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Research Paper

Quantitative analysis of nanoplastics in environmental and potable waters by pyrolysis-gas chromatography–mass spectrometry

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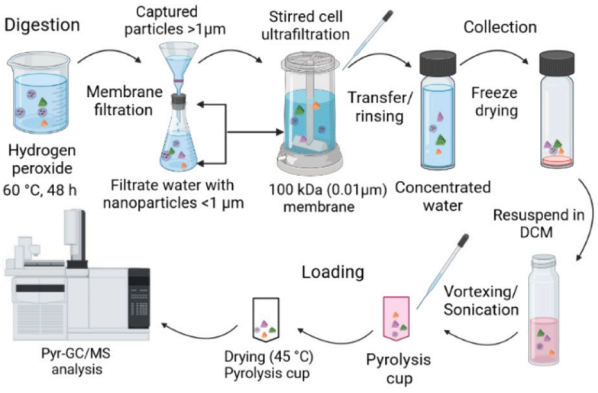
HIGHLIGHTS

- Presentation of a methodology for the quantification of nanoplastics in water.
- Mass concentrations of nanoplastics across water samples were assessed by Pyr-GC/MS.
- Eight nanoplastics (PMMA, Nylon 6, Nylon 66, PC, PS, PET, PP, PE) quantified.
- PE, PET, PP and PS were the predominant nanoplastics.
- Total nanoplastics concentrations of between 0.04 and 1.17 μg/L in water samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanoplastics are emerging environmental contaminants, but their presence in environmental and potable water remains largely understudied due to the absence of quantitative analytical methods. In this study, we developed and validated a pretreatment method that combines hydrogen peroxide digestion and Amicon® Stirred Cell ultrafiltration (at 100 kDa, approximately 10 nm) with subsequent detection by pyrolysis gas chromatography–mass spectrometry (Pyr-GC/MS). This method allows for the simultaneous identification and quantification of nine selected nanoplastic types, including poly(ethylene terephthalate) (PET), polyethylene (PE), polycarbonate (PC), polypropylene (PP), poly(methyl methacrylate) (PMMA), polystyrene (PS), polyvinylchloride (PVC), nylon 6, and nylon 66, in environmental and potable water samples based on polymer-specific mass concentration. Limits of quantification ranged from 0.01 to 0.44 μg/L, demonstrating the method's ability to quantitatively detect nanoplastics in environmental and potable water samples. Most of the selected nanoplastics were detected at concentrations of between 0.04 and 1.17 μg/L, except for PC, which was consistently below the limit of detection (<0.44 μg/L). The prevalent polymer components in the samples were PE (0.10 – 1.17 μg/L), PET (0.06 – 0.91 μg/L), PP (0.04 – 0.79 μg/L), and PS (0.06 – 0.53 μg/L) nanoplastics. The presented analytical method offers an accurate means to identify, quantify, and monitor nanoplastics in complex environmental and potable water samples. It fills gaps in our understanding of nanoplastic pollution levels, providing a valuable methodology and crucial reference data for future studies.

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1. Introduction

Over the past century, plastic production and consumption have seen significant growth, but efforts in reusing, recycling, and implementing pollution control measures have not kept pace, leading to a predominantly linear plastics economy [1,2]. Consequently, plastics have been extensively released into various environmental compartments, potentially breaking down into smaller fragments, categorized as microplastics (particles between 1 μm and 5 mm) and nanoplastics (particles < 1 μm) [1,3–21]. The occurrence and impacts of microplastics have been well studied in environmental and biological systems [22–24], however, the scientific significance of studying nanoplastics is an area of active research since they have been shown to exhibit greater bioavailability during accumulation than microplastics, thereby resulting in higher toxicity to organisms [9,12,25–32]. The detrimental impacts of nanoplastics fragments for instance in aquatic organisms, which includes oxidative stress, downregulated gene expression, and behavioral disorders [27], is of major concern since there are likely to be more nanoplastics particles in the environment than microplastic particles [33]. That said, little is known about the environmental occurrence and distribution of nanoplastics, and there are even fewer data or studies on nanoplastics in environmental and potable water samples [12,16,34,35]. This can be largely attributed to the lack of appropriate analytical methodologies for the pre-treatment, extraction or pre-concentration and quantification of nanoplastics in environmental samples, although there is mounting evidence of their existence [10,12,16,20,21,34–37]. This calls for an urgent development of analytical methods that can detect and quantify trace nanoplastics in environmental samples.

Most of the current analytical techniques, such as Raman and Fourier transform infrared (FTIR) spectroscopies, commonly employed for measuring microplastics in environment samples are only capable of reporting data for plastic particles > 1 μm [11,36,38–41]. These methods have inherent size limitations and become ineffective when dealing with particles at the nanoscale (< 1 μm) level or those falling below their optical resolution [12,42]. For instance, FTIR microscopy and Raman micro-spectroscopy/spectroscopy have reported size detection limits of approximately 20 μm and 1 μm, respectively, which significantly restricts their capability for detecting and analyzing nanoplastics. [34,43–46]. In this context, thermo-analytical techniques such as pyrolysis-gas chromatography coupled with mass spectrometry (Pyr-GC/MS), provides the potential for nanoplastics analysis (by mass concentration) because the technique is unrestricted by particle sizes and exhibits high specificity [12,20,23,24,36,37,47–52]. However, the pre-concentration of nanoplastics in environmental samples to meet the analytical requirement of determination or to achieve analysis with Pyr-GC/MS is conventionally challenging [10,20,34,37,38,53,54]. This has been largely attributed to the detection limits of nanoplastics in environmental samples and generally a complex matrix with a high content of interfering organic materials [12,17,19,37,38,55].

Few studies have to date attempted to pre-treat and pre-concentrate nanoplastics in environmental water samples for Pyr-GC/MS analysis using various approaches like membrane filtration and ultrafiltration. One study proposed a cloud-point extraction to concentrate nanoplastics (polystyrene (PS) and poly(methyl methacrylate) (PMMA)) in water samples. [37]. While the recovery rate of spiked PMMA and PS nanoplastics was high, their concentrations in real environmental samples remained below the method's detection limits (0.6 and 1.1 μg/L, respectively for PMMA and PS) [37]. Another study focused on the colloidal fraction of seawater, identifying but not quantifying polymers using a 10 kDa ultrafiltration device [36]. Zhou et al. [12]. proposed an extraction approach mediated by protein corona for the pre-concentration of nanoplastics in water samples. While the approach showed high recovery rates for PS and PMMA nanoplastics, only PS nanoplastics were quantifiable above the method's detection limits (0.08 μg/L for PS and 0.03 μg/L for PMMA) in real samples. Recently, a method combining crossflow ultrafiltration and hydrogen peroxide (H_2O_2) digestion, followed by Pyr-GC/MS [11,54] was successfully used to extract and quantify nanoplastics from water and wastewater samples.

While these studies have advanced our understanding of nanoplastic contamination in the environment, there is an urgent need for extraction and separation methodologies that meet the requirements for sensitive analysis of nanoplastics by Pyr-GC/MS [11,37]. Methods published to date have encountered challenges, such as low recoveries of nanoplastic particles, interference from organic material and only able to process small sample volumes. Due to the relatively high instrumental detection limit for nanoplastics [37], suitable enrichment methods that are highly efficient in concentrating large volumes of water are required to analyze nanoplastics in water samples. To reduce potential interference from organic materials, sample pretreatment methods such as H_2O_2 digestion is necessary.

