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Salute e Materiali di Riferimento: solide garanzie per nuove esigenze

5 Ottobre 2021

Dipartimento Laboratori di taratura

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Determinazione di micro e nanoplastiche

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5 Ottobre 2021

Micro e nanoplastiche

M**icroplastiche** particelle di materiale plastico con dimensioni comprese tra 0,1 e 5 mm

N**anoplastiche** dimensioni < 100 nm

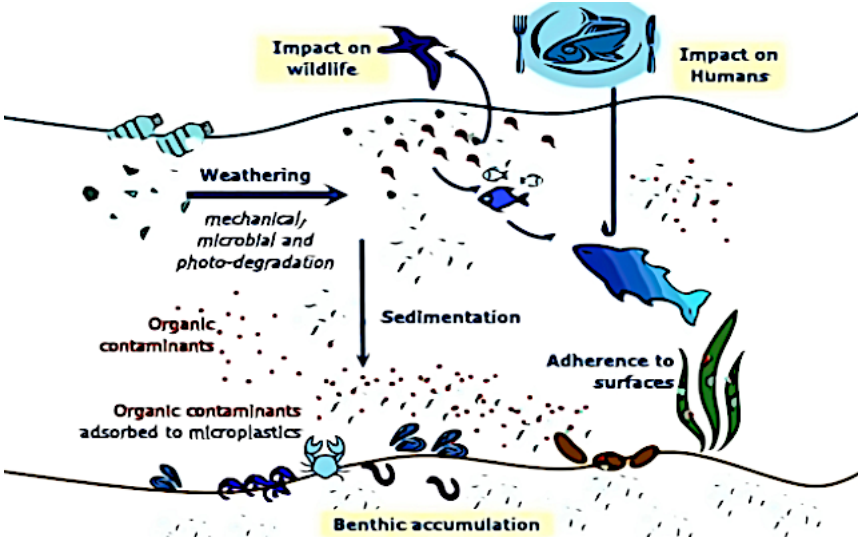
360 milioni di tonnellate di plastica prodotta ogni anno*

13 milioni di tonnellate di rifiuti plastici riversati nei mari

Agenti atmosferici

Azione meccanica di onde e correnti

Radiazione UV

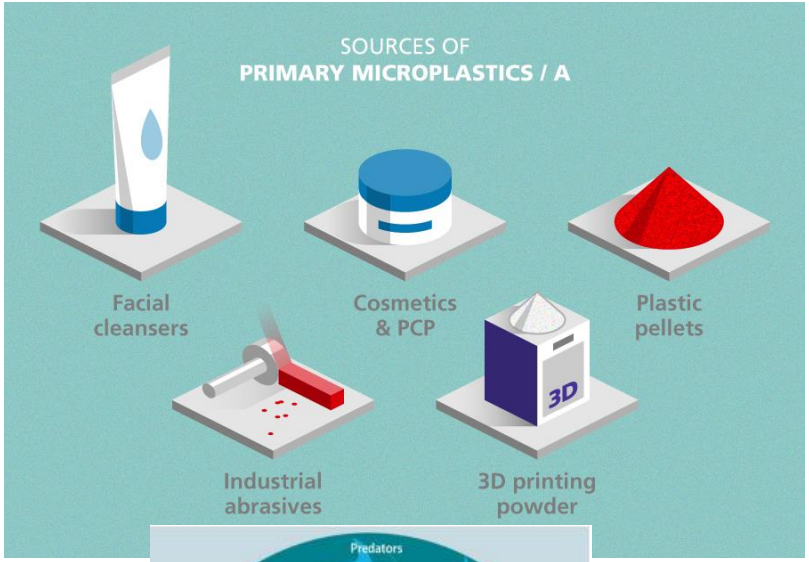


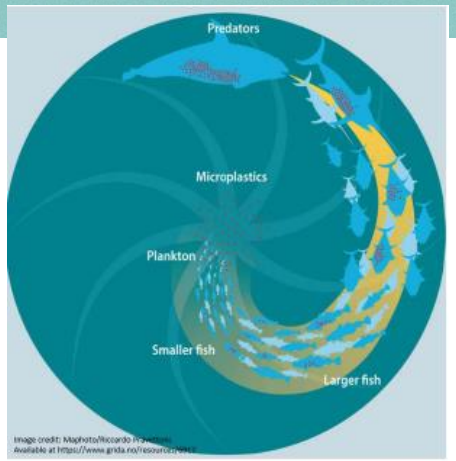
*The environmental impacts of plastics and micro-plastics use, waste and pollution:
EU and national measures, Policy Department for Citizens' Rights and Constitutional Affairs, PE 658.279 - October 2020

Definizioni

Micro e nanoplastiche primarie: deliberatamente prodotte con dimensioni millimetriche o submillimetriche. Presenti in diversi prodotti per la casa, materie prime utilizzate nella produzione di materiali plastici.

