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Understanding the surface chemistry of long term environmentally transformed nanomaterials for regulatory assessment

Laura-Jayne A. Ellis (Bradford), ^a Christian Njel,^b
Andrew J. Chetwynd ^{ac} and Iseult Lynch ^{★a}

Environmental conditions significantly influence nanomaterial behaviour and physico-chemical transformations. Surface coatings initially enhance stability but degrade under environmental conditions, exposing nanomaterial cores and increasing transformation rates. These environmental transformations will impact nanomaterial bioavailability and ecotoxicity, with smaller particles and ionic species posing heightened toxicity to aquatic organisms, while larger agglomerates settle into sediments, posing risks to benthic ecosystems. The chronic environmental transformations of silver and titanium dioxide nanomaterials with different surface coatings over four years in diverse synthetic aquatic environments designed to mimic natural conditions are investigated. These conditions include a high-hardness salt only OECD-test medium (HH combo), and two synthetic waters representing low-alkalinity (class I) and high-alkalinity (class V) conditions, reflecting regional chemistries of areas such as Norway, the Alps, southern UK, and parts of southern Europe. A suite of analytical techniques, including dynamic light scattering, single particle inductively coupled plasma mass spectrometry, transmission electron microscopy, and X-ray photoelectron spectroscopy, were employed to assess morphological and physicochemical transformations of the nanomaterials over time. This study underscores the importance of incorporating nanomaterial ageing and environmental transformation studies into regulatory frameworks to capture the dynamic lifecycle behaviours of nanomaterials and their evolving physico-chemical characteristics and consequent toxicity. Recommendations include mandating environmental testing under diverse conditions to simulate real-world scenarios, assessing coating stability, and adopting tiered risk assessment approaches. These findings provide crucial data for regulators to develop guidelines that reflect the dynamic nature of nanomaterials, enhancing our understanding of their long-term environmental persistence, mobility, and ecological impacts.

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Environmental significance

Engineered nanomaterials (ENMs) are released into complex aquatic environments where their behaviour is poorly captured by pristine laboratory testing. This study demonstrates that long-term environmental ageing fundamentally alters ENM identity, with distinct divergence between dispersion stability and surface chemical stability. Using XPS, we reveal that Ag ENMs undergo oxidation and sulfidation, while TiO₂ ENMs remain chemically stable but undergo surface modification and aggregation, driven by coating degradation, alkalinity, and natural organic matter (NOM). These transformations directly affect bioavailability, persistence, and risk. Our findings show that ENMs behave as dynamic systems rather than static materials, emphasising the need for regulatory frameworks to incorporate ageing, surface transformations, and environmentally relevant conditions.

1. Introduction

Unlike conventional chemicals, nanotechnology-enabled products incorporate engineered nanomaterials (ENMs)

that exhibit unique behaviours based on differing size, surface coatings and shapes.^{1–3} The integration of ENMs into consumer products has led to widespread concerns about their potential environmental impacts.⁴ Titanium dioxide (TiO₂) and silver (Ag) are two of the most widely used ENMs due to their unique properties, which are widely utilised for their UV-blocking capabilities and antimicrobial effects for applications in personal care items, textiles, and coatings,⁵ products which have a short life cycle and thus have widespread potential for

^a School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham B15 2TT, UK. E-mail: i.lynch@bham.ac.uk

^b Karlsruhe Nano Micro Facility (KNMF), Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

^c Centre for Proteome Research, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, UK



environmental release due to incorrect disposal or poor waste management.

The environmental implications of ENMs are far from adequately characterised in terms of identifying exposures, transformations or hazards to enable an accurate and predictable environmental risk assessment that is as robust as those we currently prepare for traditional bulk chemicals.^{5,6} Studies have evidenced that nanoscale properties influence toxicity, suggesting links between measurable particle characteristics and hazard potential such as size/shape and core material composition.^{7–11} However, few studies have identified these relationships across a broad range of ENMs,^{12,13} making it difficult to evaluate their potential for hazard prediction or assessment. The variability in how ENM properties impact hazard may be partly due to changes in their behaviour and environmental transformations such as aggregation/dissolution that affect exposure levels.^{14,15} It is also now well understood that ENMs undergo transformation reactions during their time in the environment, altering the ENM identity from the pristine condition to which organisms are typically exposed during standardised toxicity tests.

The behaviour of ENMs in aquatic environments has gained significant interest, as water serves as a primary route through which they enter natural ecosystems, and as an endpoint from surface run off.^{16–18} Once released into the environment, ENMs are exposed to various environmental conditions such as variations in pH, ionic strength, dissolved organic matter and sulfides, which drive a range of transformation processes thereby altering their structure, composition, and toxicity in ways that traditional risk assessments fail to capture, posing unknown risks to ecosystems and human health.^{19,20} These transformations include physical, chemical, and biological changes, including eco-corona formation²¹ and binding to natural organic matter (NOM).^{22,23} ENMs such as Ag can oxidize to Ag⁺ ions or form stable silver sulfide (Ag₂S) complexes, each with distinct ecological implications. Earlier studies have further demonstrated coating degradation over sort exposure time scales.^{24,25} In contrast, TiO₂ ENMs may aggregate or interact with other ions, changing their surface properties and altering their behaviour in water, soil, and air.²⁶ Therefore, exposure to ENMs in their original manufactured state is highly unlikely in many real-world release and exposure situations.²³ Ignoring these transformations will lead to substantial gaps in understanding and potential underestimation of the risks posed by these materials, particularly when released into aquatic systems that support diverse and sensitive ecosystems.

This study specifically characterises the chronic environmental transformations of a library of Ag and TiO₂ ENMs with various surface coatings over four years in synthetic waters designed to mimic natural environments with different alkalinities and ionic compositions. The aim was to track how 'pristine' ENMs transform over time in complex environmental matrices to assess their persistence and inform long-term environmental monitoring strategies. Three distinct media were selected: a high-hardness combination (HH combo) medium, commonly used in OECD chemical toxicity testing with

Daphnia,^{27–30} and two synthetic waters, class I and class V, which represent regional freshwater chemistries found across Europe.³¹ Class I water simulates low-alkalinity regions, such as Norway and the Alpine areas, whereas class V water represents the high-alkalinity conditions found in southern UK and parts of southern Europe. These synthetic waters, developed by Hammes and Gallego-Urrea,³¹ are based on geochemical data from nearly 800 European rivers, allowing for a realistic assessment of ENM behaviour in diverse environmentally relevant gradients. By examining the ENMs in the different waters, this research simulates the range of conditions ENMs may encounter and explores the transformations undergone by the different ENMs. Each medium (class I and V) presents unique chemical challenges, such as the availability of ions that can trigger aggregation, dissolution, or chemical transformations.^{32,33} The HH combo medium, in contrast, lacks natural organic matter, allowing isolation of inorganic interactions under standardised OECD conditions.³⁴ A range of advanced analytical techniques were employed to characterise the transformations of these ENMs as they aged, focussing on assessment of changes in ENM composition morphology and surface characteristics.

While previous studies have demonstrated degradation of ENMs over short timescales (hours to weeks),^{22,24,25,35} the present work provides one of the first multi-year (4-year) systematic, multi-media datasets combining dispersion (dynamic light scattering, transmission electron microscopy and single particle inductively coupled plasma mass spectrometry) with surface chemical transformation analysis (X-ray photoelectron spectroscopy). This enables us to demonstrate that (i) coating degradation is not only an initial process but continues to drive long-term divergence between dispersion stability and chemical stability, (ii) transformation pathways (e.g., oxidation *versus* sulfidation) are environment-specific and time-dependent, and (iii) long-term ageing leads to the emergence of new steady-state material identities that are not captured in short-term studies. Overall, this research provides essential data for regulatory bodies to understand the lifecycle impacts of ENMs, thus supporting the development of standards that account for their dynamic behaviour in real-world settings, which are currently overlooked in hazard and risk determination.