The objective of this study was to develop an analytical workflow for concentrating (particles in large volumes of water) and analyzing nanoplastics (particles and agglomerates of between 10 and 1000 nm) in complex environmental and potable water samples. The potential of H_2O_2 pre-treatment combined with Amicon® Stirred Cell pre-concentration, followed by Pyr-GC/MS analysis was evaluated as a reliable and practical method for simultaneously analyzing nine selected nanoplastics. Amicon® Stirred Cell ultrafiltration was found to be a suitable pre-concentration step for nanoplastics analysis in environmental samples due to its high efficiency in concentrating large volumes of water [36,56]. In this study, water samples were first pre-treated with H_2O_2 digestion, a mild and effective pre-treatment method used for extracting microplastics from organic-rich environmental matrices [4,11,57], to reduce potential interference from organic substances [56]. The nanoplastic particles in the large volume of water was then concentrated using Amicon® Stirred Cell ultrafiltration (at 100 kDa, approximately 10 nm) and subsequently analyzed with Pyr-GC/MS. The method's successful application is demonstrated through the analysis of various samples, including stormwater, wastewater, reservoir water, municipal (tap) water, bottled water, and surface water. This study provides information on the method's applicable concentration ranges, detection, and quantification limits, selected indicator compounds and signals interferences, and the recoveries of PS (nominal sizes 30, 200, and 700 nm) and PMMA (nominal sizes 70, 110, and 740 nm) nanoplastics from ultrapure water and wastewater samples. These findings are presented and critically discussed, demonstrating the feasibility and reliability of the proposed method.

2. Materials and methods

2.1. Chemical and materials

A dispersion of polystyrene (PS) nanoplastics particles (nominal sizes 30, 200 and 700 nm) and poly-(methyl methacrylate) (PMMA) nanoplastics particles (nominal sizes 70, 110 and 740 nm) were purchased from the Bangs Laboratories, Inc. (Indiana, USA). Analytical reference materials or standards of PS (CAS 9003–53–6, powder), PMMA (CAS 9011–14–7, powder), polyvinyl chloride (PVC) (CAS 9002–86–2, powder), Nylon 6 (CAS 25038–54–4, pellets) and Nylon 66 (CAS 32131–17–2, pellets) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Polypropylene (PP, powder) was supplied by LyondellBasell (Melbourne, Australia). Low-density polyethylene (LDPE, referred to as PE in this study) (CAS 9002–88–4) was obtained from Thermo Fisher Scientific (Waltham, MA). Deuterated polystyrene (d_5-PS, powder), polycarbonate (PC, powder), and polyethylene terephthalate (PET, powder) were supplied by Polymer Source, Inc. (Dorval, Canada). The 9 different plastics or polymer types were selected for analysis as these polymers have been widely reported in water samples [11,12,54]. Whatman glass fiber filters (47 mm in diameter, 1.0 and 0.7 μm pore sizes) were supplied by Thermo Fisher Scientific (Waltham, MA). Glass fiber membrane filters (21 mm, 1 μm) was supplied by Advantec Co.,

Ltd. (Japan). Ultrapure water obtained from (i.e., purified with a MilliQ system (Millipore, 0.22 μm filtered, 18.2 MΩ cm^{-1}, Bedford, USA)), was additionally filtered through a 0.7 μm pore size (47 mm) glass fiber filter prior to use (referred as MilliQ water in this study). Hydrogen peroxide (H_2O_2, (30%)) was supplied by Sigma-Aldrich (Australia). Analytical grade dichloromethane (DCM) and acetone were supplied by Merck (Darmstadt, Germany) with 1,1,1,3,3,3-Hexafluoro-2-propanol (≥99%, CAS 920–66–1) purchased from Merck (Australia). All reagents and solvents were obtained in glass bottles and used as received without additional purification.

2.2. Environmental and potable water sampling

Wastewater influent (1 L each) and effluent (2 L each) samples from three plants (A, B and C), as well as surface water from a dam (2 L), reservoir water (2 L), and stormwater (2 L) samples were collected in Australia following validated microplastics sampling approaches [58,59]. Bottled (processed) water samples (1.2 L each from two different brands, A and B) were purchased from a retail store in Queensland, Australia. Municipal water samples (2 L each from two different locations, A and B) were collected in Queensland, Australia. The influent water samples were collected directly by submerging a pre-rinsed 1-L stainless-steel bottle (with acetone, DCM and MilliQ water) into the WWTPs influent water. For the effluent, surface water, reservoir water, and stormwater samples, dedicated pre-rinsed 1-L stainless-steel bottles (one for each site) were used for sampling. The bottles were immersed or submerged two times, and the water collected was subsequently transferred into 2-L stainless steel bottles that had been prepared in advance. The municipal water samples were directly collected into 5-L pre-rinsed prepared glass bottles while the bottled water was purchased in PET bottles. After sampling, all sample bottles were sealed with DCM pre-cleaned aluminum foil and metal lids to prevent contamination. To assess for contamination during sampling, field blanks ($n = 2$) (MilliQ water) were collected. All samples were collected through grab sampling, transported to the laboratory, and stored at − 4 °C until analysis.

2.3. Pretreatment and extraction of nanoplastics

The pretreatment and nanoplastic particle extraction procedures for the water samples were established based on previous studies with considerable modifications [11,36,54]. As shown in Fig. 1, all samples were pre-treated with H_2O_2 and concentrated using an Amicon® Stirred Cell to extract nanoplastics. Each water sample was first transferred to a DCM pre-cleaned glass beaker (1.5 L) and H_2O_2 (100 mL at 30%) added, mixed, and incubated at 60 °C for 48 h in a Thermoline Orbital incubator shaker (Thermoline Scientific, Wetherill Park, NSW). To prevent possible contamination and spillovers, the samples were covered with DCM pre-cleaned aluminum foil during incubation. The H_2O_2 digestion steps (i.e., the solution volume and incubation time) were carefully selected to ensure the complete removal of interfering organic materials (i.e., effectively minimizing any potential interference from organic substances), while preserving the integrity of plastic particles, as previously reported [4,60]. After digestion, the samples were vacuum filtered through 1 μm pore size membrane glass fiber filters (47 mm) on a glass filtration unit. To ensure all adhered particles were removed or transferred, filtration funnels and glass beakers were washed and rinsed three times with MilliQ water. Importantly, it should be noted that any nanoplastics agglomerates that were filtered out during the initial membrane filtration step (>1 μm) were categorized as microplastics and thus omitted from the subsequent nanoplastics analysis, which was consistent with findings from prior research [11,54].

The < 1 μm filtrate water was subjected to concentration using an Amicon® Stirred Cell equipped with a 100 kDa poly (ether sulfone) (PES) membrane (Merck, Australia, Table S1). N_2 gas at 75 psi was used to push the samples through the membranes. Before usage, all membranes were washed and soaked in MilliQ water. Similarly, the Amicon® Stirred Cell was washed with MilliQ water and then dried upside-down in a fume hood on a DCM pre-cleaned aluminum foil, wrapped to prevent contamination or possible airborne plastic particles. The total volume of the Stirred Cell was 50 mL. Subsequently, during the concentration and ultrafiltration of 1 L of water or wastewater, the ultrafiltration setup was stopped, the cell was opened, and then refilled with the sample. This operation was repeated 20 times and lasted for 2 h. The beakers and ultrafiltration unit were rinsed three times with MilliQ water to ensure all possible adhered particles were transferred. The resulting retentate, which contained the concentrated nanoplastics (i.e., up to the limit of the filtration procedure or slightly exceeding the void volume of the Amicon® Stirred Cell), was collected and carefully transferred into DCM pre-rinsed 15 mL glass centrifuge tubes using glass pipettes that were also pre-rinsed with DCM. To reduce and minimize any potential sample loss, 50 mL of MilliQ water was introduced into the Stirred Cell, and the walls of the cell and membrane surfaces were gently washed. This step was repeated six times (5 min each time), and the retentate washing liquid was combined with the previous retentate. This process ensured that all adhered particles were transferred and also purified the sample by removing or reducing interfering substances, while simultaneously pre-concentrating the potential nanoplastics content. The same procedure was applied to all samples, resulting in a final collected and transferred retentate volume of 10 ± 2 mL.