Micro e nanoplastiche secondarie: derivanti dalla frammentazione di plastiche a maggiori dimensioni per azione chimica, meccanica, biologica.
Problema globale che coinvolge tutti gli ecosistemi. Rischi per la salute umana
Interazioni con elementi in tracce, inquinanti organici… possibile creazione di artefatti, rischio intereferenze





Fattori chiave

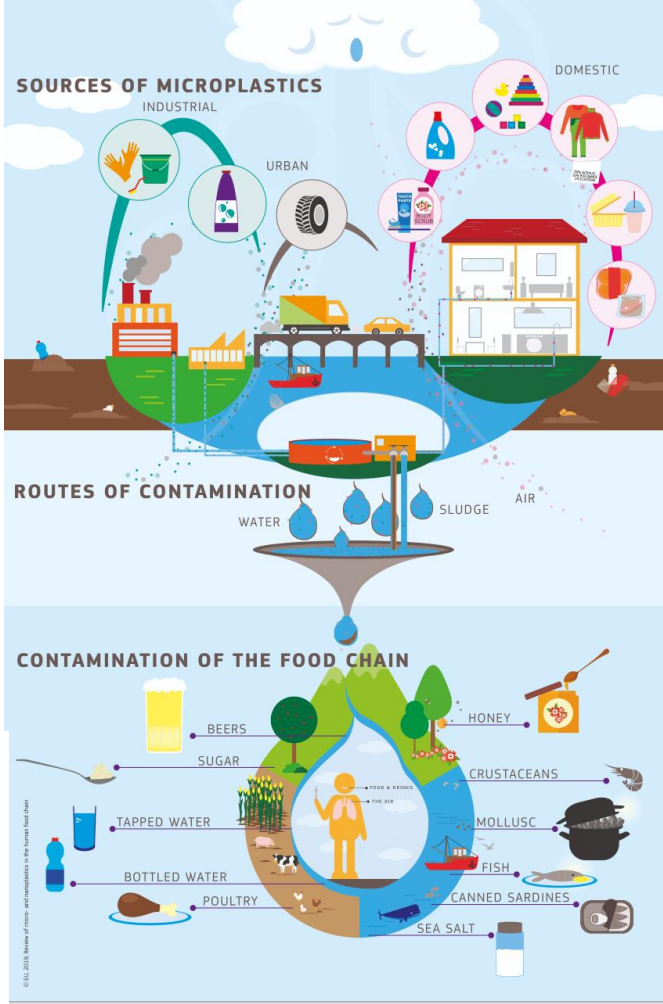
Proprietà chimico-fisiche
(forma, dimensione, composizione e idrofobicità)

influenzano trasformazione, interazione e biodisponibilità

Nanomateriali

Forma irregolare o uniforme

Dispersi come tal quali o come aggregati/agglomerati



Cosa succede a livello di nanoscala?

Proprietà e comportamento delle particelle possono cambiare a livello di nano-scala se confrontate con quelle a dimensioni maggiori

Dimensione piccola = elevata area superficiale = elevata reattività per peso equivalente

Rischi associati all’assunzione di nanomateriali

Esposizione a particelle insolubili o scarsamente solubili

Maggiore assunzione e biodisponibilità di nanoforme se confrontate con le forme convenzionali

Migrazione delle nanoparticelle dal tratto gastrointestinale ad altre parti del corpo

Interazione delle nanoparticelle con cellule

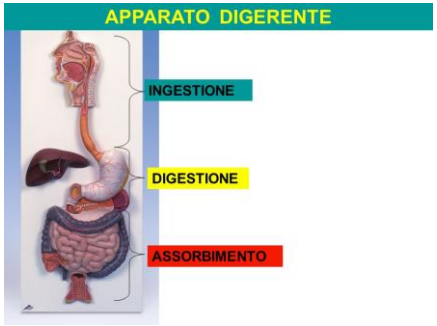
Effetti avversi

Nanoparticelle

Inalazione

Contatto

Ingestione



Importanza della nanometrologia e standardizzazione dei nanomateriali

Nanometrologia fondamentale per:
Standardizzazione
Assicurazione qualità
Risk assesment

Enti regolatori: importante verificare la presenza di nanomateriali
Industria: commercializzazione di prodotti di qualità tramite controllo sulla produzione
Consumatori: Informazione sulla presenza/rischi