2. Experimental

2.1. Materials

Commercially available chemicals, ionic standards, solvents and humic acid for natural organic matter (NOM) were purchased from Sigma Aldrich and were of analytical reagent grade. Ultrapure water (UPW) with a maximum resistivity of 18.2 M Ω cm⁻¹ was used throughout. ICP-MS grade water was purchased from Fisher Scientific.

2.2. Nanomaterials

The ENMs selected for this study include Ag and TiO₂ particles (detailed descriptions in Table S1), chosen for their widespread



use in consumer products ranging from personal care to uses in industry and agriculture, and include a variety of coatings.^{36–39} The TiO₂ ENMs used included uncoated TiO₂, and the same TiO₂ particles coated with polyvinylpyrrolidone (PVP), Dispex, Solplus, and Pluronic polymers (all supplied by Promethean Particles Ltd., <https://www.prometheanparticles.co.uk>).

The Ag ENMs included uncoated Ag (citrate from both Promethean Particles Ltd. and AppNano Ltd., Spain), Ag₂S PVP coated (AppNano Ltd., Spain), PVP Ag Nano Ink AX-JP-60n and paraffin-coated Ag (Amepox Ltd., Poland <https://www.axpsa.eu>), and PEG-thiol Ag ENMs (Promethean Particles Ltd.) all of which were obtained from the EU H2020 funded project NanoFASE. Each coating (with a different core material of Ag/Ti) was picked to understand its influence on the stability, reactivity, and transformation of the ENMs and thus its influence on their environmental behaviour and risk profile. For instance, PVP and PEG-thiol are known for providing steric stability in aqueous solutions and thus reducing agglomeration, while uncoated or sulfide-coated particles will behave differently under environmental stressors and have different degrees of persistence in the environment.

2.3. NM transformations

ENMs were aged in high hardness combo media (HH combo),²⁷ class I and class V synthetic natural water standards.³¹ Stock solutions (1000 mg L⁻¹) of the ENMs were prepared in the HH combo, class I and class V synthetic river water and were stored for 4 years in a refrigerator at 4 °C throughout the ageing process to mimic the average temperatures in natural waters.

2.4. Media and representative waters

The HH combo medium is designed to match the total hardness of water found in the environment without any natural organic matter (NOM), and is commonly used for OECD standard chemical toxicity testing with the model organism *Daphnia magna*.^{28,29} NOM can complex with metal ions, influence aggregation, and alter the fate of NMs in the environment. By excluding NOM, the HH combo media provide a controlled environment to isolate the effects of ionic strength and water hardness on ENM stability. In comparison, the class I

and class V synthetic waters represent standardised geochemical categories derived from a Europe-wide classification of freshwater chemistry based on alkalinity, ionic composition, and dissolved constituents.³¹ Class I water reflects low-alkalinity, low ionic strength systems, typical of northern and upland regions (e.g., Norway), whereas class V water represents high-alkalinity, ion-rich systems, characteristic of southern and more mineralised catchments (e.g., southern UK, Poland, Greece, France, the Balearic countries and the Iberian Peninsula). A description of the water compositions is presented in Tables S2, S2A and S2B.

2.5. ENM characterisation

ENMs were characterised in the pristine forms, and then in the three media weekly for the first 4 weeks, monthly for the first 6 months and then again after 2 years (DLS, Zeta, TEM and spICP-MS) and in year 4 with XPS.

2.5.1. Dynamic light scattering (DLS) and zeta potential (ZP). DLS and ZP were used to measure the hydrodynamic diameter size and size distribution of the 'pristine' and 'aged' ENMs over 2 years in each of the test media. Measurements of the polydispersity index and zeta potential were also recorded using a Malvern Nanosizer 5000 instrument (Tables 1 and S3–S6, Fig. S1), with results reported as the average mean size of 10 runs.

2.5.2. Transmission electron microscopy (TEM). TEM analysis of the ENMs (Table 2, Fig. 1 and 2, and Tables S7 and S8) was performed using JEOL 1200EX 80 kV and JEOL 1400EX 80 kV microscopes. ENMs were prepared by the drop casting method, depositing a 20 µL drop of the ENM suspension onto a 300 mesh carbon-coated copper TEM grid (Agar Scientific, UK) and air drying. Grids were stored in a Petri dish covered with tinfoil to avoid dust contamination.

2.5.3. Single particle inductively coupled plasma mass spectrometry (spICP-MS). spICP-MS was used to quantify the dissolved and ENM concentrations (Table S9) using a Perkin Elmer NexION 300D. Operating conditions were optimized to produce maximum Ag/Ti intensity using Ag/Ti ENM and ionic standards. Instrument calibration was achieved by analysis of a blank and 3 dissolved Ag/Ti solutions ranging from 0 to 1

Table 1 DLS characterisation of pristine and 2 year 'aged' Ag and TiO₂ ENMs (hydrodynamic sizes)

ENM identifier	Pristine	2 years aged in HH combo individual size (nm)	2 years aged in class I individual size (nm)	2 years aged in class V individual size (nm)
Ag prom uncoated	323 ± 73	11 170 ± 2	789 ± 392	1274 ± 616
Ag PVP	44 ± 2	7024 ± 9327	405 ± 100	1741 ± 1945
Ag ₂ S	219 ± 17	249 ± 46	118 ± 3	309 ± 360
Paraffin Ag	120 ± 3	3163 ± 1829	288 ± 293	284 ± 182
Ag AppNano uncoated	82 ± 7	2688 ± 1733	78 ± 6	2744 ± 1960
PEG-thiol Ag	128 ± 3	532 ± 257	120 ± 19	321 ± 104
TiO ₂ -uncoated	207 ± 11	305 ± 139	508 ± 42	8001 ± 1453
TiO ₂ -PVP	311 ± 43	305 ± 176	202 ± 74	225 ± 79
TiO ₂ Dispex	462 ± 36	413 ± 71	343 ± 196	1894 ± 813
TiO ₂ Pluronic	858 ± 188	237 ± 191	94 ± 25	73 ± 127
TiO ₂ Solplus	569 ± 70	8862 ± 2160	133 ± 171	2384 ± 1144



Table 2 TEM characterisation of pristine and 2 year 'aged' Ag and TiO₂ ENMs (individual particles sizes)

ENM identifier	Pristine	2 years aged in HH combo individual size (nm)	2 years aged in class I individual size (nm)	2 years aged in class V individual size (nm)
Ag prom uncoated	61 ± 36	40 ± 27	54 ± 41	48 ± 24
Ag PVP	18 ± 11	55 ± 29	679 ± 495	27 ± 13
Ag ₂ S	44 ± 14	57 ± 36	63 ± 28	53 ± 21
Paraffin Ag	22 ± 19	58 ± 31	36 ± 16	11 ± 3
Ag AppNano uncoated	81 ± 83	52 ± 24	62 ± 24	72 ± 32
PEG-thiol Ag	42 ± 15	88 ± 239	57 ± 32	71 ± 35
TiO ₂ -uncoated	9 ± 2	8 ± 2	9 ± 4	11 ± 8
TiO ₂ -PVP	9 ± 2	9 ± 3	9 ± 3	10 ± 9
TiO ₂ Dispex	9 ± 3	12 ± 2	13 ± 5	10 ± 3
TiO ₂ Pluronic	9 ± 3	9 ± 3	9 ± 3	9 ± 2
TiO ₂ Solplus	3 ± 1	3 ± 1	6 ± 2	39 ± 14

µg L⁻¹. Transport efficiencies were determined using three standards of citrate stabilized gold ENMs with diameters of 20, 40 and 80 nm. The single particle element was calibrated using ionic and particulate gold individually for both Ti and Ag analyses with an R^2 value of 0.999 and 0.945 respectively for the Ti and 0.998 and 0.947 for the Ag. Ionic calibrations for Ag and Ti were also performed and returned R^2 values of 0.999 and 0.999, respectively. All ionic standards were determined at concentrations of 1, 2 and 3 parts per billion (ppb). These gave transport efficiencies of 7.61 and 6.75%, respectively, for the Ag and TiO₂ analyses. For the Ag analysis, the flow rate was 0.339 mL min⁻¹ and for TiO₂ it was 0.299. In both cases, the pump speed was set to -10 rpm, a scan time of 60 s and a dwell time of 100 µs. Time resolved signals were acquired using the Syngistix nano application module in the NexION software. All concentrations and particle diameters were determined from triplicate samples. To

ensure no deviations due to instrumental drift over time, dissolved Ag/Ti calibration check standards were also run in between samples. Experimental procedures were performed according to the Perkin Elmer application note for ENMs.⁴⁰

