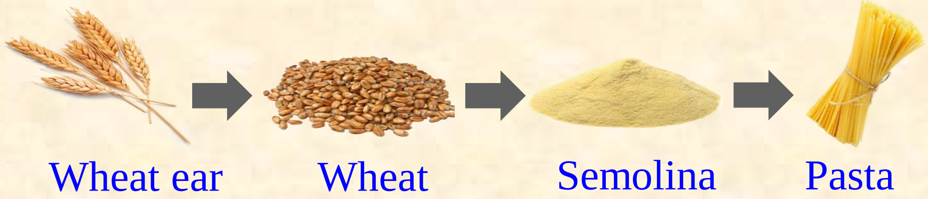
- From agronomical/genetic point of view Aureo is similar to Svevo because it has been developed from Svevo by progressive cultivars selection

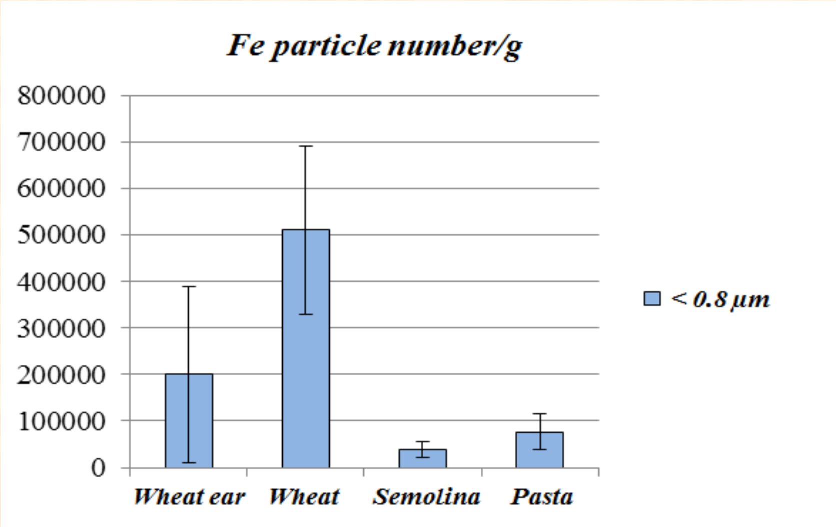
- Exposure time (about 50 days) under atmospheric conditions was the same for all the varieties but Svevo/Aureo generally grow quickly achieving "mature age/dimension" of ears about 2 weeks before Pigreco/Normanno

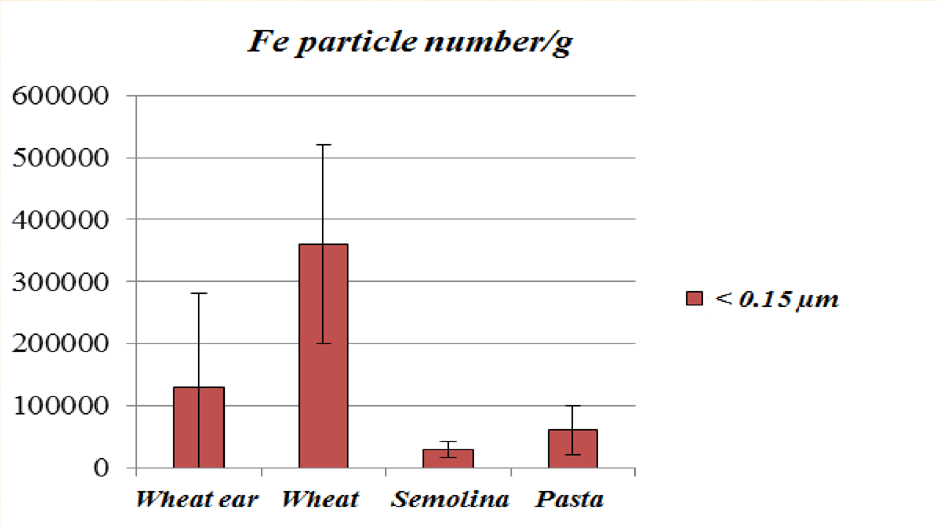
- Svevo/Aureo plants (Svevo in particular) are longer and more extended than Pigreco/Normanno ones