EFSA Guidance 2021

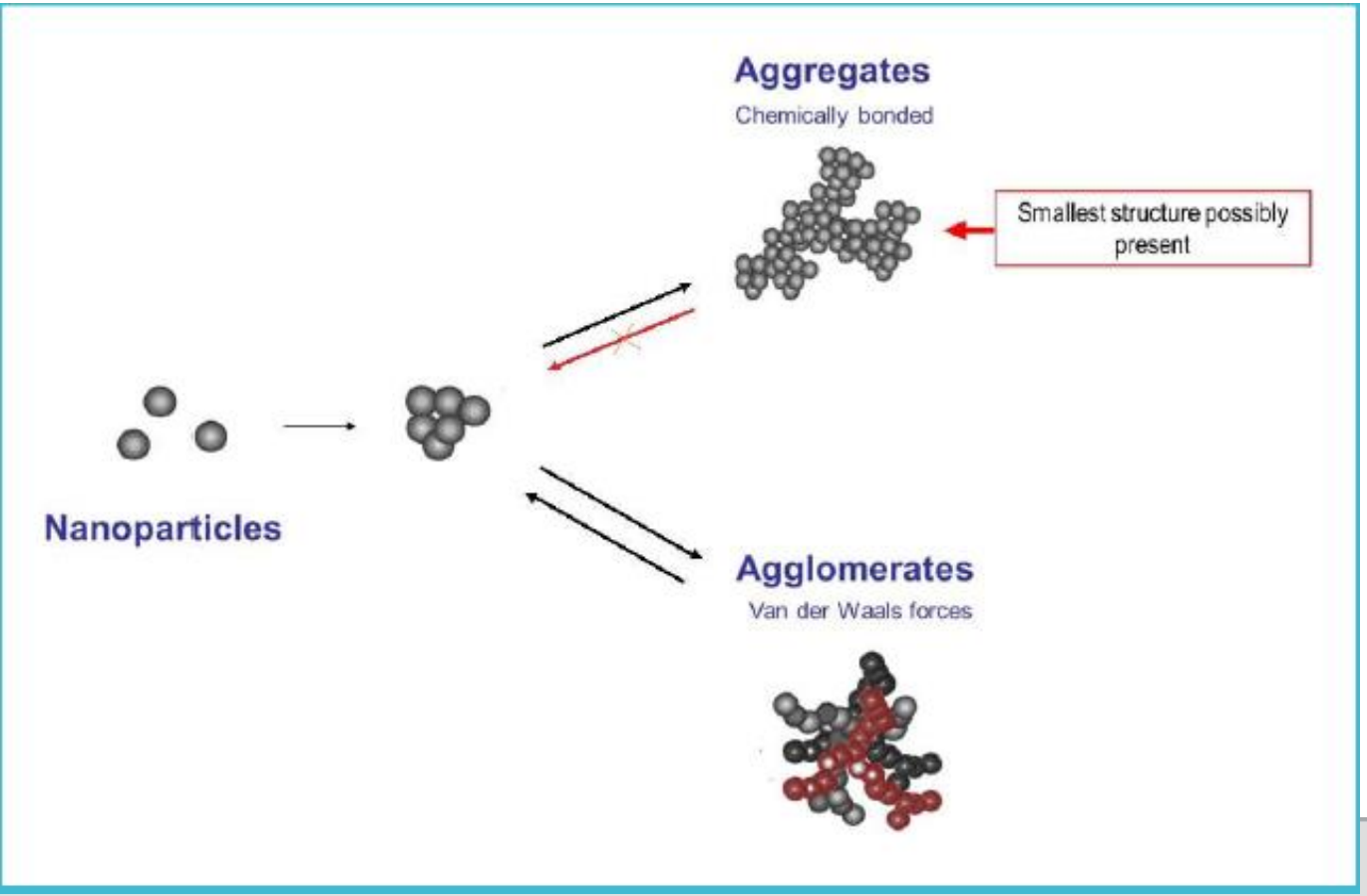
- Detailed characterisation required for 1) pristine nanoparticles as produced; 2) as added to the cosmetic product, and 3) as present during toxicological investigations;
- Key physicochemical aspects include chemical composition/identity; purity/impurities; primary, & secondary particle sizes; agglomeration/ aggregation state; physical form and morphology; crystalline phase/shape; particle and mass concentration; specific surface area; surface chemistry; coating/surface modifications; surface charge; redox potential; solubility and partition properties; pH and viscosity; density, pour density and dustiness; chemical reactivity, catalytic activity.

NANOPARTICELLE

Analisi chimica	+	**Analisi morfologica**	+	**Analisi funzionalità**
Composizione Identità chimica		Dimensione Morfologia Area superficiale Caratterizzazione superficiale ….		Biochimica Immunologica …

La dimensione è **uno fra i tanti** parametri che devono essere determinati
(si tratta di una distribuzione di valori).
Differenza da analisi di composti target non nanomateriali (identità e concentrazione)

Aggregati ed agglomerati



Aggregati:
si formano durante la sintesi

Riduzione degli agglomerati necessaria per una corretta valutazione

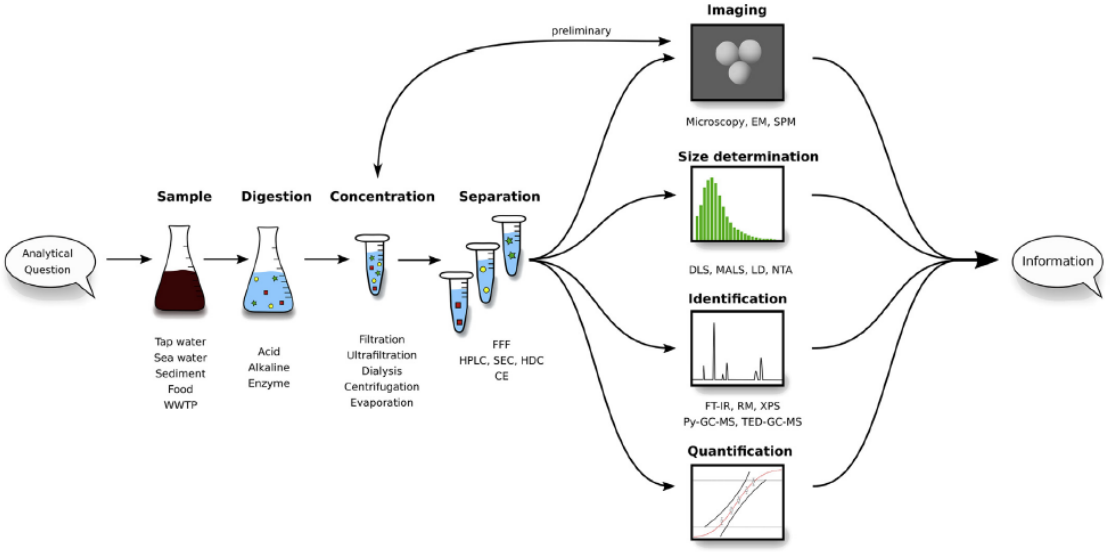
Quali protocolli utilizzare???