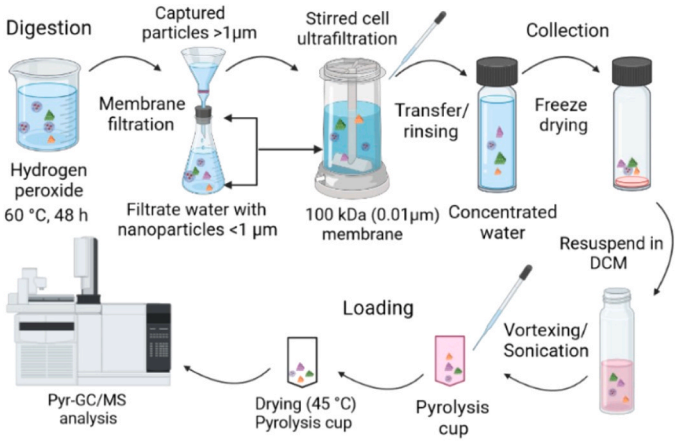

Fig. 1. Pretreatment processes of water samples for nanoplastics detection by Pyr-GC/MS.

The H_2O_2 treated and concentrated sample in the 15 mL glass centrifuge tubes was then freeze-dried for 24 h to obtain a powder containing the nanoparticles. Subsequently, 2 mL of DCM was added to the dried sample, which was then subjected to sonication and vortex-mixing for 30–40 min to resuspend the nanoparticles. The final resuspended and redispersed sample was repeatedly transferred into pyrolysis cups (80 μL, Eco-Cup LF, Frontier Labs, Japan), while being placed on a heating plate at a temperature below 45 °C to evaporate the DCM and ensure all the nanoplastics were loaded for Pyr-GC/MS analysis. To ensure complete transfer of all adhered particles, this step was repeated six times, with the glass centrifuge tubes rinsed six times with DCM. All samples were covered with aluminum foil during this process. Before analysis by Pyr-GC/MS, all cups were spiked with 0.02 μg of d_5-PS internal standard.

2.4. Pyrolysis gas chromatography–mass spectrometry

The quantification of the nine selected nanoplastic types (i.e., PP, PE, PS, PET, Nylon 6, Nylon 66, PC, PMMA and PVC) was carried out using a Multi-Shot Micro-furnace Pyrolyzer EGA/PY-3030D equipped with an Auto-Shot Sampler (AS-1020E) from Frontier Laboratories, Fukushima, Japan, coupled to a Shimadzu GC/MS-QP2010-Plus (Shimadzu Corporation, Japan). The Pyr-GC/MS analysis parameters were selected based

on those used in our previous studies [24,47,61,62]. In brief, the samples underwent a double-shot pyrolysis analysis and were injected with a split of 5:1. The first pyrolysis shot (from 100 °C to 300 °C) served as a clean-up step to remove interfering volatile and semi-volatile organic materials and compounds co-extracted from the water samples. The second pyrolysis shot (at 650 °C for 0.20 min (12 s)) was used for quantitative measurements of the identified plastics. The Pyr-GC/MS operating system was composed of 30 m, 0.25 mm I.D., 0.25 μm film thickness Ultra Alloy 5 capillary column from Frontier Laboratories Japan; helium as the carrier gas (at a flow rate of 1.0 mL/min with a constant linear velocity); oven temperature program: 40 °C (held for 2 min) to 320 °C at 20 °C/min (held for 14 min); pyrolyzer interface and GC injection port temperatures of 320 °C and 300 °C, respectively; ion source temperature of 250 °C with an ionization voltage of 70 eV and a full scan mass range of m/z 40–600 [24,47,61,62].

2.5. Indicator compound selection

To identify and quantify single nanoplastic polymer types in environmental samples using Pyr-GC/MS, specific indicator compounds/ions are required. The individual analytical standards or reference materials of the target nanoplastics were therefore analyzed using Pyr-GC/MS to identify their characteristic components and ions **(**Table S2**,** Fig. S1 and S4**)**. The obtained pyrograms were then cross-checked with literature data and a customized in-house database, and the specific indicator ions for the nine target nanoplastics were selected following criteria's or recommendations from Fischer and Scholz-Böttcher [49], Hermabessiere, et al. [63], Okoffo, et al. [47], Rauert, et al. [61] and Tsuge, et al. [64]. (details on the chosen indicator ions are discussed in the Results and Discussion section).

2.6. Potential matrix interferences

The use of selected pyrolysis products or ions for identification and quantification of polymers by Pyr-GC/MS can present challenges when the chosen products are not specific to distinct polymers or are not exclusive to specific polymers and can also be produced from natural organic materials and other matrix interferences present in the samples [11,17,47–49,65]. To address this issue in this study, the selectivity of the indicator ions used for identifying and quantifying the target nanoplastics were evaluated for their specificity, potential interferences, and co-formation from a variety of biogenic polymers and organic materials that may be present in the water samples due to incomplete sample digestion or removal with H_2O_2 and the first pyrolysis-shot (thermal desorption) clean-up steps or due to secondary contamination. The assessment included materials such as wood (lignin), fir needle (tree gum, terpene), fish filet (proteins, fat), leaf (cellulose, organic matter), engine oil (hydrocarbons), prawns (chitin, proteins), sunflower oil (polyunsaturated fat), cellulose (filter paper/lab tissue), triglyceride analytical standard (monounsaturated fat), humic acid (organic matter), and food-grade coconut oil (saturated fat) as previously reported [11,18,47,54,61]. Each of these materials constitutes common components found in environmental matrices, such as cellulose, organic matter, oils, proteins, and fats. The bias for the selected nanoplastic polymer types induced by 1 g or 1 mL of the organic materials after digestion with H_2O_2 and the first pyrolysis-shot (thermal desorption) clean-up step is provided in Table S3 (**see** supplementary information **for details on sample preparation and analysis**). The principal goal was to remove/reduce potential interferences with the selected indicator ions in the water samples, with digestion with H_2O_2 and the first pyrolysis-shot (thermal desorption) clean-up step before nanoplastic quantification (details on potential interferences and their removal are discussed in the Results and Discussion section).