- Svevo/Aureo were exposed in the direction of the motorway/thermal incinerator and oriented vs general wind direction









- Identification of particles containing mainly **Fe**, low number of particles containing Ti, Al, Cr, Mn, Ni, Zn
- Similar trends for particles < 0.8 µm and < 0.15 µm
- The majority of particles of Fe with dimensions < 0.15 µm

ICP-MS confirmation of element identified by ESEM-EDS only qualitatively because their measured concentration was always below limits of quantification (LODFe = 75 ng/g, LODMn = 12 ng/g, LODCr = 20 ng/g, LODZn = 80 ng/g, LODAl = 30 ng/g, LODNi = 30 ng/g, LODCu = 15 ng/g, LODAs = 60 ng/g, LODSb = 20 ng/g, LODSn = 25 ng/g, LODTi = 9 ng/g).

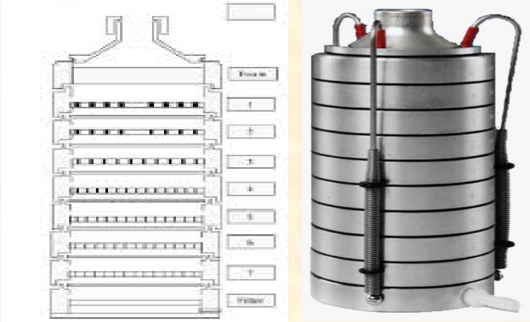
Depletion of micro- and nanoparticles along production chain

Let's continue with focus on air contamination…

Airborne particles sampling in the same area…

→ First experiment through air sampling for 72h along 5 weeks. Then 4 consecutive seasonal monitoring periods, starting from Spring2016 to Winter2017

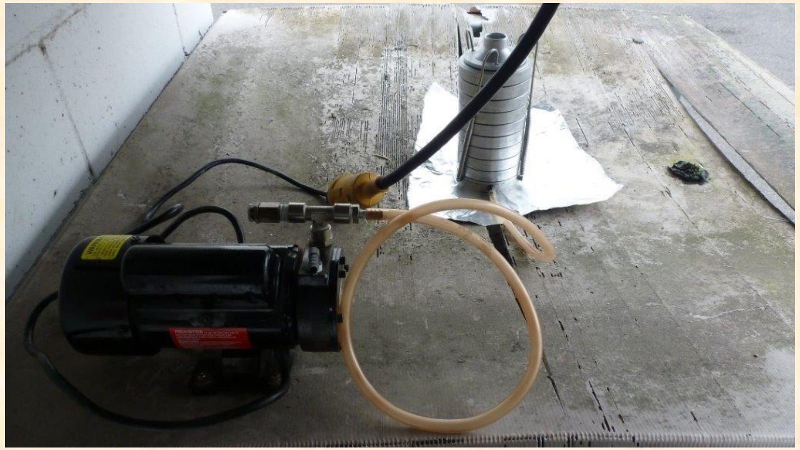
→ All the filters related to the different stages were conditioned 48h in a desiccator analyzed in comparison to a further "blank filter" (not exposed): ESEM-FEG, ICP-MS, Gravimetric Analysis (accurate mass balance / 3 replicates each)



Eight-stage cascade impactor

Size-fractionated sampling was performed by an **eight-stage cascade Andersen impactor** (polycarbonate filters, 0.4 µm from I to VII stages, final stage (F): 0.1 µm). Nominally, F stage should collect particles <0.7 µm, whereas stage VII in the 1.1-0.7 µm range). Five weeks sampling.