I metodi di analisi

Estrazione/concentrazione

Rivelazione/quantificazione

Caratterizzazione/ identificazione composizione chimica



Trends in Analytical Chemistry 112 (2019) 52–65

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Trends in Analytical Chemistry

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Methods for the analysis of submicrometer- and nanoplastic particles in the environment

Christian Schwaferts, Reinhard Niessner, Martin Elsner, Natalia P. Ivleva*

Le sfide ancora aperte…

Necessità di pre-concentrazione

Incertezza nel campionamento

Necessaria riduzione interferenze dovute a presenza di biofilm e materiale organico

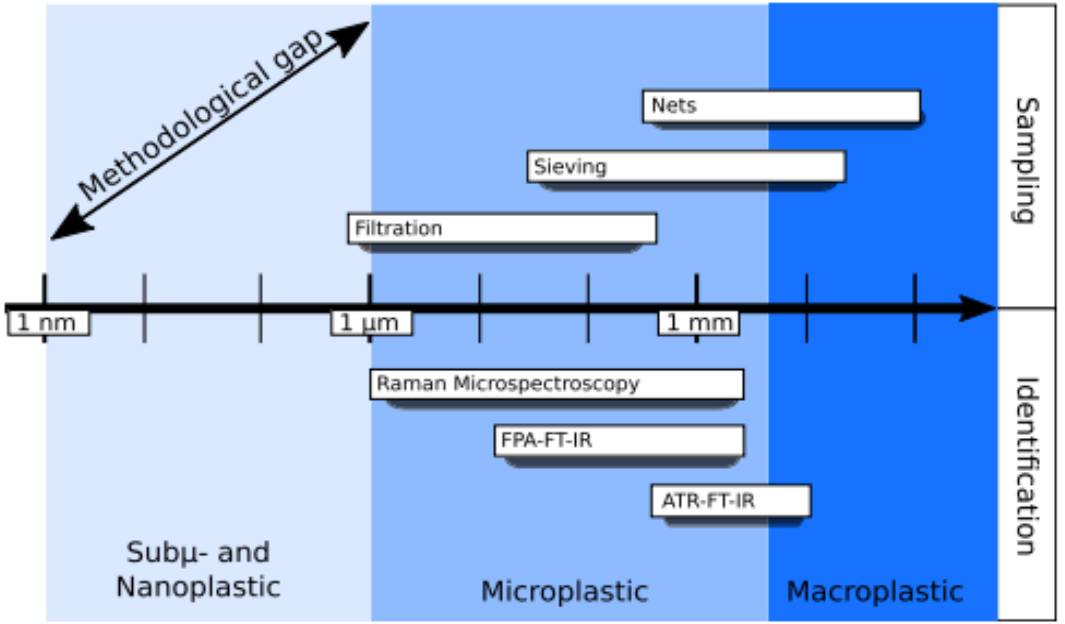

Fig. 1. The analysis of MP is established for particles down to 1 µm. Below, there is a methodological gap.

Trends in Analytical Chemistry 111 (2019) 173–184

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Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Micro(nano)plastics – Analytical challenges towards risk evaluation

João Pinto da Costa [a, b, *], Vanessa Reis [b], Ana Paço [b], Mónica Costa [c], Armando C. Duarte [a, b], Teresa Rocha-Santos [a, b]

Trends in Analytical Chemistry 112 (2019) 52–65

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Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Methods for the analysis of submicrometer- and nanoplastic particles in the environment

Christian Schwaferts, Reinhard Niessner, Martin Elsner, Natalia P. Ivleva*

Table 1
Methods for the preconcentration and separation (detectors are listed in Table 2) of subμ- and nanoplastic particles. References are divided whether they have been applied for subμ- and nanoplastic (left) and by documents from other fields (right).

Task	Technique	Range	Advantages	Disadvantages	References
Preconcentration	Membrane filtration	>10 nm	• Easily available • Cheap	– Low flow rates with small pores – Small volumes	[1]/–
	UF	10–100 kDa ca. 5–50 nm	• Large volumes • Little sample damage/aggregation • Little membrane clogging/fouling	– Interaction with membrane – Setup not plastic free	[2,25,41,43]/–
	Dialysis	Similar to UF	• Mild conditions	– Slow – Large volume of counter dialyzing medium – Risk of microbial contamination	–/[42]
	UC	Any	• Simple • Washing of particles with centrifugation and redispersing	– Harsh conditions – No separation from particulate matrix – Difficult to obtain complete separation	–/[21,42]
	AUC	1 nm–1 μm	• High resolution • Can provide many information • Multiple detectors	– Best for small particles (1–10 nm)	-/[49,50,67]
	Evaporation of solvent	Any	• Cheap, easy	– Does not remove dissolved matter – Superheating	[51]/[42]
Separation	AF4	1 nm–1 μm	• No stationary phase • Sample focusing • Online coupling	– Operation difficult – Interaction with membrane – Steric inversion	[32,41,55]/[53]
	HDC	5 nm–1.2 μm	• Less interaction with stationary phase • Coupled detectors	– Little used	–/[21]
	SEC	1 nm–100 nm	• Coupled detectors	– Stationary phase – Small range	–/[62]
	HPLC	1 nm–40 nm	• Coupled detectors	– Stationary phase – Small size range	–/[61]
	CE	5 nm–500 nm	• High separation resolution • Coupled detectors • Fast	– Charge required – Electrolyte/surface modification – Interaction with capillary/clogging – Might damage sample – Complex matrices difficult	–/[23,65,66]