2.7. Recoveries of spiked nanoplastics in MilliQ water and wastewater samples

Recoveries of PS (nominal sizes 30, 200 and 700 nm) and PMMA (nominal sizes 70, 110 and 740 nm) nanoplastics from spiked ultrapure water (MilliQ water) and wastewater samples were assessed with the combined techniques as explained above. Briefly, three MilliQ water samples (1 L each) and three influent wastewater samples (1 L each) were simultaneously spiked with the PS and PMMA nanoplastic solutions at the three spiking concentrations of between 22 and 41 μg/L **(**Table S6**)** and then concentrated with the same procedure and made ready for Pyr-GC/MS analysis as described above. Before spiking, both MilliQ water and wastewater samples were filtered through a 1 μm pore size (47 mm) filter. Following the spiking of the nanoplastic particles, the samples were subjected to the same processing steps as the real water samples, including sample digestion, ultrafiltration concentration, freeze-drying, and transferring and loading into Pyr-GC/MS cups. To evaluate quantification error and reproducibility, three replicates of the spiked MilliQ water and wastewater samples were prepared at each spiking level and subsequently analyzed. Additionally, three MilliQ water samples (1 L each) and three wastewater samples (1 L each) without PS and PMMA nanoplastics spiking were concentrated and analyzed as blank samples. These samples were analyzed to establish and determine the background concentration levels of the spiked nanoplastic types. Hence, the masses of the spiked PS and PMMA nanoplastics in the standard spiked samples were calculated as Δcompounds = mass of compound detected in the spiked sample - mass of compound established in the background or blank sample. The recoveries (R) were then calculated as R = mass of Δcompounds / mass of compounds added. Furthermore, the recovery rates of the spiked PS and PMMA nanoplastics in the MilliQ water and wastewater samples were compared to the analytical results of unconcentrated standards analyzed on Pyr-GC/MS (i.e., the same concentration of nanoplastics that were spiked into MilliQ and wastewater samples were analyzed on Pyr-GC/MS) that would represent 100% recovery.

2.8. Limits of detection and quantification

An eight-point injection external calibration standard/curves for all polymers ranging from 0.08 to 33 μg/cup was performed for the selected nanoplastic types. The calibration curves had a regression coefficients (R^2) of $\geq$ 0.96 (Table S8). To perform calibrations, the PE, PP and PET analytical standards were extracted using a Pressurized Liquid Extraction (PLE) technique with DCM as used in our previous studies [24,47,61], while the PVC, PS, PMMA, and PC standards were dissolved in DCM at room temperature. For nylon 6 and 66, the analytical standards were first dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol [66], then an aliquot was then redissolved in DCM to perform the calibrations. Individual measurements and mixtures of the selected plastics in one pyrolysis cup were used for creating calibration curves. The calibration curves were generated by plotting the peak area ratio of the indicator compound (quantifier) of the target plastic or polymers to the styrene-d_5-monomer (d_5-PS internal standard) (Table S2) against the concentration of each target plastic. Quantification was then carried out using the integration results obtained from these curves. The d_5-PS internal standard was employed for all the target polymers, and all reported values in this study were adjusted for the recovery of the internal standard.

The limit of quantification (LOQ) for each nanoplastics type was determined based on a peak with a signal-to-noise ratio of 10:1 or 10 times the baseline noise. Similarly, the limit of detection (LOD) was calculated using concentrations measured in laboratory and procedural blanks, which involved calculating the mean concentration plus three times the standard deviation of detected concentrations (**see** Table S4). In cases where the concentrations were below the LOQ, ½ LOQ was used for calculating average concentrations and standard deviations in the

blanks. For nanoplastics types where all blanks were below the LOQ, the LODs are reported as the LOQ. All reported data were adjusted for blank values. Accordingly, the method successfully reached the LOQs, demonstrating its sensitivity and suitability for nanoparticle detection.

2.9. Method application to environmental and potable water samples

To assess the suitability of the method, we collected and analyzed 3 influent and 3 effluent wastewater samples, 1 surface water sample (potable water from a dam), 1 reservoir water sample, 1 stormwater sample, 2 bottled (processed) water samples, and 2 municipal water samples. The analysis was conducted to determine the mass concentration or amounts of the target nanoplastics, following the described procedures above.

2.10. Particle size distribution of nanoplastic particles

Particle size distribution of nanoparticles in the water samples were characterized using the Microtrac Sync 5000 system with the FlowSync (wet) module (Microtrac Retsch GmbH, Haan/Duesseldorf, Germany) to support the Pyr-GC/MS identification and quantification measurements above. All water samples after the digestion pretreatment with H_2O_2 and membrane filtration step (>1 μm) and after the Amicon® stirred cell ultrafiltration concentration were analyzed. Prior to analysis, the concentrated water samples were sonicated for 15 min to minimize errors caused by sample concentration and agglomeration of particles. Briefly, 50 mL subsamples of the collected water samples were prepared for the FlowSync analysis. The measurements were conducted in triplicate for each water sample owing to the heterogeneity of potential plastic particles in environmental sample. Expanded details on the FlowSync instrumental conditions, and analytical methods used can be found in SI.

2.11. Quality assurance and quality control (QA/QC)

Throughout the entire process of nanoparticle sample collection, pretreatment, extraction, and analysis, strict adherence to quality assurance and control procedures was maintained with particular emphasis on minimizing and preventing the possibility of contamination from the surrounding environment [41,67]. The sample digestion and ultrafiltration concentration steps were conducted within a fume hood and precautions were taken to prevent or reduce the risk of air contamination. Samples were covered with DCM pre-cleaned aluminum foils whenever they were not actively being processed. To avoid potential contamination, all glassware and apparatus used in the study (i.e., glass pipettes, spatulas, centrifuge tubes, funnels, beakers, flasks, forceps, vials, etc.) were meticulously rinsed three times with DCM and MilliQ water before use. Surfaces and working areas were cleaned with ethanol (70%) prior to use. Personal protective equipment, including nitrile gloves and 100% cotton laboratory coats, were worn during sample preparation and analysis. Metal and glass materials were used during sample processing and laboratory procedures to minimize the risk of contamination. To assess potential contamination during sample processing and preparation, MilliQ water samples (1 L, $n = 3$) were placed in the fume hood as deposition samplers (they are referred to as Lab blanks 1, 2, and 3). These Lab blanks underwent the same pre-treatment, concentration, and analysis procedures as the real sample. Procedural blanks, consisting of MilliQ water (1 L, $n = 3$), were included in each batch of samples and subjected to the same processing steps as the real samples, including sample digestion, ultrafiltration concentration, freeze-drying, and transferring and loading into Pyr-GC/MS cups. These were analyzed alongside the real samples to monitor for any processing and extraction contamination. To prevent plastic contamination from the ultrafiltration membrane, all membranes were washed and soaked in MilliQ water before use. The Amicon® Stirred Cell and its assembling parts were washed three times with MilliQ water and dried upside-down in a fume hood on DCM pre-cleaned aluminum foils to avoid contamination before and between treatment of different samples. For the bottled water samples, the polymer content of the plastic containers and lids were also investigated. To achieve this, small slivers of each container and lid (~1 mg) were carefully collected using a pre-cleaned scalpel (duplicate subsamples). These subsamples were then placed in pyrolysis cups, along with a spike of 0.02 μg of d_5-PS internal standard and subjected to analysis using Pyr-GC/MS for polymer identification. The results of this analysis can be found in Table S7.

Before conducting the Pyr-GC/MS analysis, system cleans were carried out, which involved running no pyrolysis cups to ensure that no plastic contamination was present in the system. All pyrolysis cups used in the study were new and underwent washing with DCM before any sample was added to prevent any chances of contamination. Furthermore, instrumental blanks ($n = 5$), which consisted of blank runs with no pyrolysis cups, were included and analysed within each batch of samples, specifically injected after every 5 samples. These served the purpose of confirming the absence of secondary contamination, cross-contamination, carryovers, background response, or any potential instrumental contamination. In both procedural and laboratory blanks, the analyzed nanoplastics were either not detected or found to be below the method detection limits (LODs) (Tables S4, Fig. S3). This indicates that the pretreatment processes employed in this study did not introduce plastic contamination, as rigorous cleaning measures were implemented to prevent any such interference. Furthermore, to assess shifts in instrument performance and sensitivity over time, midpoint calibrations and internal standard checks (d_5-PS) were regularly injected (after every 10 samples) throughout the analytical run. These calibrations yielded acceptable recoveries, ensuring the reliability and accuracy of the results. The average internal standard recoveries in the analysed samples ranged from 83% to 92%, further confirming the robustness of the analytical methodology.