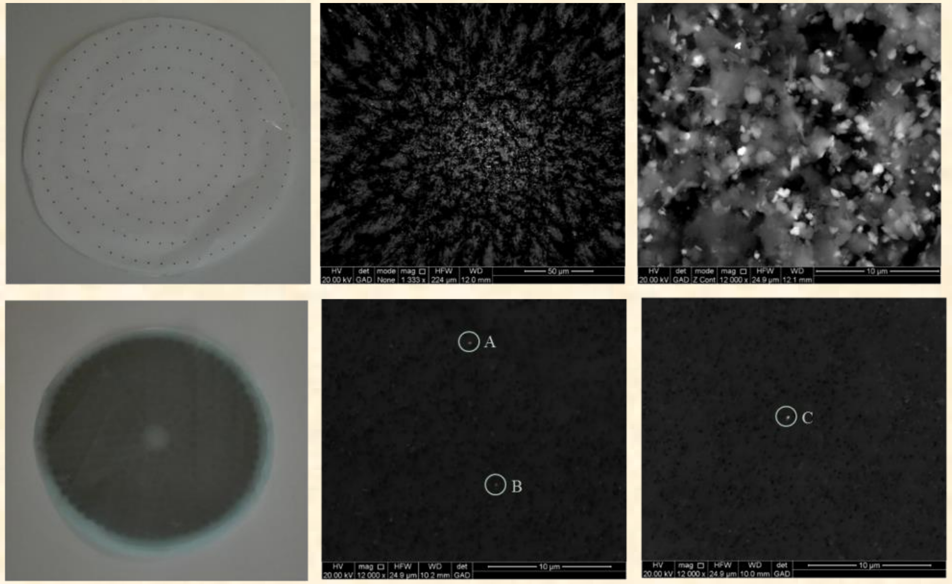
For ESEM-EDS analysis: polycarbonate filters; exposition time: 72 h.
For gravimetric analysis: QM-A quartz filter; exposition time (spring, summer, autumn, winter): 1 month.



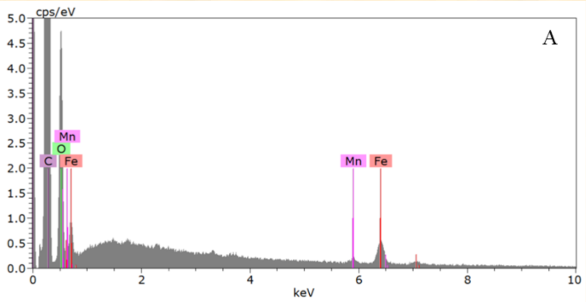
Air contamination...

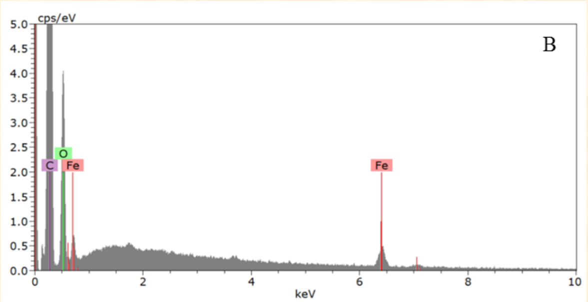
Stage VII

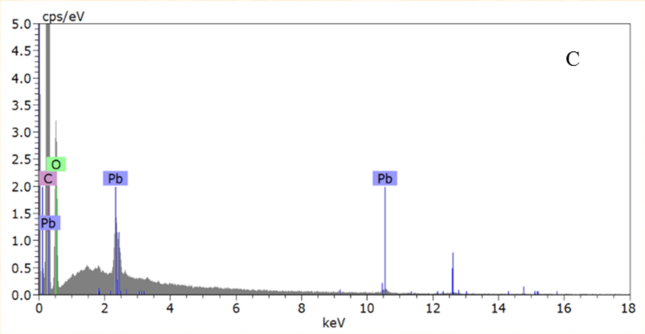
Stage F



Images of filters from Stage VII and F and corresponding ESEM micrographs (BSE)