2.5.4. X-ray photoelectron spectroscopy (XPS). XPS characterisation was carried out at the Karlsruhe Institute of Technology (KIT) in Germany (Tables S10–S12). Samples were prepared by placing a drop of the ENM dispersion on the surface of a silicon wafer, which was allowed to air-dry overnight. XPS measurements were performed using a Thermo Scientific K-alpha+ spectrometer. The monochromatic AlK α line was used as X-ray excitation (1486.68 eV) with a pass energy of 50 eV to obtain high resolution spectra. The samples were analysed using a microfocused, monochromated Al K α X-ray source (400 µm spot size). XPS spectra were fit with one or more Voigt profiles (binding energy uncertainty: ±0.2 eV) and Scofield sensitivity factors were applied for elemental surface

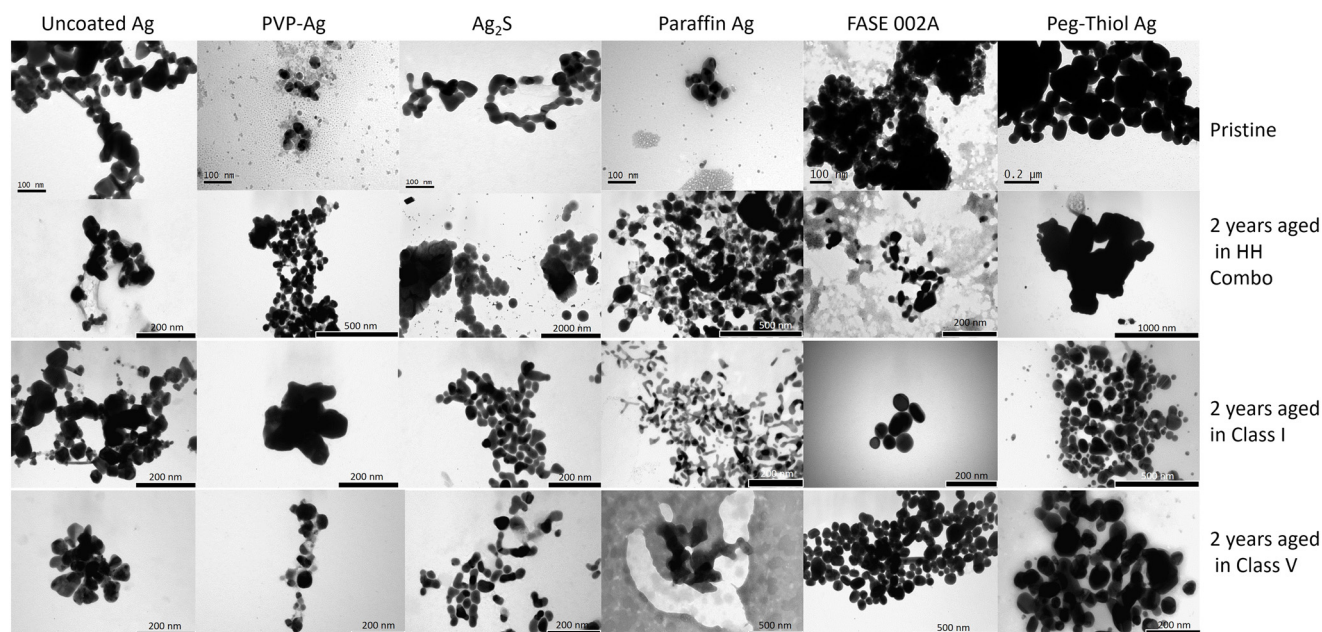


Fig. 1 TEM images of the Ag ENMs shown as a series of pristine ENMs, and 2 years aged in each of the HH combo, class I and class V media. This image is reproduced under a Creative Commons License from Varsou, Ellis.⁴²



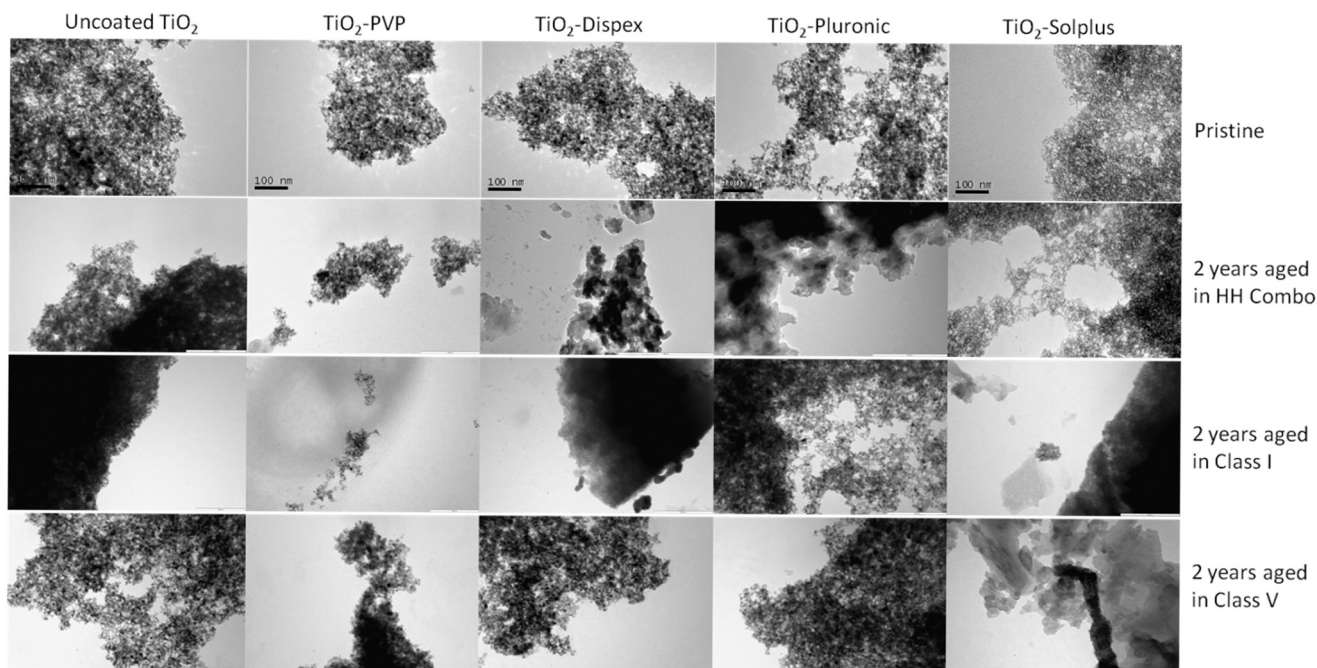


Fig. 2 TEM images of the TiO₂ ENMs shown as a series of pristine ENMs, and 2 years aged in each of the HH combo, class I and class V media. This image is reproduced under a Creative Commons License from Varsou, Ellis.⁴²