3. Results and discussion

3.1. Indicator compound selection

The specific pyrolysis products, including the selected quantifier and qualifier compounds/ions, used for the analysis of nanoplastics in this study are summarized in Table S2. For PMMA, methyl methacrylate (m/z 100) was chosen as the quantifier indicator compound due to its specificity and high sensitivity as a pyrolysis product. For PP, 2,4-dimethyl-1-heptene (m/z 126) was chosen as the quantifier indicator ion. Regarding PS, its pyrolysis produces three main products: styrene (monomer) (m/z 104), its dimer (3-butene-1,3-diyldibenzene, m/z 91 and 130), and its trimer (5-hexene1,3,5-triyltribenzene, m/z 91 and 312). Although styrene has the highest abundance and would be an ideal indicator for PS quantification in matrix-free samples, it may not be suitable for quantification in matrices with potential interference from environmental constituents or natural products such as albumin, chitin, fish protein and wood (lignin) which can release or generate styrene monomers during pyrolysis [12,37,38,49,65]. Instead, in this study the less intensive styrene dimer and styrene trimer were chosen as the quantification and qualification compounds, respectively because their generation is unambiguously linked to the presence of PS in natural matrices [12], making them more reliable indicators [11,38,49,65].

For PET, vinyl benzoate (m/z 105) was chosen as the quantifier indicator ion, while benzoic acid (m/z 122) was selected as qualification indicator ion. Bisphenol A (m/z 213) and isopropenylphenol (m/z 134) served as the quantification and qualification indicator ions, respectively for PC. ε-Caprolactam (m/z 113) was identified as a specific indicator ion for Nylon 6 and was therefore used for quantification. Its *N*-(5-cyanopentyl)− 6-hexanamide (m/z 114) and *N*-(5-cyanopentyl)hex-5-enamide (m/z 154) were chosen for qualification purposes. Regarding Nylon 66, cyclopentanone (m/z 84) was found to be the most abundant pyrolysis product and was selected as the primary quantification

indicator ion. However, as cyclopentanone can also be a pyrolysis product of other nylon polymers such as nylon 4,6, nylon 12,6, nylon MXD6, or their containing copolymers (e.g., nylon 6/66) [64], it was designated as a pyrolysis product representative of the total polymerized cyclopentanone derived from all cyclopentanone-containing nylon blends, composites or copolymers (abbreviated here as ∑Nylon 66). For qualification purposes, 1,8-diazacyclotetradecane-2,7-dione (*m/z* 112) was selected.

The pyrolytic analysis of PE results in the generation of various long-chain alkanes, alkenes, and alkadienes, which are monitored and quantified to determine PE concentrations in environmental samples. However, it should be noted that other substances or chemicals containing hydrocarbon chains, such as surfactants, natural fats (e.g., fish protein), lipids, oils, and waxes, can also break down into the same pyrolysis products, specifically the *n*-alkenes, producing significant interferences when this pyrolysis product is used for the sole quantification of PE in matrices with medium to high content of hydrocarbon chains containing chemicals, natural fats or biogenic materials (**see potential matrix interference for details**) [47,49,61,65,68]. Therefore, precautionary measures are essential for the identification and quantification of PE in *n*-alkenes containing environmental samples. The PE *n*-alkadienes are reported to be the most indicative pyrolysis products of PE, although the dienes are shown to have low sensitivity or signal intensity compared with the other *n*-alkanes and *n*-alkenes pyrolysates of PE [49,68]. Previous studies have indicated that *n*-alkadienes may not be significant or are negligible in samples with interferences from fatty acids/stearates, as dienes are typically produced from the thermal decomposition of long-chain parent compounds like PE [68]. In this study, to minimize matrix interference, we opted for the more indicative and less affected or interfered pyrolysis product, *n*-alkadienes from C_{18} to C_{21} [49] for PE quantification (**see potential matrix interference for details**). Additionally, their corresponding *n*-alkenes from C_{18} to C_{21} were assessed and monitored for qualification purposes, confirming the results obtained via *n*-alkadienes.

For PVC, although 1-metylnapthalane (*m/z* 142) and 2-metylnapthalane (*m/z* 142) were highly unspecific, they were chosen for quantification purposes due to the lack or absence of a more specific alternative compound. Naphthalene (*m/z* 128) was selected as the qualification indicator ion due to its high peak intensity and sensitivity, as compared to the other components or products which exhibited lower sensitivity levels. However, the analysis of biogenic polymers/organic materials (**see potential matrix interference for details**) showed that the quantification of PVC with the selected indicator compounds were not feasible for the water samples due to potential interference (although these were shown to be below their respective LOD's Table S3 and S4). In fact, the concentrations of PVC were way too high in the samples (**See** Table S5 **for PVC data**). Accordingly, PVC data is currently excluded from further discussions.

3.2. Removal of potential matrix interferences

The selected indicator and qualification compounds/ions for PMMA, PET, PP, PC, Nylon 6, Nylon 66, and PS were not affected or impacted by the pyrolysis products of the tested natural materials (all were below their respective LODs, Table S3 and S4). However, as anticipated, the pyrolysis products of PE and PVC were influenced by the natural materials. Previous studies have reported on the formation of various indicator compound interferences for PE during the pyrolysis of different natural products or biogenic material [11,47,49,61], such as fish protein (natural fats) and waxes (which are rich in long alkyl chains), and hydrocarbon chains containing chemicals, including surfactants, oils, and lipids leading to the production of *n*-alkanes and *n*-alkenes during pyrolysis [47,49,61,65]. Most of the materials tested (i.e., sunflower oil, triglyceride, engine oil, prawn, wood, fir needle, humic acid, and leaf) produced levels of interference for all the *n*-alkenes pyrolysis products monitored (typically above their respective LODs) from the untreated materials and after their digestion treatment with H_2O_2 and thermal desorption pyrolysis (Fig. S5). However, their generations accompanied by background interferences for the *n*-alkadienes pyrolysis products monitored were negligible after the digestion treatment with H_2O_2 and thermal desorption (typically below their respective LODs, Table S3 and S4**,** Fig. S5). Accordingly, the *n*-alkadienes from C_{18} to C_{21} were chosen for calibration and PE analysis.