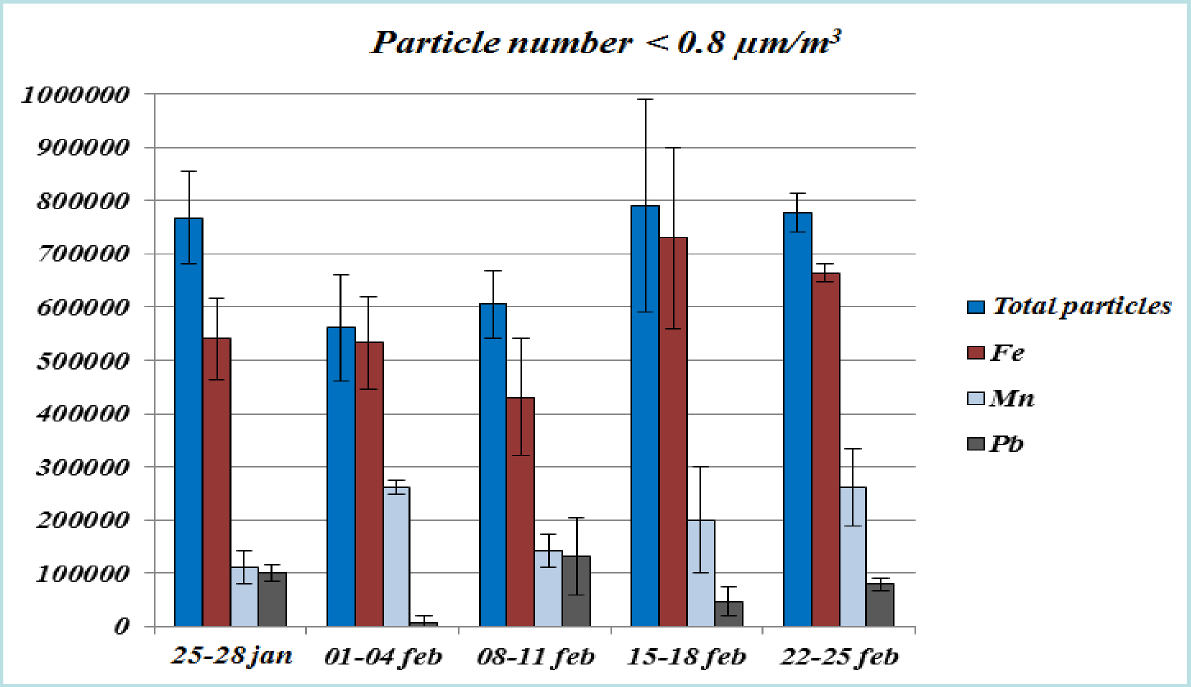


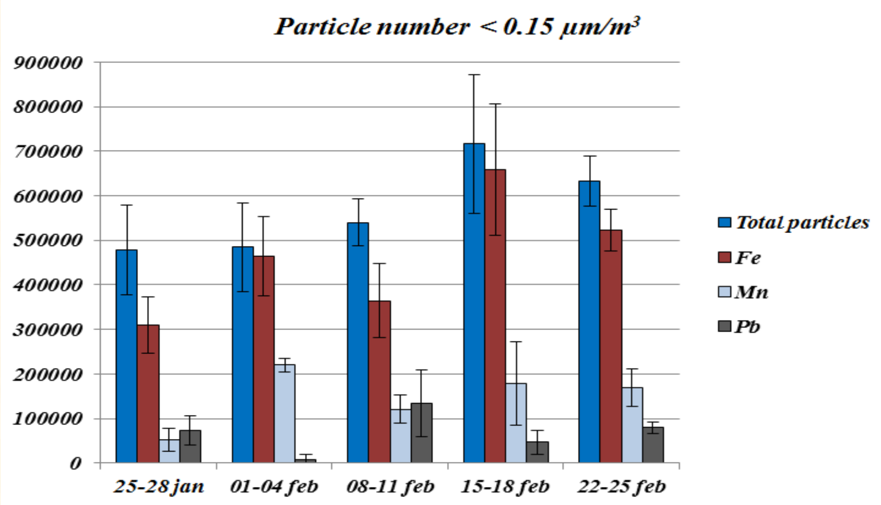


EDS spetctra of selected particles

Air contamination…

- Identification of particles containing mainly **Fe**, but significant presence also of **Mn** and **Pb**
- **Similar composition for particles <0.8 and <0.15 µm**