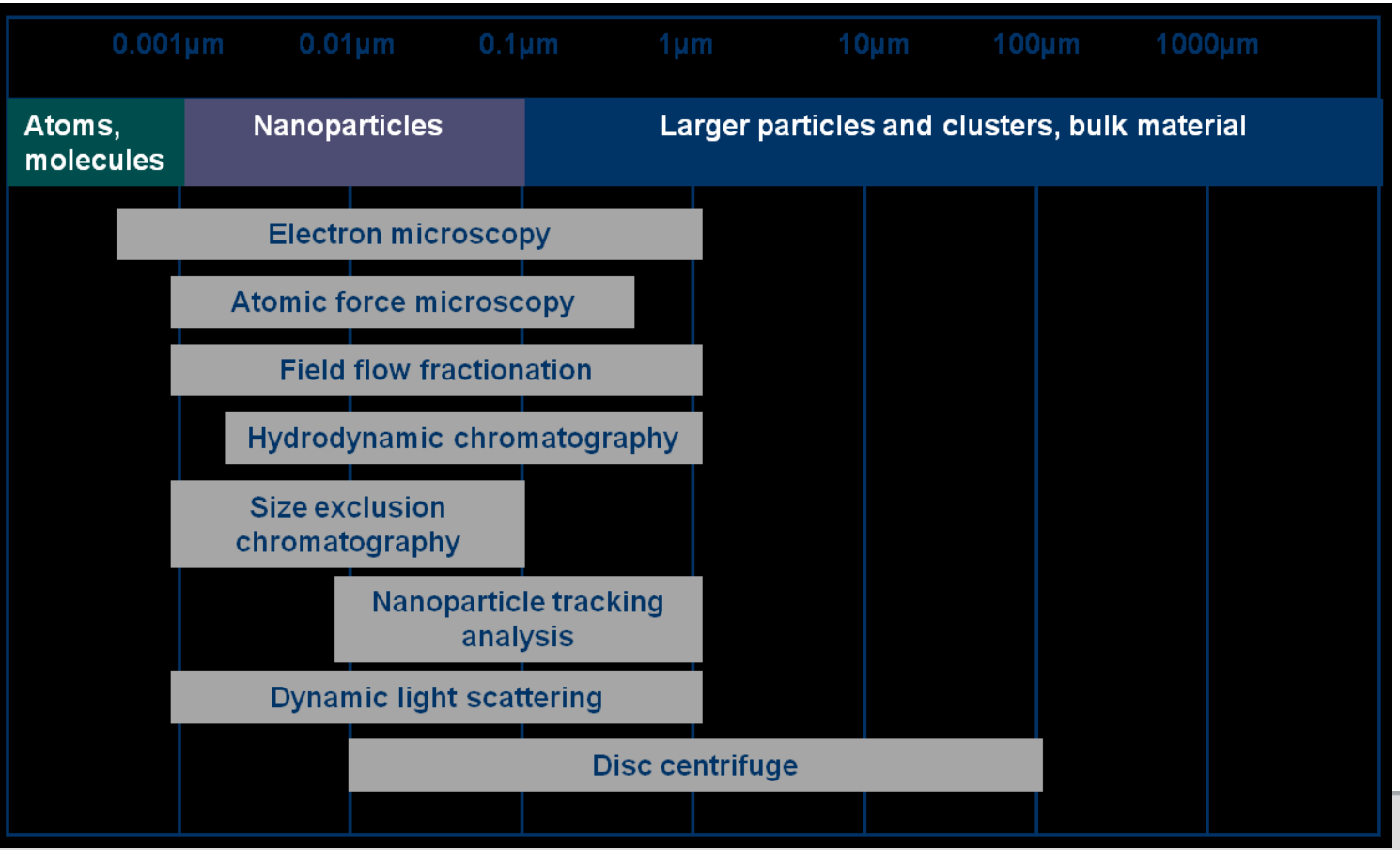
Pre-trattamento del campione

Table 1
The most commonly used methods for the separation/digestion of plastic materials from environmental samples, including biota.