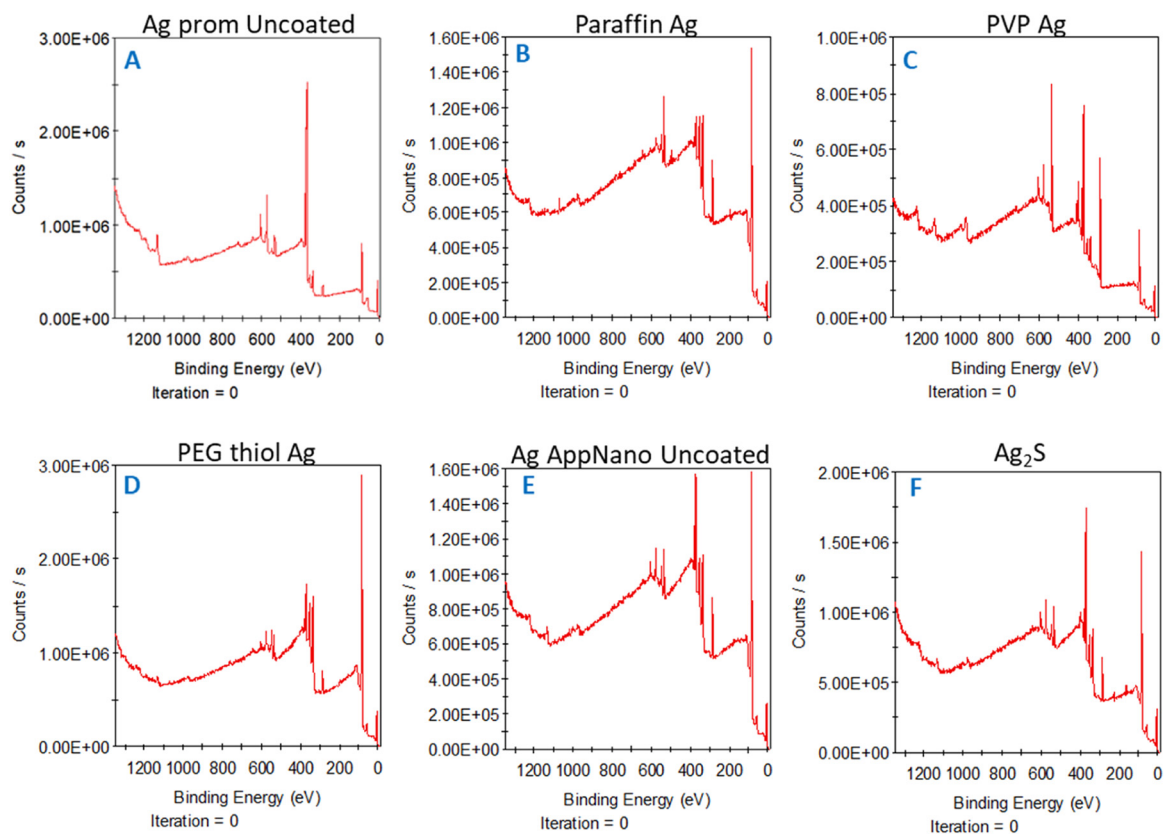


Fig. 3 The X-ray photoelectron spectroscopy graphs show the binding energy spectra of the pristine Ag ENMs with different surface coatings; A) Ag prom uncoated, B) paraffin coated Ag, C) PVP coated Ag, D) PEG-thiol coated Ag, E) AppNano uncoated Ag and F) Ag₂S. The binding energy of electrons corresponds to the specific elements and their electronic states, and the counts represents the number of electrons detected at each binding energy, which are indicative of the abundance of specific elements or states in the sample. Key to the peaks Ag⁰ (~368 eV and ~374.2 eV), Ag⁺ (~367.7–368.0 eV), C 1s (~285 eV), O 1s (~532 eV), S 2p (~162 eV) where present, and N 1s (~400 eV) for coatings.



composition.⁴¹ All spectra were referenced to the C 1s peak (C–C, C–H) at 285 eV binding energy controlled by means of the photoelectron peaks of metallic Cu, Ag, and Au, respectively. Carbon (C 1s) spectra were recorded at the beginning and after each resolution analysis to check/confirm the absence of any sample degradation under irradiation.

3. Results and discussion

3.1. Characterisation of the pre-environmental ageing of ENMs

Characterisation of the pristine ENMs establishes baseline data on their manufactured form, providing a reference for assessing environmental ageing and transformations. Pre- (and post-environmental exposure) characterisation of the ENM properties included size determination by DLS, surface charge determined as zeta potential (ZP) (Tables 1 and S3–S6), size and morphology determined by transmission electron microscopy (TEM) (Table 2, Fig. 1 and 2, and Tables S7 and S8), quantification of the dissolved and ENM concentrations by single particle inductively coupled plasma mass spectrometry (spICP-MS) (Table S9) and analysis of surface chemistry by X-ray photoelectron spectroscopy (XPS) (*pristine Ag ENMs*: Fig. 3 and S2 and Tables S10 and S11; *pristine TiO₂ ENMs*: Fig. 4 and S3 and Tables S12 and S13; *aged Ag ENMs*: Fig. 5, 6, S4, S5, S8, S9, S12 and S13 and Tables S14–S16; and *aged TiO₂ ENMs*: Tables S14 and S16 and Fig. S6, S7, S10, S11, and S14).

Observations from XPS identified that each pristine Ag ENM exhibits a distinct chemical signature, reflecting its coating composition and variability in the oxidation state (Fig. 3 and S2 and Tables S10 and S11). PVP coatings are often associated with carbon (C) 1s (~285.0 eV), nitrogen (N) 1s (~399–400 eV), and oxygen (O) 1s (~531–533 eV) peaks, confirming the presence of the organic molecules that help stabilise the ENMs and reduce oxidation.⁴³ For the Ag ENMs, the Ag 3d region shows peaks at ~368.2 eV (Ag 3d_{5/2}) and ~374.2 eV (Ag 3d_{3/2}), characteristic of metallic Ag⁰, while slight shifts to lower binding energies (~367.7–368.0 eV) indicate the presence of oxidised Ag⁺ species. Variations in the relative intensity and position of these peaks indicate differences in the oxidation state across coatings. Samples exhibiting a higher proportion of Ag⁰ (*i.e.*, stronger, well-defined peaks at ~368.2 eV) suggest that certain coatings provide greater resistance to surface oxidation (excluding the nominally uncoated, citrate-stabilised ENMs).

The XPS spectra (Fig. 4 and S3 and Tables S12 and S13) of the pristine TiO₂ ENMs with various surface coatings also highlight the differences in chemical stability and surface composition depending on the surface coating. For the uncoated TiO₂ ENMs, the spectra show clear peaks corresponding to Ti 2p and O 1s regions, with Ti 2p_{3/2} observed at ~458.5 eV and Ti 2p_{1/2} at ~464.3 eV, consistent with Ti⁴⁺ in TiO₂. The O 1s peak at ~529.5–530.0 eV further