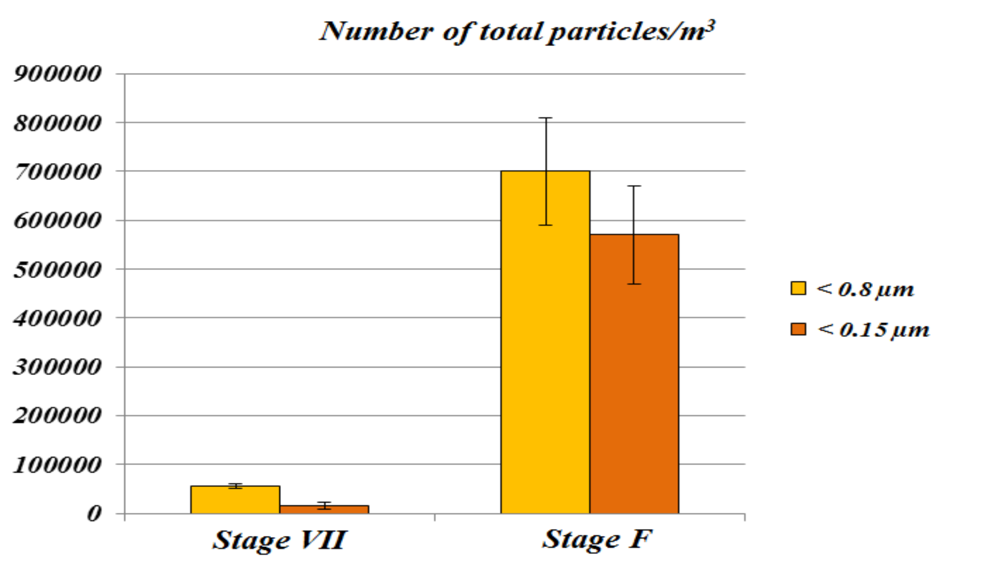




*Inductively coupled plasma mass spectrometry (**ICP-MS**) confirmation of element identified by ESEM-EDS*

Air contamination…

- Multi-stage impactor accumulate progressively smaller dimension particles. Nominally stage VII should collect 1.1-0.7 um range and stage F should include only < 0.7 um particles
- Effectively, the number of particles <0.8 and <0.15 µm measured in this way is prevalent in stage F with respect to stage VII
- Furthermore, **particles <0.15 µm represent around 80% of the total**