	Method	Matrix	Recovery/Efficacy	Observations	Limitations
Separation	MPSS*	Sediments	95.5 ± 1.8% 97.1% ± 2.6% 13%–39%	For particles <1 mm Artificial reference particles Real marine particles	Expensive; suited for larger sediment samples; quantitative analyses require validation
	Overflow	Sediments	65–95.1% (SD: 3–24.8%)	Only HDPE• was considered	Highly variable recovery rates; requires validation, including for other polymers
	Density separation ($ZnCl_2$)	Sediments	39.8% ± 16.6% 95.8% ± 1.6%	Particles <1 mm Used a PVC+ separator	Low recovery rates The use of PVC (plastic) may cause sample contamination; requires validation
	Density separation ($CaCl_2$/$SrCl_2$)	Surface seawater	–	Only precision (>1%) was reported	The process was not fully detailed; toxicity risks due to $SrCl_2$
	Elutriation/Density separation (NaI)	Sediments	98%	Used a PVC separator	The use of PVC (plastic) may cause sample contamination; expensive (NaI)
Digestion	KOH @ 10%	Biota	98.2%–99.8%	Significant degradation of CA∅ observed	Induced changes in either shape or size of some of the particles
	NaOH	Mussel tissue	94% ± 10%	–	Affected the integrity of the polymers tested
	Enzymatic	Biota (plankton) rich seawater	88.9 ± 1.5%	Non-optimized protocol	Expensive; Partial destruction of nylon fibers, melding of PE and yellowing of PVC granules
		Mussel tissue	93% ± 11%	Industrial enzyme	Expensive
	HNO_3 @ 22.5 M	Mussel tissue.	93.6%–98.3%	Followed by 1 h of boiling and warm filtration	Effects on morphology not provided; efficiencies highly variable, depending on polymer type
	H_2O_2 @ 35%	Sediments	92%	The noted efficacy includes bleached biogenic material	Some polymer types underwent degradation as a result of H_2O_2 oxidation
	Fenton's reaction	Soil and Sludge	79–88%	Carried out at pH 3	Some mass and size changes observed;

*MPSS – Munich Plastic Sediment Separator; +PVC – Polyvinylchloride; •HDPE – High density polypropylene; ∅CA – cellulose acetate.

Tecniche di analisi



La scelta di una tecnica dipende dalla natura del materiale, ma anche dalle dimensionalità delle nanoparticelle

Le tecniche di caratterizzazione/identificazione

Dynamic light scattering (DLS)
Single particle-inductively coupled plasma mass spectrometry (spICP-MS)
Atomic force microscopy (AFM)
Scanning electron microscopy (SEM)
Transmission electron microscopy (TEM)
MicroFourier transform infrared (MicroFTIR)
MicroRaman spectroscopy (MRS)
Pyrolysis gas chromatography coupled with mass spectrometry (Py-GC-MS)
Thermal extraction and desorption coupled with mass spectrometry (TED-GC-MS)

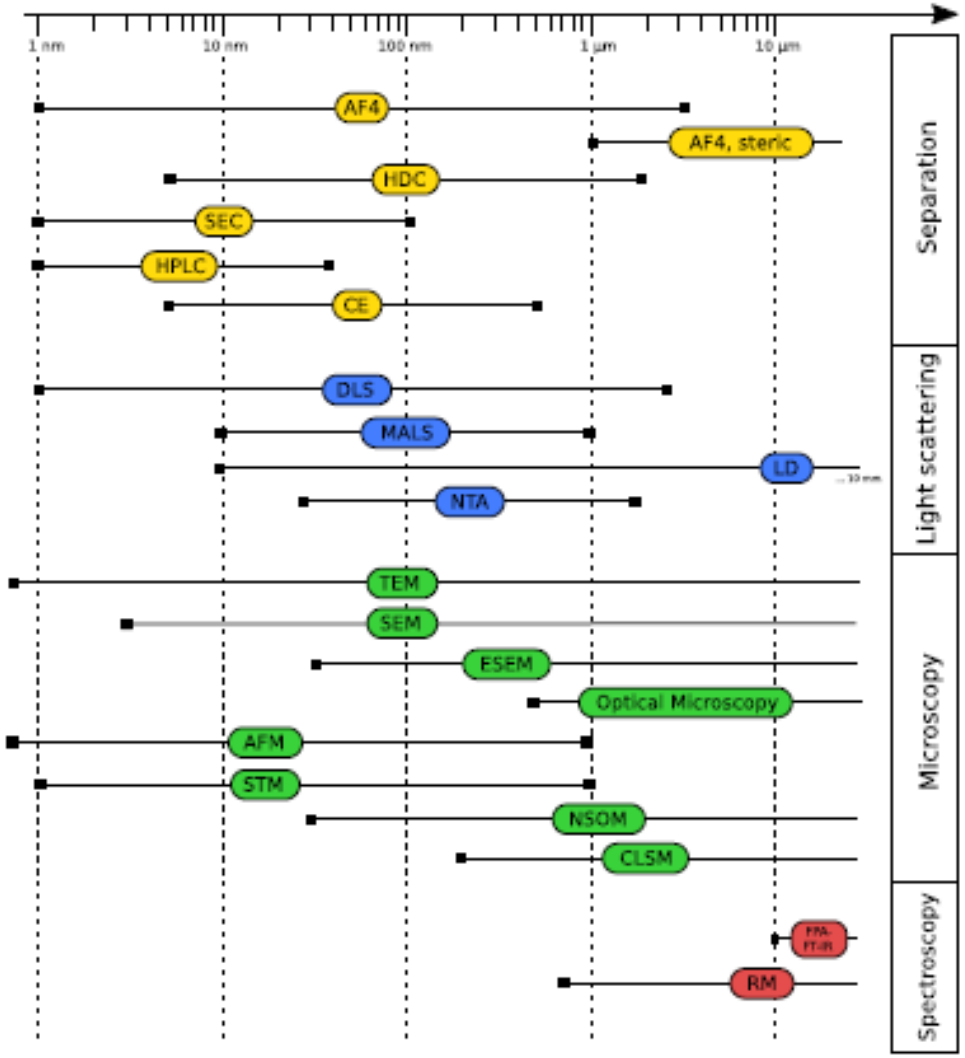

1 nm
10 nm
100 nm
1 µm
10 µm
AF4
AF4, steric
HDC
SEC
HPLC
CE
DLS
MALS
LD
NTA
TEM
SEM
ESEM
Optical Microscopy
AFM
STM
NSOM
CLSM
FPA-FT-IR
RM
Separation
Light scattering
Microscopy
Spectroscopy