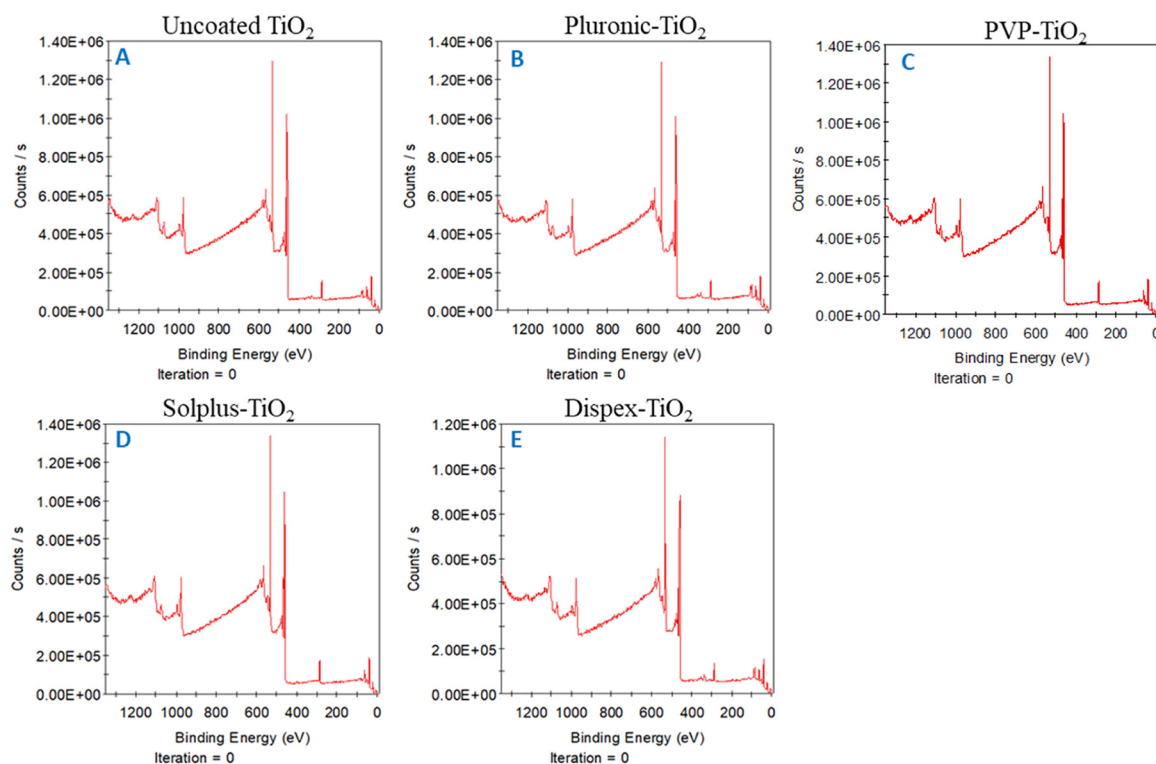


Fig. 4 The X-ray photoelectron spectroscopy graphs show the binding energy spectra of the pristine TiO₂ ENMs with different polymer surface coatings; A) uncoated TiO₂, B) Pluronic coated TiO₂, C) PVP coated TiO₂, D) Solplus coated TiO₂, and E) Dispex coated TiO₂. The binding energy of electrons corresponds to the specific elements and their electronic states, and the counts represents the number of electrons detected at each binding energy, which are indicative of the abundance of specific elements or states in the sample. Key to the peaks 458.5 eV and ~464.3 eV are consistent with Ti⁴⁺ in TiO₂. The O 1s peak at ~529.5–530.0 eV (O in TiO₂).



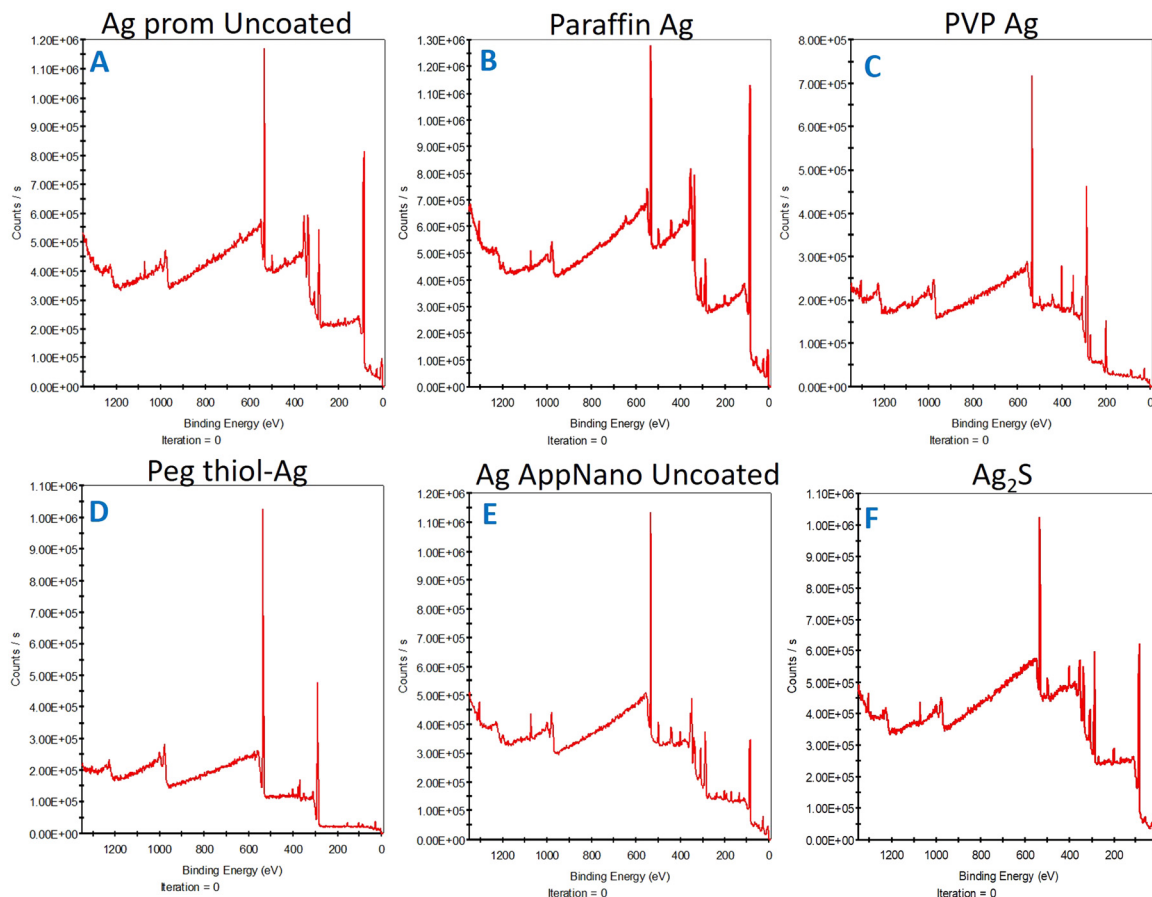


Fig. 5 XPS graphs showing the binding energy spectra of the various Ag ENMs with different surface coatings (A) Ag prom uncoated, B) paraffin coated Ag, C) PVP coated Ag, D) PEG-thiol coated Ag, E) AppNano uncoated Ag and F) Ag_2S aged over 4 years in the HH combo medium. The binding energy of electrons corresponds to the specific elements and their electronic states, and the counts represents the number of electrons detected at each binding energy, which are indicative of the abundance of specific elements or states in the sample.

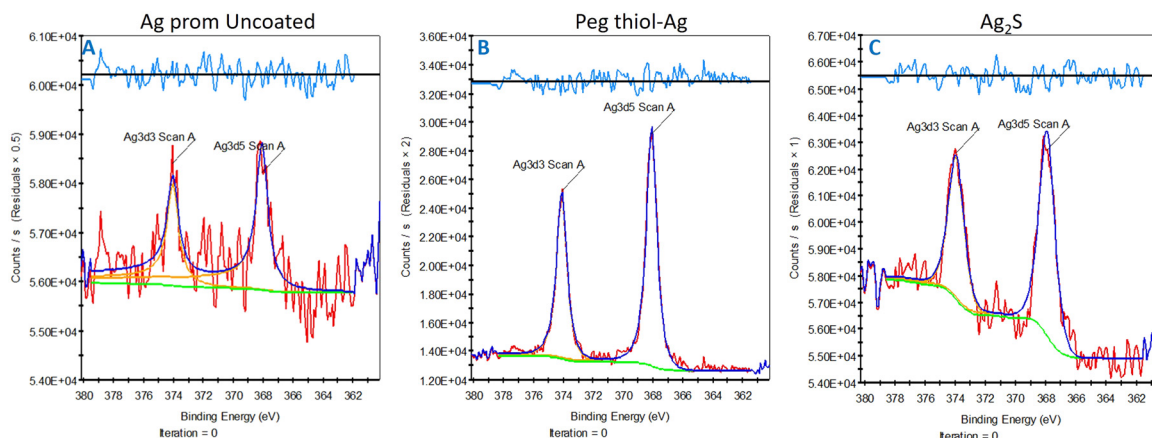


Fig. 6 High-resolution XPS spectra of the Ag 3d region for representative Ag ENMs aged for 4 years in the HH combo medium; A) prom uncoated Ag, B) PEG-thiol coated Ag, and C) Ag_2S the experimental spectra (red) are shown with fitted peak envelopes (blue), and individual fitted components/background contributions (green). The upper traces represent the residuals (difference between experimental data and fit), where a near-flat profile indicates a good fit. Broader and less symmetric peaks suggest increased chemical heterogeneity and the presence of transformed species (e.g. Ag^+ or AgS), whereas sharper, well-defined peaks indicate a greater contribution from metallic Ag^0 .

confirms the presence of lattice oxygen in TiO_2 . The sharp and well-defined Ti^{4+} peaks indicate a stable TiO_2 structure without significant interference from organic species.