Similarly, unspecific is the generation or formation of benzene and naphthalene (which are the most abundant pyrolysis indicator ions of PVC) from the untreated and treated fish filet, sunflower oil, triglyceride, engine oil, prawn, wood, fir needle, humic acid, and leaf under the given pyrolysis conditions. It has been previously reported that the complete removal of interference for PVC with benzene is difficult due to its abundant in most natural matter [11]. Being aware of the importance of caution for future PVC quantification in the water samples using either benzene or naphthalene, we carefully monitored and screened for other pyrolysis products of PVC with low signal intensity and sensitivity (such as chlorobenzene and 1-metylnapthalane/2-metylnapthalane). These products have been reported to exhibit minimal background interference, making them suitable candidates for PVC analysis. It is important to highlight that chlorobenzene was not detectable in the pyrolysis products of the PVC analytical standards analyzed and was consequently disregarded. The focus was then directed solely to 1-methylnaphthalene and 2-methylnaphthalene. After the digestion treatment with H_2O_2 and the thermal desorption step, all the tested materials (except for engine oil) exhibited minimal or negligible 1-methylnaphthalene and 2-methylnaphthalene signals, falling below their method detection limits (LODs), as shown in Table S3 and S4. This indicates that the precursors of these compounds were effectively removed during the digestion and thermal desorption process. However, due to the high lack of specificity in the concentrations obtained with 1-methylnaphthalene and 2-methylnaphthalene for PVC, we decided to exclude PVC from further discussions as there may be potential interferences (refer to Table S5 **for PVC data**). The specificity of the selected indicator compounds/ions is facilitated by the digestion treatment with H_2O_2 followed by thermal desorption to remove or reduce potential matrix interferences **(**Fig. S2**).**

3.3. Recoveries of spiked PS and PMMA nanoparticles

Nanoplastic particles of various size and composition are expected to occur in environmental and potable water samples [11,54], however analytical standards for many nanosized polymer types are not yet commercially available [10–12,18,20,36–38,54]. The available nano-sized PS (nominal sizes 30, 200 and 700 nm) and PMMA (nominal sizes 70, 110 and 740 nm) nanospheres were therefore used as near to representative as possible to represent all the target nanoplastic types and for evaluating their recoveries. The available standards were used to determine particle recoveries, which we expect to be a size and density dependent controlled concentration process. Even if polymer matched nano-sized reference materials were available they too may not be representative of the particles being extracted since there is a paucity of characterization data for environmental plastics < 10 μm with very little known about their shapes, size distribution, surface physico-chemical properties and potential agglomeration state (i.e., nanoparticles that have associated into a cluster composed of two or more nanoparticles). Quantifying nanoplastic contamination is the first step in developing a though understanding of the environmental occurrence of this contemporary contaminant. Once environmental occurrence and distribution has been ascertained, further characterization should address the above listed factors [2].

Reproducible and quantitative recoveries were obtained for each nanoplastic standard (i.e., PS and PMMA) at various spiking concentrations between 22 and 41 μg/L (Table 1 and S6). The extraction efficiencies of spiked nanoplastics ranged from 58.3 ± 2.3–68.1 ± 2.3% in MilliQ water and from 54.6 ± 2.9–64.2 ± 3.1% in wastewater samples.

Although the recovery rates for the spiked nanoplastic particles were < 70% in the present study, it was deemed acceptable and similar to previously reported rates [11,54]. For example, studies combining large volume crossflow ultrafiltration nanoplastic extraction or pre-concentration method and H_2O_2 digestion, followed by Pyr-GC/MS, reported recovery rates of spiked PS nanoplastic (200 nm) to be 61.4 ± 13.5% [11] and 50.1–55.9% [54] in surface and wastewater samples, respectively. Our results are especially significant when there are no other validated or well-established quantitative methods for analyzing nanoplastics in environmental and potable samples [11,37,54,69]. This said, it's important to highlight that the initial sample pre-treatment procedures/processes, encompassing digestion, microfiltration, ultrafiltration (i.e., pre-concentration with the Amicon® stirred cell), freeze drying, and sample loading or transfer into Pyr-GC/MS cups, may have led to the unavoidable loss of spiked nanoplastic particles, which could potentially lead to an underestimation of their true concentration. This loss could primarily stem from particles physical adherence onto the surfaces and walls of containers, membranes, and pipettes used during sample transfers. Additionally, the nanoplastic particles that might have aggregated or adhered onto the surfaces of larger particles, or other nanoplastic particles, were more likely to be eliminated during the 1 μm membrane filtration step.

Results are based on the selected indicator compounds for the PS and PMMA as presented in Table S2. Polystyrene (PS), Poly(methyl methacrylate) (PMMA). n = number of analysed samples. Spiking concentrations between 22 and 41 μg/L (Table S6).

It should be noted that though natural aging processes and its impacts on the selected nanoplastic standards (i.e., PS and PMMA) were not explored in this study, previous studies have shown that extensive weathering (of plastic particles) can influence the relative signal of the indictor compound (i.e., either decrease or fluctuate with UV exposure period) used for plastic quantification by Pyr-GC/MS [70,71]. We therefore speculate that the quantitative analysis of nanoplastic particles in water samples that have been subjected to weathering under harsh conditions could be influenced, especially when virgin standards of the plastics were used for calibration. As stated above, the paucity of existing surface characterisation data for environmental plastics introduces a degree of uncertainty to our analyses through our limited understanding of the physcio-chemical status of the particles being analysed. This can only be resolved through improved understanding of how weathered environmental nanoplastics are and how this affects quantification by Pyr-GC/MS.

3.4. Method performance

Parameters including linearity of calibration range, relative standard deviation (RSD), and limit of detections (LOD) were evaluated to investigate the analytical performance of the proposed analytical workflow. The calibration curves of the nine target nanoplastic polymer types were positive and linear in the range of 0.08 – 33 μg/cup with acceptable determination coefficient values (R^2) ≥ 0.96 (Table S8). The relative standard deviations (RSDs) of the quantitative ion areas of each polymer standard with five replicates were used to evaluate the precision of the Py-GC/MS measurements. The RSDs of the nine target polymers were determined to be 5.1–13.8% for PVC, 6.5–12.5% for PMMA, 7.4–16.1% for PP, 8.2–15.2% for PS, 3.5–13.1% for PE, 4.4–13.9% for Nylon 6, 5.9–14.6% for Nylon 66, 6.9–12.6% for PC and 5.6–18.6% for PET, suggesting an acceptable reproducibility of the proposed method. The limit of detections (LODs), which were calculated using the concentrations measured in the laboratory and procedural blanks were 0.07 μg/L for PE, 0.04 μg/L for PP, 0.05 μg/L for PS, 0.04 μg/L for PET, 0.10 μg/L for PMMA, 0.44 μg/L for PC, 0.01 μg/L for Nylon 6, and 0.03 μg/L Nylon 66. The low LODs allow for the quantification of the mass concentrations of nanoplastic particles in the water samples by the proposed method. The LOD values reported in this study were similar to that reported in literature for nanoplastics in environmental waters, which was between 0.02 and 0.07 μg/L for PS and 0.03 and 0.10 μg/L for PMMA [20], 0.08 μg/L for PS and 0.03 μg/L for PMMA [12], but were lower when compared to the 0.6 and 1.1 μg/L, reported for PMMA and PS nanoplastics, respectively [37].

Table 1
Summary of method performance or recovery of selected nanoplastics (μg/L).