Table 2
Commonly used methods for the characterization of microplastics. Potential advantages and drawbacks are described.

	Technique	General advantages	General disadvantages
Visual sorting	Microscope-assisted	Inexpensive; adequate for pre-sorting of samples for subsequent analyses (e.g., FTIR); easy	Over-estimation owing to misidentification; underestimation of smaller and transparent items; no chemical composition
Spectroscopic techniques	FTIR-ATR/μFTIR	Adequate identification in complex matrices; less sensitive to autofluorescence than Raman; better size resolution (>1 μm); chemical composition; detection of down to 10 μm sized plastics; amenable to semi-automation (FPA)	May become time consuming; organic matter may contribute to misidentification of plastics; better results require dry samples
	Raman/μRaman	Better spatial resolution than FTIR - detection of down to 1 μm sized plastics	Time consuming; autofluorescence strongly affects identification
Thermal analysis	Pyr-GC/MS	Simultaneous analysis of polymer type and additives	Expensive; time consuming; requires pre-selection of particles to be examined
	TED-GC/MS	Pre-selection of particles unnecessary; some polymers successfully identified in complex matrices; allows higher sample masses than pyr-GC/MS	Demonstrated to only accurately identify some polymers (mainly PE and PET); expensive; time consuming

Table 3
Effects (when observed) of micro- and nanoplastics, in isolation or combined with chemical pollutants, on numerous organisms. Major finding(s) of each study is (are) described.

Organism(s)	Plastic material	Experimental conditions exhibiting effect(s) (if any)	Major(s) finding(s)	Parameter assessment	Ref.
Chlorella sp. and *Scenedesmus* sp.	20 nm PS* nanoparticles	>0.55 g L^{-1}	Adsorption interfered with photosynthesis and promoted reactive oxygen species ROS♦ production	Carbon dioxide depletion; ROS kit	[64]
Scenedesmus obliquus	70 nm PS particles	>30–103 mg L^{-1}	Inhibited growth and reduced chlorophyll cellular concentration	Cell density; Chlorophyll-a extraction and measurement	[65]
Daphnia magna	70 nm PS particles		Lowered reproduction rate and reduced body size	Body size measurement; number of neonates	
	50 nm to 10 μm PS particles + phenanthrene	50 nm PS 5 mg L^{-1} + 0.34 mg L^{-1} phenantrene	Physical damage; increased mortality	Visual assessment of abnormalities; assessment of immobility	[62]
Amphora sp., *Ankistrodesmus angustus* and *Phaeodactylum tricornutum*	23 nm PS particles	10–100 μg L^{-1}	Induced significant acceleration in EPS+ assembly	Assay kits; elemental analyses; chromatographic quantification	[66]
Pomatoschistus microps	PE spheres 1–5 μm + Cr(VI)	0.184 mg L^{-1} PE + 18.9 mg L^{-1} Cr(VI)	Simultaneous exposure resulted in decreased predatory performance (≤67%) and significant inhibition of enzymatic activity (≤31%)	Assessment of feeding activity; quantification of specific biomarkers (spectrophotometric)	
Tigriopus japonicus	500 nm PS particles	1.25 and 25 mg L^{-1}	Decreased fecundity	Counting of nauplii	[67]
Mytilus edulis and *Crassostrea virginica*	100 nm PS particles	1.3×10^7 particles. L^{-1}	Nanoparticles accumulated in the digestive tract	Feces and digestive glands examination (dissection)	[68]
Crassostrea gigas larvae	70–20 μm PS particles	$<10^5$ particles. L^{-1}	No measurable developmental or feeding effects	Feeding and growth assessment	[69]
Mytilus spp.	2 and 6 μm PS spheres + fluoranthene	32 μg L^{-1} (PS) + 30 μg L^{-1} fluoranthene	High affinity of fluoranthene for polystyrene; did not lead to bioaccumulation in exposed individuals	Morphological and functional analyses	[70]
Mytilus edulis	30 nm PS particles	0.1, 0.2 and 0.3 g L^{-1}	Reduced filtering activity; production of pseudofaeces	Assessment of dispersed dissolved organic carbon; dissection	[71]
Mytilus galloprovincialis	200 nm PS particles	50 mg L^{-1}	Induced pre-apoptotic processes	Enzyme quantification (spectrophotometric); staining	[72]
	<100 μm PE or PS powders + pyrene	20 g L^{-1} PE/PS + 50 mg L^{-1} pyrene	Alterations of immunological responses; neurotoxic effects, onset of genotoxicity and changes in gene expression	Staining; evaluation of phagocytosis activity and DNA damage; quantification of specific biomarkers (spectrophotometric)	[73]
Oryzias latipes	39.4 nm latex particles	10 mg L^{-1}	Particles accumulated in gills, intestine, brain, testis, liver and blood	Fluorescence microscopy observations	[74]
Oryzias latipes embryos and larvae	50 and 500 nm latex particles	10 mg L^{-1}	Decreased survival rate	Visual assessment	[75]
Lumbricus terrestris	<150 μm PE particles	1.2% (w/w) in bulk soil	Decreased growth rate and increased mortality	Counting; visual measurement and assessment	[76]
Tripneustes gratilla	10–45 μm PE spheres	1, 10, 100, and 300 spheres.mL^{-1}	No significant effects	Optical microscopy; counting of larvae; assessment of growth and survival	[77]
Paracentrotus lividus embryos	~90 nm PS particles	>3.85 mg L^{-1}	Caused severe developmental defects	Staining; visual evaluation (confocal microscopy)	[78]
Eisenia andrei Bouché	PE particles >250 and < 1000 μm	62.5, 125 and 500 mg kg^{-1}	No effects on survival, number of juveniles and final weight of adults; histopathological effects were observed	Visual assessment; weight measurement; spectroscopic and histological methods	[79]
Carassius carassius	24 and 27 PS particles	9.3×10^{15} particles.L^{-1}	Induced alterations in the behavior, physiology and metabolism	Monitoring of activity, inter-fish distance and fish exploration; dissections and NMR* analyses	[80]
	28 nm PS particles	10 g L^{-1}	Induced behavioral and fat metabolism	Evaluation of feeding activity; assay kits	[81]