In contrast, the Pluronic, PVP, Solplus, and Dispex-coated TiO_2 ENMs (Fig. 5 and S3) exhibit pronounced C 1s signals at ~ 285.0 eV, alongside broader O 1s contributions at ~ 531 – 533



eV associated with surface hydroxyl groups and organic oxygen species. These features indicate the presence of a surface-bound organic layer. This coating layer likely reduces direct interaction between the TiO₂ surface and environmental components such as natural organic matter or sulphides, thereby helping to maintain the Ti⁴⁺ oxidation state and limiting surface transformation.

3.2. Characterisation of the post-environmental ageing of ENMs

To simulate environmentally relevant conditions and evaluate ENM transformation, persistence, and ecological implications, the behaviour of the Ag and TiO₂ ENM library was assessed across three media (HH combo, class I and class V). Here, “stability” is considered in two complementary contexts: (i) dispersion stability, referring to the tendency of ENMs to remain suspended or undergo aggregation/agglomeration and (ii) chemical stability, referring to changes in composition, oxidation state, or dissolution.

Dispersion stability was assessed through changes in hydrodynamic size, polydispersity, and surface charge using DLS and zeta potential measurements, supported by TEM and particle size distributions obtained from spICP-MS. Chemical stability was evaluated through XPS analysis of the surface composition and oxidation state, alongside spICP-MS quantification of dissolved metal fractions. Together, these approaches provide an integrated assessment of ENM behaviour, linking physicochemical transformations to environmental persistence, bioavailability, and potential toxicity.^{44–46}

3.2.1. Long-term transformations in the HH combo medium. In the simple HH combo medium, Ag ENMs exhibited substantial increases in the Z-average size over time, with the Ag AppNano uncoated ENMs exceeding 1000 nm (DLS) after two years, indicating significant particle aggregation and dispersion instability (Table S3). Over longer-term storage (4 years), XPS reveals pronounced changes in the chemical stability of the Ag ENMs (Fig. 5, 6, S4 and S5 and Tables S11 and S13). Comparison of the pristine and aged spectra shows a reduction in the intensity of the metallic Ag⁰ signal (Ag 3d region ~368.2 eV; Fig. 6), alongside the emergence and/or increased prominence of sulfur-associated peaks in the S 2p region (~161–163 eV), consistent with the formation of Ag–S species (Ag₂S). In some cases, coating-related signals (e.g. C 1s and N 1s) are also attenuated, suggesting degradation or displacement of the original surface coatings during ageing.

These spectral changes indicate that sulphur-containing species present in the HH combo medium react with Ag⁰ over time, driving sulfidation and transformation of the ENMs. This also demonstrates that polymeric surface coatings become less effective over prolonged environmental exposure, allowing increased interaction between the ENM core and surrounding ions. These transformations reflect the chemical interactions between Ag and the ionic composition of the HH combo

medium, reinforcing coating instability under high ionic strength conditions. Such processes are consistent with transformations observed in sulphide-rich environments, including those influenced by decaying organic matter or sulphate-reducing conditions,^{47,48} and suggest a shift toward more aggregated, chemically transformed, and potentially less bioavailable Ag forms.^{49,50}

For the TiO₂ ENMs, single particle concentrations remained relatively high even after 4 years in the medium, indicating that the core structure is largely preserved (Table S9), although coating integrity is altered over time. XPS analysis (Fig. S6 and S7) reveals increased surface hydroxylation, with enhanced contributions from surface-bound –OH groups and Ti–O species, indicating interactions with hydroxide ions in the medium. This hydroxylation modifies surface charge and reactivity, influencing particle–particle interactions.

These changes are reflected in reduced dispersion stability, as evidenced by increases in hydrodynamic diameter measured by DLS (Table S3). For example, uncoated TiO₂ ENMs increased from ~207 nm (pristine) to around 305 nm after 2 years in the HH combo medium, while TiO₂ Solplus exhibited a substantial increase from 569 nm to 8862 nm (approximately 15-fold), indicating extensive agglomeration. In contrast, TEM analysis (Table S8) shows that primary particle sizes remain largely unchanged (typically between 3 and 12 nm), confirming that aggregation occurs without structural transformation of the TiO₂ core. These trends are further supported by shifts in zeta potential toward less negative values over time (Fig. S1 and Table S6), indicating reduced electrostatic stabilisation and an increased propensity for aggregation.

These findings indicate that, unlike Ag ENMs, TiO₂ ENMs in the HH combo medium do not undergo substantial chemical transformation into new phases (e.g., sulphides) but instead experience surface modifications that reduce dispersion stability. Such changes are likely to reduce photocatalytic activity and alter environmental reactivity.⁵¹ Overall, TiO₂ ENMs remain chemically stable but undergo significant physicochemical changes that influence their environmental behaviour and long-term persistence.

3.2.2. Long-term transformations in the class I water. In the class I water (low alkalinity and low NOM content), the polydispersity index (PDI) values of all Ag ENMs fluctuated over 2 years but generally indicated gradual increases in particle size distribution over time, and showing significant aggregation over prolonged exposure (Table S4). After 4 years in the class I environmental water, significant chemical transformations were observed by XPS for the Ag ENMs compared to the pristine and HH combo medium conditions (Fig. S2 (pristine) and Fig. S8 and S9, Table S15 – aged). The Ag ENMs exhibit a higher level of oxidation, with an increased contribution of Ag⁺ species relative to metallic Ag⁰. This is supported by spICP-MS measurements (Table S9), which show an increase in the dissolved Ag fraction after ageing, indicating oxidative dissolution of Ag⁰. The presence of Ag₂S is also more pronounced in the class I-treated samples with an increase in sulphur content due to



interactions with sulphur-containing compounds in the class I water (Table S2). Ag_2S is relatively stable and less soluble, potentially reducing acute toxicity but contributes to long-term environmental persistence through sediment accumulation.

Surface coatings further influence the Ag ENM stability in class I water, with PEG-thiol and PVP-coated Ag ENMs showing greater resistance to oxidation (lower relative Ag^+ and Ag_2S signals), whereas uncoated ENMs oxidise more readily. These findings highlight the role of surface functionalisation in modulating oxidative dissolution and transformation pathways.⁵² Overall, the shift toward more oxidised species (Ag^+ and Ag_2S) suggests that ENMs in class I-like environments may contribute to both immediate toxicity (*via* Ag^+ release) and longer-term environmental persistence (*via* Ag_2S formation).⁵³

The XPS data for polymer coated TiO_2 ENMs after four years of ageing in the class I water (Fig. S10 and S11 and Table S16) also show medium-specific transformations compared to the pristine TiO_2 ENM dispersions. There is an increase in surface-bound hydroxyl ($-\text{OH}$) groups, leading to enhanced surface hydroxylation. The oxidation states are also consistent with TiO_2 , with Ti^{4+} remaining predominant. This is shown by the Ti 2p region, which is split into two peaks due to spin-orbit coupling, the first is Ti 2p_{3/2}, which is observed at ~ 458.5 eV for Ti^{4+} , and the second is Ti 2p_{1/2}, which is observed at ~ 464.3 eV for Ti^{4+} . Other oxidation states of titanium are also present (Ti^{3+} or Ti^{2+}) for the uncoated TiO_2 ENMs, which is shown by the lower binding energies (~ 457.0 eV) compared to Ti^{4+} due to reduced oxidation. This evidences that the TiO_2 ENMs largely retain their chemical composition but undergo surface chemistry changes due to interactions with water.