Polymer	Size (nm)	Recovery (in MilliQ water %), $n = 3$	Recovery (in wastewater %), $n = 3$
PS	700	60.1 ± 1.6	58.1 ± 2.7
PS	200	58.7 ± 5.1	57.9 ± 1.4
PS	30	58.3 ± 2.3	54.6 ± 2.9
PMMA	740	68.1 ± 2.3	64.2 ± 3.1
PMMA	110	65.4 ± 3.8	61.1 ± 4.3
PMMA	70	66.7 ± 8.1	57.8 ± 3.2

3.5. Particle size distributions of nanoplastic in environmental and potable water samples

The particle size distribution and counts for each water sample measured are presented in Table 2 with their d10 (i.e., the diameter of particles that 10% of the population have that size or smaller), d50 and d90 values. The particles measured in the samples were mostly between 100 and 930 nm in size with a total measured particle count of between 220 and 5260 particles/L, indicating that the nanoplastics in the water samples were dominated by particles or agglomerates of particles in this size range (Table 2, See Fig. S6 for an example of the images of particles obtained by FlowSync analysis). Using a dynamic light scattering (DLS) technique after sample concentration with cross-flow ultrafiltration and pretreatment with hydrogen peroxide, Xu et al., [11] reported the particles size distribution of nanopalstic particles (between 10 nm and 1 μm) in both surface water and groundwater to be mostly between 200 and 800 nm, which were similar to the distributions reported in the current study. It's important to note that the sample pre-treatment procedures and membrane filtration step (>1 μm) may have remove aggregated/agglomerated particles larger than 1 μm, categorizing them

Table 2
Particle size distribution (mean diameter, percentile distribution: d10, d50 and d90) of particles in the water samples.

Sample ID	Particle size (nm)				Particle number * **
	Mean Diameter	Percentile distribution* *			Per L
		d10	d50	d90	
Wastewater Influent A	600	200	500	900	5260
Wastewater Influent B	300	300	400	800	4760
Wastewater Influent C	400	300	400	900	3260
Wastewater Effluent A	400	200	500	600	1380
Wastewater Effluent B	400	200	300	500	1120
Wastewater Effluent C	300	100	300	500	920
Municipal Water A	200	100	200	300	440
Municipal Water B	200	100	300	400	380
Bottled Water A	300	200	400	600	360
Bottled Water B	200	100	200	300	220
Surface Water	600	200	600	700	2860
Reservoir Water	700	500	600	800	880
Stormwater	800	300	700	900	1320

Values are averages of n = 3 measurements. * * The d number (normally written as just d10, d50 or d90) is the diameter – d10 for example is the diameter of particles that 10% of the population have that size or smaller. * **The number of particles determined/counted for each of the 50 mL water subsample analyzed and converted to particles per L.

as microplastics. This exclusion may result in a minor underestimation of nanoplastic particles in the water samples, both in terms of mass concentration and number concentration.

3.6. Mass concentration of nanoplastics in environmental and potable water samples

The applicability of the hyphenated method was demonstrated by analyzing different environmental and potable water types collected from various sources in Australia (Fig. S4). Table 3 shows the mass concentrations of the various nanoplastics quantified in the samples. PE, PET, and PP were detected in the influent wastewater < 1 μm fraction at concentrations of between 0.20 and 1.17 μg/L, 0.65–0.91 and 0.51–0.79 μg/L, respectively, followed by PS (0.15–0.53 μg/L), PMMA (0.21–0.39 μg/L), Nylon 6 (0.09–0.12 μg/L), and Nylon 66 (0.05–0.08 μg/L). As expected, the nanoplastics concentrations in the effluent wastewater samples were lower than those in the influent samples, and several nanoplastics were below their quantification limits (LODs) (Table 3). PE (0.17–0.21 μg/L) and PET (<LOD–0.08 μg/L) remained the detectable nanoplastics in the effluent samples. The concentration of PP (<LOD–0.04 μg/L), PS (<LOD −0.06 g/L), PMMA (<LOD–0.14 μg/L), and Nylon 6 (<LOD–0.04 μg/L) were low compared to the influent samples. PE, PET, PS, PP, and Nylon 66 nanoplastics were detected in the stormwater and reservoir water samples with concentrations ranging from < LOD–0.76 μg/L, 0.18–0.19 μg/L, 0.32–0.51 μg/L, < LOD–0.59 μg/L, and < LOD–0.04 μg/L, respectively. Similarly, PE, PP, PS, PET, and Nylon 6 were quantified in the municipal water samples from the two sources analyzed at concentrations of 0.10–0.21, 0.12–0.24, 0.08–0.09, < LOD–0.25, and < LOD–0.07 μg/L, respectively. PET, PP, PS and PE were the only nanoplastics detected in the bottled water samples. The concentrations were in the range of 0.10–0.26 μg/L, < LOD–0.12 μg/L, < LOD–0.08 μg/L and < LOD–0.11 μg/L, respectively. This is not surprising since the plastic bottles were made of PET and the caps were made of PE/PP (Table S7). The findings indicate that the packaging itself may be releasing nanoparticles into the water [46]. For the surface water sample, PE (0.66 μg/L), PS (0.39 μg/L) Nylon 6 (0.27 μg/L), and PET (0.25 μg/L) were quantified. The results obtained in this study demonstrate the utility of the proposed approach for the quantitative trace analysis of nanoplastics in complex or natural environmental and potable water samples. Studies have shown that wastewater, surface water, tap water, stormwater, reservoir water and bottle water samples are polluted with microplastics (>1 μm), which mainly consisted of PE, PP, PET, PS, and PMMA [4,46,54,57,72–77] and the plastic profiles of the nanoplastics detected in the present study agrees with that of the microplastics data.

WWTPs play a crucial role as central hubs for the accumulation of plastic particles originating from industries, households, and trade, thus serving as a secondary source of environmental plastic pollution. The limitations of conventional treatment methods have become evident as they prove ineffective in adequately removing the substantial loads of plastic particles entering WWTPs through the influent [4,7,78,79], with more than 90% of the plastic particles retained in the sewage sludge that is produced during treatment (treated as biosolids) [4,62,80]. Despite the partitioning of plastics into biosolids, substantial quantities of plastic particles that enter WWTPs have the potential to be discharged into the environment through wastewater effluent [57]. Of particular concern is the continuous release of persistent plastic particles, occurring through the continual release of treated effluent into water bodies, as well as the use of biosolids for agricultural purposes [1,4,13,62,80,81]. This continuous release poses a risk of contaminating surface and groundwaters, which are commonly utilized as sources of potable water, thus potentially exposing humans to these plastic particles. The analyzed nanoplastic types are commonly used in various applications, including catering products (e.g., plates, lids, disposable cutlery, potable straws and cups), packaging materials for liquid foods, cosmetics, waste bags and packaging, agricultural and horticultural products (e.g., mulch films), clothing, textiles, toys, films, consumer electronics, and more [82,83]. A considerable number of these plastic products have relatively short service lives and could have entered the studied WWTPs through usage, degradation, and abrasion [7]. As a result, there is a possibility that nanoplastics are being released into water bodies through effluent discharges from the WWTPs [4,57].

Although several studies have attempted to detect nanoplastics in water samples, there are only a few that report mass-based concentrations [11,12,18,37,38,54]. Due to the limited data available in the literature, direct comparison of the quantitative results from this study with plastic particle counts reported in other studies is not feasible. Table 4 provides a direct comparison of studies quantifying nanoplastics in environmental and potable water samples with the results obtained from this study. As can be seen from Table 4 the concentrations reported

Table 3
Concentrations of nanoplastics in several environmental and potable water samples (μg/L).