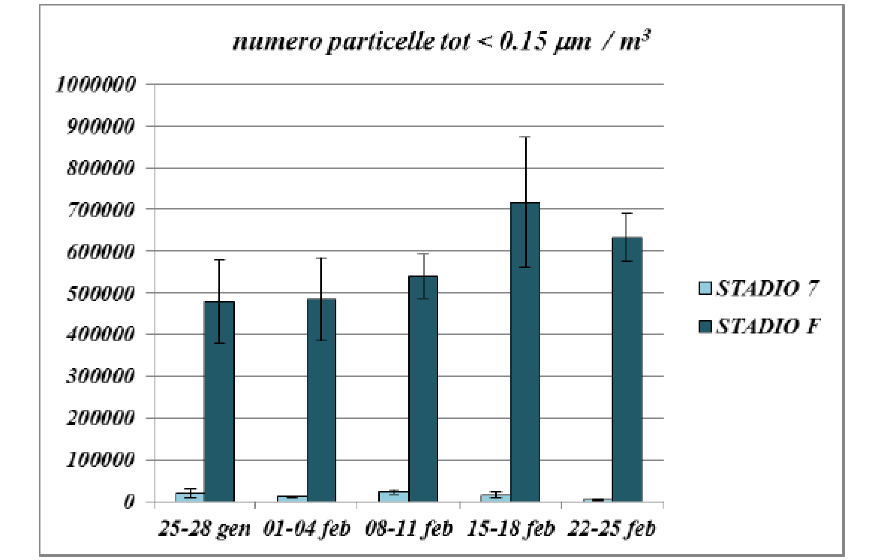
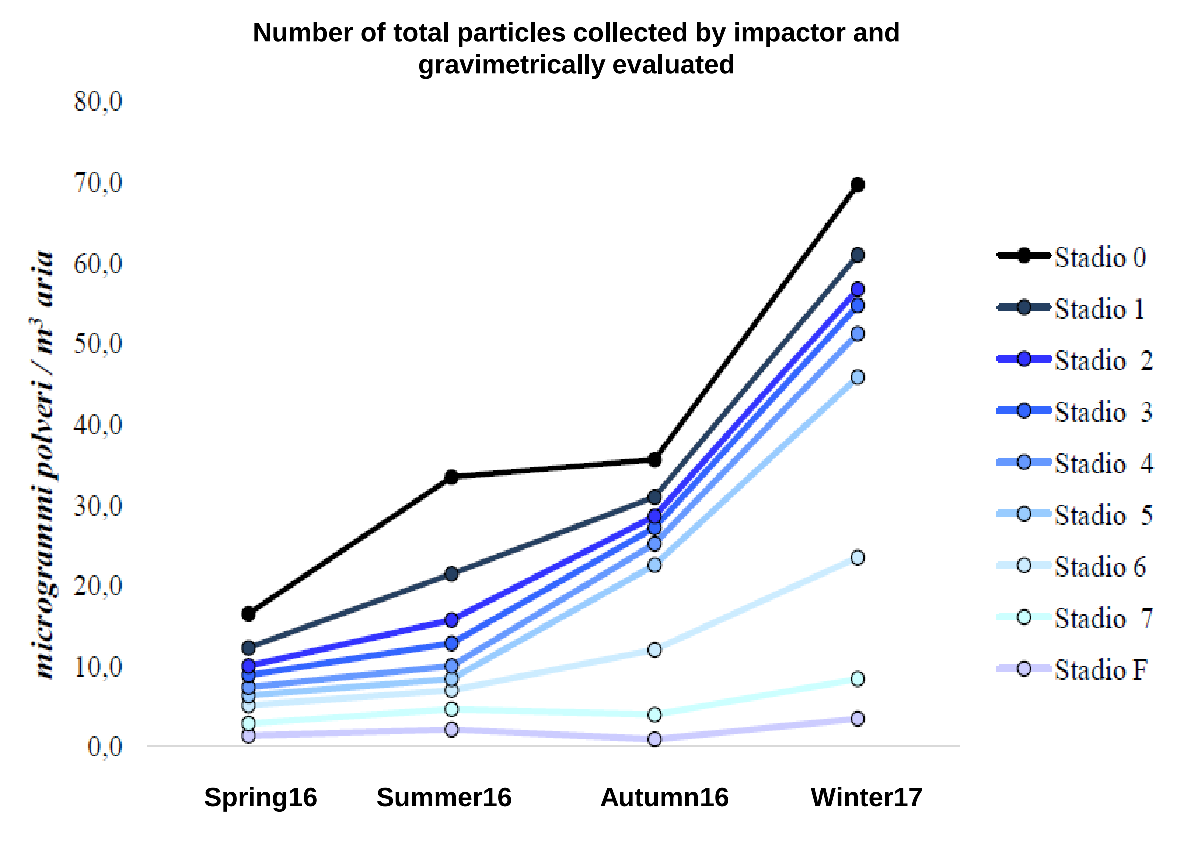
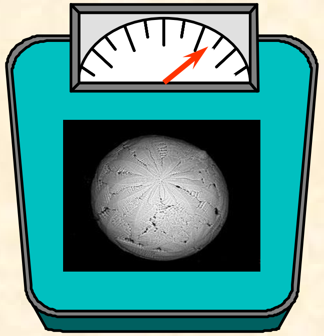