Compared to the pristine engineered TiO_2 ENMs, the surface-coated ENMs show clear evidence of coating modification and adsorption of organic matter from the class I water. This is supported by the XPS survey spectra (Fig. S10), which show an increased and broadened C 1s signal (~ 284 – 289 eV), indicating the presence of additional oxygenated carbon species (*e.g.*, C–O, C=O, and O–C=O) consistent with adsorbed natural organic matter. Additionally, the carbon signal becomes less defined relative to pristine materials, suggesting partial coating degradation or masking by an environmentally derived organic layer. The Ti 2p spectra (Fig. S11) confirm that Ti remains predominantly in the Ti^{4+} oxidation state, indicating that these changes are confined to surface chemistry rather than transformation of the TiO_2 core. These transformations lead to an increase in aggregation (as evidenced by the DLS and TEM morphology data), and altered surface charges (due to hydroxylation and organic matter adsorption) likely promoting particle–particle interactions and thus agglomeration.^{54,55}

3.2.3. Long-term transformations in the class V water. The class V standard water (high alkalinity and higher NOM content) caused the ENMs to exhibit significant size variations,

with some Z-averages exceeding 1000 nm by week 3 for the uncoated and Ag_2S ENMs (Table S5). Initially, Ag ENMs had large negative ZP values, but after two years, these values trended toward less negative or even slightly positive values, signalling a gradual decline in dispersion stability; likely due to aggregation and/or coating degradation. Surface coatings also played a key role in Ag ENM stability. The PVP Ag and PEG thiol Ag ENMs exhibited more moderate shifts in ZP over time, suggesting that these coatings helped maintain dispersion stability (Table S6). For example, uncoated ENMs and those with less stable coatings, such as paraffin, exhibited rapid size increases (TEM/spICP-MS: Table 2 and S9) and elevated PDI values, reflecting instability and aggregation. However, all Ag ENMs ultimately displayed reduced stability, as evidenced by the overall decline in the magnitude of negative ZP values over time (*i.e.*, their tendency towards zero).

Compared with the class I-treated and pristine ENMs, the class V water environment promoted further oxidation, with elevated levels of Ag^+ detected for the Ag ENMs. This increased oxidation indicates that class V water has stronger oxidizing conditions and higher ionic content, driving more of the Ag^0 to Ag^+ , which is then bioavailable and known to be toxic.⁵⁶ Similarly to the class I water, the class V aged ENMs also show the formation of Ag_2S as shown by the Ag 3d_{5/2} and Ag 3d_{3/2} peaks shifting to ~ 368.0 eV and ~ 374.0 eV (Table S16; Fig. S12 and S13), respectively. This transformation from metallic silver to Ag_2S stabilises the particles in a less soluble, but environmentally persistent, form, reducing immediate toxicity but further contributing to long-term environmental accumulation risks.^{47,48} The different surface coated Ag ENMs respond uniquely under class V conditions. More protective coatings such as paraffin and PVP result in the ENMs showing fewer transformations, while less stable coatings (and the uncoated) Ag ENMs display higher levels of Ag^+ and Ag_2S transformations. This reinforces the importance of coating choice in managing ENM stability and potential environmental release of Ag^+ .

Overall, compared to pristine Ag ENMs, both the class I and class V treatments lead to significant transformations, with class V showing a more pronounced effect. While class I led to some oxidation and sulfidation, the harsher conditions in class V amplify these changes, suggesting that ENMs exposed to environments like those of the class V environment (Table S2) could have higher environmental impacts. This progression underscores the role of environmental conditions in shaping ENM transformation pathways, which has implications for environmental risk assessments and the need for tailored regulations to protect environmental and ecosystem health.

Under the class V environmental conditions (Fig. S14), the TiO_2 ENMs remain chemically stable (Ti^{4+} species dominate) over long periods, suggesting high environmental persistence. The degradation of the surface coatings like PVP and Pluronic highlights the need to evaluate stabiliser effectiveness over time under relevant environmental conditions. Degraded coatings in this instance led to increased ENM aggregation. These transformations are significant as they lead to reduced ENM mobility in the water column, promoting sedimentation.



3.3. Key environmental interactions and transformation mechanisms

3.3.1. Transformation and environmental behaviour, with implications for risk assessment. The results demonstrate that ENMs do not retain their pristine manufactured form under environmentally relevant conditions but instead undergo dynamic transformations that fundamentally alter their physicochemical properties, behaviour, and potential risks. For Ag ENMs, processes such as dissolution, oxidation and sulfidation (*e.g.*, formation of Ag₂S) lead to changes in particle size, speciation, and bioavailability, as previously investigated with these particles.^{35,55–57} These transformations influence toxicity pathways, as smaller ENMs and released Ag⁺ ions are more bioavailable and capable of penetrating biological membranes,^{7,8} whereas larger aggregates may reduce immediate bioavailability but enhance accumulation in sediments and exposure to benthic organisms.⁵⁷ In contrast, TiO₂ ENMs remain largely chemically stable, retaining Ti⁴⁺ as the dominant oxidation state, but undergo significant surface modifications, including hydroxylation and adsorption of environmental organic matter, which alter dispersion stability, aggregation behaviour, and environmental interactions.

Surface coatings are applied to enhance ENM stability, performance, and controlled application; however, they add complexity to environmental behaviour. The present study shows that these coatings are not environmentally stable over longer timescales. Progressive coating degradation or displacement exposes the ENM core, facilitating processes such as oxidation, sulfidation, and other chemical speciation reactions. These transformations modify particle–particle interactions and promote aggregation, leading to changes in transport, behaviour, and toxicity. In this study, coatings such as PVP initially enhanced dispersion and chemical stability, potentially reducing immediate risks, but their long-term effectiveness appeared limited. This degradation may also contribute to environmental concerns such as microplastic pollution. As a result, ENM behaviour evolves over time, with implications for both short-term toxicity and long-term environmental persistence. In particular, transformation to more stable forms such as Ag₂S in sulphur-rich environments reduces acute toxicity associated with dissolved ions but increases environmental persistence and accumulation in sediments and biota.³⁵

Environmental persistence is therefore a key outcome of the transformations observed in this study. Aggregated ENMs exhibit altered transport and fate, and while reduced dispersibility may limit exposure in the water column, it may increase bioavailability within benthic ecosystems.⁵⁷ These findings highlight that ENMs should be considered as dynamic systems rather than static materials, with properties that evolve depending on environmental conditions such as ionic strength and dissolved organic content. These dynamic transformations challenge traditional risk assessment frameworks, which typically assume a static material form and are therefore inadequate for evaluating ENMs.^{20,58} As ENMs undergo physicochemical changes, their

interactions with organisms, exposure pathways, and hazard profiles also change. For example, Ag ENMs may dissolve into Ag⁺ ions, suggesting that risk assessments based solely on pristine ENMs may underestimate real-world exposure risks, as also demonstrated with these particles.^{59,60}

Without studies such as the present work, regulatory bodies lack the necessary data to establish effective guidelines and testing protocols for ENMs. Conventional testing approaches may misrepresent environmental risks by failing to account for transformation processes occurring under diverse environmental conditions. Standardised OECD test conditions, while useful for assessing intrinsic material toxicity, may not fully capture environmentally relevant ageing, surface modification, or speciation changes. If a material remains stable under OECD conditions, observed toxic effects may reflect intrinsic ENM properties rather than environmentally driven transformations. Therefore, future regulatory frameworks should explicitly incorporate ENM ageing, transformation pathways, and environmentally derived surface modifications to improve predictions of long-term environmental behaviour and risk.

3.3.2. Recommendations for environmental testing and regulation

3.3.2.1. Include ageing and transformation studies in risk assessments. Regulatory bodies should mandate environmental risk assessments to include evaluation of ENMs under simulated environmental ageing conditions, reflecting the diverse aquatic or soil/sediment environments these materials may encounter. These tests should assess changes in particle size, surface coating integrity, chemical form, particle concentration and long-term transformations.