Fondamentale determinazione della natura chimico-fisica

Possibile trasformazione / degradazione delle nanoparticelle durante manipolazione, trattamento del campione, ….

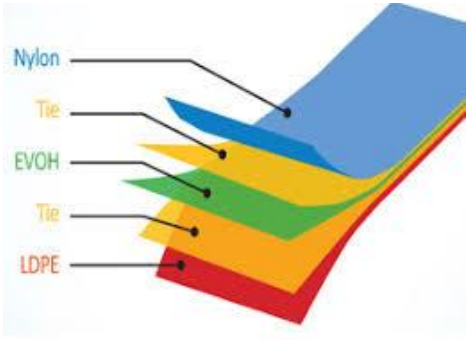
Risultati non riferibili allo stato originale

Mancanza di metodi di analisi validati

Approccio multi-metodologico (fit-for purpose)

Nanomateriali da classificare in funzione del numero e **NON** del peso, poichè aumentando le dimensioni, il peso aumenta drasticamente

Ulteriori sfide…



- Particelle monotipologia

Monocomposizione
Multicomposizione
Compositi

- Particelle multi-tipologia

Differenti tipologie contemporaneamente presenti nel campione

Disponibilità di MR e CRM

Particelle caratterizzate da diversi costituenti (multistrato) difficili da analizzare

For screening methods:	For confirmatory methods:
• determination of the limit of detection (LoD), • precision for quantitative methods, • selectivity, • ruggedness/robustness;	• determination of LoD, • limit of quantification (LoQ), • linearity/working range, • trueness/ recovery, • precision, • selectivity, • ruggedness/robustness.

Linsinger T.P.J., Q. Chaudhry, V. Dehalu, P. Delahaut, A. Dudkiewicz, R. Grombe, F. von der Kammer, E.H. Larsen, S. Legros, K. Loeschner, R. Peters, R. Ramsch, G. Roebben, K. Tiede and S. Weigel, 2012. Validation of methods for the detection and quantification of engineered nanoparticles in food. Food Chemistry Volume 138 (2–3): 1959–1966.

EFSA Journal

GUIDANCE

ADOPTED: 30 June 2021

doi: 10.2903/j.efsa.2021.6769

Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles

EFSA Scientific Committee,
Simon More, Vasileios Bampidis, Diane Benford, Claude Bragard, Thorhallur Halldorsson, Antonio Hernández-Jerez, Susanne Hougaard Bennekou, Kostas Koutsoumanis, Claude Lambré, Kyriaki Machera, Hanspeter Naegeli, Søren Nielsen, Josef Schlatter, Dieter Schrenk, Vittorio Silano (deceased), Dominique Turck, Maged Younes, Jacqueline Castenmiller, Qasim Chaudhry, Francesco Cubadda, Roland Franz, David Gott, Jan Mast, Alicja Mortensen, Agnes G. Oomen, Stefan Weigel, Eric Barthelemy, Ana Rincon, Jose Tarazona and Reinhilde Schoonjans

Caratterizzazione chimico-fisica dei materiali nella Guida EFSA

Completa caratterizzazione dei nanomateriali sia puri che negli alimenti

Identificazione dei cambiamenti durante l'uso o lo stoccaggio

Identificazione dei cambiamenti dopo ingestione

Aspetti futuri

Riduzione delle discrepanze derivanti dall'uso di diverse tecniche di analisi non standardizzate

Approfondimenti riguardanti i potenziali effetti ecotossicologici

Miglioramento delle tecniche di estrazione

Automazione/miniaturizzazione

Valutazione dell'esposizione umana

Nuovi protocolli

Grazie per l’attenzione