Fig. 4. Confronto tra stadio VII e stadio F in termini di numero di particelle totali con dimensioni <0.15 µm, per m³ di aria

Air contamination…

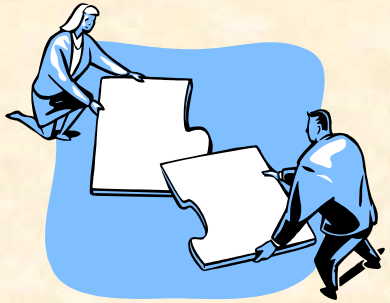




→ **During Winter the contribute** to the finest fraction of the total airborne particles **is doubled** than in Summer

→ The ratio between particles collected within *5-6-7-F* stages and particles collected overall (1←→F) [which means the formal **PM2.5/PM10**] increase **from *0.4-0.5 Spring/Summer* to *0,7-0,8 Autumn/Winter***.

COMPLETING THE PUZZLE…



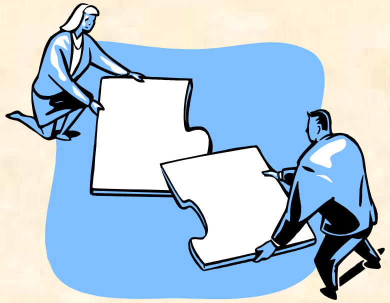
1 An essential issue for **future exhaustive risk assessments** is to correctly measure inorganic **micro- and nanoparticles both in environment & complex food matrices** with reproducible and accurate protocols

2 An **integrated approach based on the use of ICP-MS & (E)SEM-EDAX** techniques for qualitative/morphological characterization and quantitative analysis of metal nano-microparticles in complex food matrixes **was devised and validated**

3 ICP-MS data as well as (E)SEM measurements showed a **decrease in the concentration of metal particles** from wheat to flour/semolina samples, thus indicating a **substantially "external" contamination of grains**

4 Working for an entire year divided into 4 **seasonal periods, parallel airborne sampling** through multi-stage impactor combined with ESEM / ICP-MS & Gravimetric **analytical information confirm this scenario**

COMPLETING THE PUZZLE…



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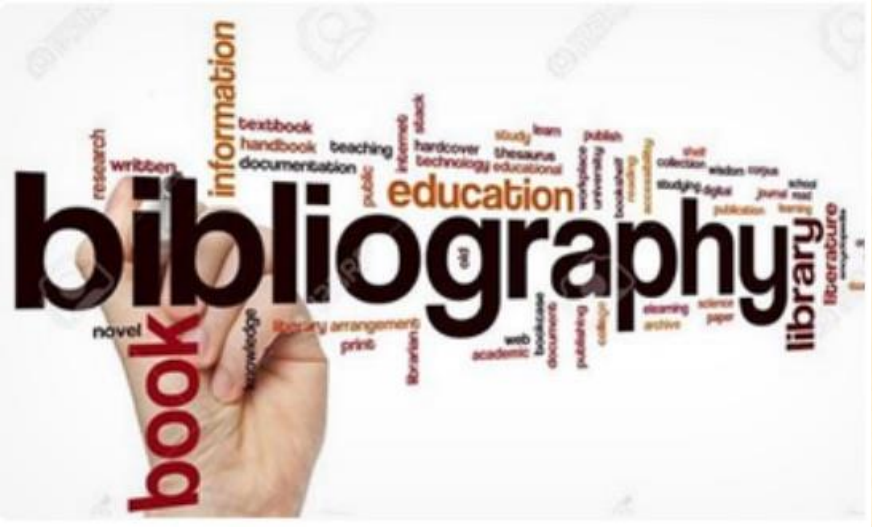

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Thanks for your kind attention!