Simulated conditions should encompass a range of environmental scenarios, including freshwater, seawater, and soil, as ENMs exhibit unique transformation pathways in each setting due to differences in salinity, ionic strength, pH, organic matter content, redox conditions and the presence of competing ions, as these factors significantly influence ENM transformations and interactions^{22,23,61–64} as observed in the present study. For example, Ag ENMs have shown to undergo sulfidation in freshwater and seawater, forming Ag₂S, a less bioavailable and less toxic species, whereas in soils, interactions with organic matter and clay minerals can lead to stabilisation or increased aggregation.^{47,48,64} Each environmental condition necessitates a distinct risk profile that reflects the specific transformation and ecological impacts of ENMs, as their fate and bioavailability are highly context-dependent.⁶⁵ To ensure accurate environmental safety evaluations, regulatory frameworks must incorporate these dynamic transformations into risk assessments. This includes requiring manufacturers to provide data on the environmental fate of their products over their lifecycle to improve our understanding of persistence, mobility, and ecological impacts, ultimately supporting the design of safer ENMs.^{4,66,67}

3.3.2.2. Coating stability testing. The critical role of coatings in determining ENM behaviour demands rigorous testing of coating stability across various environmental settings. Coatings



like PVP, PEG and citrate, used to enhance ENM dispersion and reduce aggregation, degrade over time due to UV radiation, microbial activity, and chemical interactions in natural environments.^{24,68–70} Previous studies have demonstrated that commonly used surface coatings, including citrate, PVP, and PEG, can lose stability in ecotoxicological media over relatively short timescales, leading to increased aggregation and changes in ENM behaviour.^{24,25,33,71,72} These studies have established coating instability as an important factor influencing nanomaterial fate under controlled laboratory conditions. However, they are typically limited to short-term exposures and do not resolve how coating degradation progresses under environmentally relevant conditions over extended timescales, nor how this interacts with evolving surface chemistry. In contrast, the present study extends this understanding by providing a multi-year (4-year), multi-media assessment of coating stability, integrating dispersion-based measurements with surface chemical characterisation. This combined approach demonstrates that coating degradation is not a discrete or short-term process, but a progressive and environment-dependent driver of ENM transformation. Specifically, the results show that coating loss contributes to a divergence between dispersion stability and chemical stability, facilitates environment-specific transformation pathways (e.g., oxidation *versus* sulfidation), and leads to the emergence of new, environmentally conditioned material states that are not captured in short-term studies.

Building on these findings, the critical role of coatings in determining ENM behaviour demands rigorous testing of coating stability across various environmental settings. Coatings like PVP, PEG and citrate, used to enhance ENM dispersion and reduce aggregation, degrade over time due to UV radiation, microbial activity, and chemical interactions in natural environments.^{24,68,69} Regulatory bodies should require manufacturers to provide data on the degradation rates of coatings and their potential effects on the surface properties and reactivity of ENMs. Standardised tests for coating persistence could be modelled using similar protocols used for evaluating persistent organic pollutants (POPs),^{73,74} incorporating assessments of coating degradation kinetics, secondary transformations, and interactions with environmental matrices.⁴ Such tests would enable regulators to predict how coatings influence long-term ENM environmental behaviour, aiding in the development of grouping and safer-by-design of ENMs.^{66,67}

3.3.2.3. Tiered testing approach. Implementing a tiered framework for ENM risk assessment is essential for addressing the complexities of their environmental interactions.^{75,76} This approach should begin with fundamental physicochemical characterisations under pristine conditions as shown in the present study to establish a baseline understanding of the ENM properties. The second tier should involve ageing studies under simulated environmental conditions, as discussed in section 3.3.1.⁷⁷ Finally, the third tier should incorporate ecological impact assessments within simulated natural environments (e.g., mesocosms or field studies) to evaluate the broader

consequences of these transformations on ecosystem health, including impacts on trophic interactions and sediment deposition.^{57,78,79} This tiered approach ensures a holistic understanding of ENM behaviour and impacts, addressing the complexities introduced by environmental ageing and transformations.

3.3.2.4. Ecological impact monitoring. Long-term environmental monitoring programs should be implemented to track the presence, transformations, and accumulation of ENMs in ecosystems where they are likely to be released. Monitoring should involve periodic sampling and analysis of environmental compartments such as water, soil, and sediments, using both qualitative and quantitative techniques including spICP-MS and XPS to identify ENM forms and transformation products.⁸⁰ Such data enable early identification of risks associated with transformed ENMs and help to support adaptive management strategies to mitigate environmental and ecological impacts. By linking monitoring programs with predictive modelling,^{2,42,81} regulators can develop dynamic risk profiles and ensure that safety measures evolve alongside new scientific findings.

4. Conclusions

This study demonstrates that long-term environmental ageing fundamentally alters the physicochemical identity of Ag and TiO₂ ENMs, with transformations governed by core composition, surface coatings, and environmental conditions. Across all media, clear and consistent differences emerged between dispersion stability (aggregation behaviour) and surface chemical stability (composition and oxidation state), highlighting the need to treat these as distinct but interlinked processes in environmental risk assessment. A key finding is the utility of X-ray photoelectron spectroscopy (XPS) in resolving environmentally relevant chemical transformations. XPS analysis revealed that Ag ENMs undergo progressive oxidation and sulfidation, with a decline in metallic Ag⁰ and the formation of Ag⁺ and Ag₂S species over time, while TiO₂ ENMs largely retain their Ti⁴⁺ core but exhibit increased surface hydroxylation and adsorption of environmental components. These results demonstrate that XPS provides critical mechanistic insight into surface speciation changes that cannot be captured by size-based techniques alone.

Surface coatings were shown to play a dual but time-dependent role. Initially, coatings such as PVP and PEG-thiol enhanced dispersion and reduced reactivity; however, progressive coating degradation or displacement during ageing exposed the ENM core, accelerating chemical transformations and reducing dispersion stability. This highlights that coating loss is a key driver of long-term ENM behaviour, with implications for both transformation pathways and environmental exposure. Environmental conditions strongly influenced transformation trajectories, with alkalinity and natural organic matter (NOM) emerging as major drivers. High ionic strength and alkalinity promoted aggregation and reduced dispersion stability, while class I and class V waters enhanced oxidative dissolution and sulfidation of Ag ENMs, increasing



the relative abundance of Ag⁺ and Ag₂S species. NOM-rich conditions facilitated surface modification, corona formation, and coating masking or replacement, particularly for TiO₂ ENMs. These findings demonstrate that ENMs do not behave as static materials but instead evolve dynamically, with divergent outcomes for dispersion stability (aggregation and sedimentation) *versus* chemical stability (speciation and reactivity). For example, TiO₂ ENMs remained chemically stable but became increasingly aggregated, whereas Ag ENMs underwent substantial chemical transformation that directly alters bioavailability and toxicity.

These transformations significantly alter ENM stability, bioavailability, and toxicity, raising critical concerns for environmental risk assessments. Current regulatory frameworks, which primarily evaluate ENMs in their pristine, manufactured forms, fail to capture these dynamic and long-term changes and may therefore underestimate real-world impacts. To address these gaps, it is essential to incorporate environmental ageing into regulatory testing strategies, explicitly considering both dispersion and chemical stability, alongside coating persistence, across environmentally relevant gradients of water chemistry, including alkalinity, ionic strength, and NOM content. Ultimately, this research demonstrates that ENM hazard cannot be defined by pristine properties alone and that mechanistic tools such as XPS are essential for capturing transformation pathways. By integrating ageing, coating stability, and environment-specific transformations into regulatory frameworks, more accurate, predictive, and protective assessments of ENM risks can be achieved. These insights support the development of safer-by-design materials and more comprehensive guidelines aimed at minimizing the ecological and human health impacts of ENMs, ensuring their responsible use and management.

Author contributions

L. J. E. (B): writing of the original draft, writing including review and editing, visualisation, resources, methodology, investigation, formal analysis, data curation, conceptualization. C. N.: writing including review and editing, methodology, formal analysis and data curation. A. J. C.: writing including review and editing, methodology, formal analysis and data curation. I. L.: writing of the original draft including review and editing, supervision, validation, and conceptualization.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

All data are available on request.

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