Samples	PE	PP	PS	PET	PMMA	PC	Nylon 6	Nylon 66	Sum plastics
Wastewater Influent A	1.17	0.51	0.15	0.91	0.27	< LOD	0.10	0.06	3.2
Wastewater Influent B	0.76	0.71	0.46	0.81	0.21	< LOD	0.09	0.05	3.1
Wastewater Influent C	0.20	0.79	0.53	0.65	0.39	< LOD	0.12	0.08	2.8
Wastewater Effluent A	0.20	0.04	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.24
Wastewater Effluent B	0.21	< LOD	< LOD	0.08	< LOD	< LOD	< LOD	< LOD	0.53
Wastewater Effluent C	0.17	< LOD	0.06	0.06	0.14	< LOD	0.04	< LOD	0.47
Municipal Water A	0.10	0.12	0.08	0.25	< LOD	< LOD	< LOD	< LOD	0.55
Municipal Water B	0.21	0.24	0.09	< LOD	< LOD	< LOD	0.07	< LOD	0.61
Bottled Water A	< LOD	0.12	0.08	0.26	< LOD	< LOD	< LOD	< LOD	0.46
Bottled Water B	0.11	< LOD	< LOD	0.10	< LOD	< LOD	< LOD	< LOD	0.21
Surface Water	0.66	< LOD	0.39	0.25	< LOD	< LOD	0.27	< LOD	1.6
Reservoir Water	0.76	< LOD	0.51	0.18	< LOD	< LOD	< LOD	0.04	1.5
Stormwater	< LOD	0.59	0.32	0.19	< LOD	< LOD	< LOD	< LOD	1.1
Lab Blank 1 (MilliQ water)	< 0.07	< 0.04	< 0.05	< 0.04	< 0.10	< 0.44	< 0.01	< 0.03	
Lab Blank 2 (MilliQ water)	< 0.07	< 0.04	< 0.05	< 0.04	< 0.10	< 0.44	< 0.01	< 0.03	
Lab Blank 3 (MilliQ water)	< 0.07	< 0.04	< 0.05	< 0.04	< 0.10	< 0.44	< 0.01	< 0.03	
Field Blank 1	< 0.07	< 0.04	< 0.05	< 0.04	< 0.10	< 0.44	< 0.01	< 0.03	
Field Blank 2	< 0.07	< 0.04	< 0.05	< 0.04	< 0.10	< 0.44	< 0.01	< 0.03	
Procedural blank 1 (MilliQ water)	< 0.07	< 0.04	< 0.05	< 0.04	< 0.10	< 0.44	< 0.01	< 0.03	
Procedural blank 2 (MilliQ water)	< 0.07	< 0.04	< 0.05	< 0.04	< 0.10	< 0.44	< 0.01	< 0.03	
Procedural blank 3 (MilliQ water)	< 0.07	< 0.04	< 0.05	< 0.04	< 0.10	< 0.44	< 0.01	< 0.03	
LOD	*0.07 **	*< 0.04 **	*0.05 **	*0.04 **	*0.10 **	*0.44 **	*0.01 **	*0.03 **	

< LOD: below method detection limits in a sample, Compound specific LODs are listed as "<LOD", Polystyrene (PS), Polyethylene (PE), Polyvinyl chloride (PVC), Polypropylene (PP), Polycarbonate (PC), Poly(methyl methacrylate) (PMMA) and Polyethylene terephthalate (PET). '* ' Indicates where an analyte was not detected in the blanks, the LOD is reported as the calculated LOQ.

in the previous studies were in line or align with the findings in the current study. Although not directly comparable, a study using thermal desorption–proton transfer reaction–mass spectrometry (TD-PTR-MS) identified and (semi)quantified nanoplastics types in snow pit and surface snow samples, with only PET nanoplastics detected at concentrations ranging from 5.4 to 27.4 μg/L [45]. These concentrations were however much higher than those found in any of the water samples in this study. Differences in analytical techniques, sampling methods, sample pre-concentration/treatment, and nanoplastics sources in the various environmental samples may account for these variations.

4. Conclusions

Extensive attention and discussion have been focused on the environmental fate and human exposure risks associated with nanoplastics [9,15–18,20,42,52,54]. However, the occurrence and concentration of nanoplastics in water samples have remained largely uncharacterized. This study aimed to address this gap by developing a practical analytical workflow capable of pre-concentrating, identifying, and quantifying levels of nanoplastics in complex environmental and potable water. Of the nine targeted nanoplastics (PE, PP, PET, PS, PMMA, PC, PVC, Nylon 6, and 66), eight were successfully identified and quantified, with PE, PET, PP, and PS being the most prevalent. The findings of this study provide important reference data for assessing the actual pollution levels of these target nanoplastics in environmental and potable water samples and demonstrate the feasibility of the proposed method. Previous research exploring the transport, fate, and effects of nanoplastics in environmental media suggests that these particles can have detrimental impacts on organisms that encounter them [84–89]. However, it should be noted that the concentrations of nanoplastics detected in the water samples analyzed in this study were lower than those typically associated with adverse effects, as they were found at concentrations below the high mg/L range used [84–89]. The future implementation of the proposed analytical workflow in ecotoxicological tests will enhance the accuracy and reliability of confirming mass-based exposure concentrations. We anticipate that this analytical approach is not confined solely to identifying and quantifying the targeted nanoparticles in environmental and potable water samples but can be expanded to encompass a wide range of commercially relevant polymer types and other environmental systems, such as sediments, soils, biosolids, air, among others. Utilizing the presented analytical workflow to quantify nanoplastics concentrations in diverse environmental systems will contribute to a better understanding of the impacts of nanoplastics in receiving environments.

While this study has successfully identified and quantified trace nanoplastics in water samples, it is important to acknowledge that there is still room for improvement. Specifically, it should be noted that the prevalent nanoparticles observed in this study were PE, PP, and PET, whereas PS and PMMA only constituted a small fraction. It is worth mentioning that although PS and PMMA analytical nanospheres are widely used as representatives of nanoplastics for studying their environmental behaviours and effects [19], the findings of this study indicate the prevalence of other nanoplastics types. Also, the recovery rates of nanoplastics of the proposed method were only successfully evaluated by PS and PMMA nanoplastics but may vary for different nanoplastics types. For the accuracy of the analytics to be improved there is an urgent need for analytical reference standards that reflect what is present in the environment. These standards need to reproduce the physiochemically dynamic complex mixture of plastic particles of different plastics, sizes, shapes, and surface properties– much of which remains largely uncharacterised [2]. Likewise, employing deuterated polymer analytical standards for identifying specific products of polymer degradation is strongly recommended, as it enhances confidence in polymer identification and subsequent quantification [24]. Consequently, further validation of the proposed method using other types of nanoplastics when they become commercially available will be vital and is recommended.

The combination of H_2O_2 pre-treatment and Amicon® Stirred Cell pre-concentration in the analysis of nanoparticles may result in sample loss, leading to a potential underestimation of their true concentration. However, considering the high concentration ratio achieved and the absence of alternative mature quantitative methods, this approach remains acceptable for the quantification of trace nanoplastics in water samples. Nevertheless, there is still a need for further improvements to enhance the extraction and treatment efficiency and minimize sample loss. In this study, we have proposed a practical method for detecting trace nanoplastics in water samples. However, it is important to expand our understanding of nanoplastics contamination levels in other complex environmental systems, such as sediments, air, and soils. The H_2O_2 pre-treatment and pre-concentration workflow presented in this study offers several advantages, including ease of operation, suitable sensitivity, efficient treatment, and minimal interference from real-sample matrices. It provides a promising alternative for investigating and monitoring the accumulation and distribution of trace nanoplastics in environmental systems. This research represents a crucial step towards assessing the potential environmental risks associated with nanoplastics [12,37].

Environmental implication

The environmental fate and potential human exposure risks associated with nanoplastics have garnered significant attention, but the occurrence and concentrations of nanoplastics in environmental and potable water samples remain largely understudied due to analytical complexities. In this study, we developed an analytical workflow capable of pre-concentrating, identifying, and quantifying levels of nanoplastics present in complex environmental and potable waters. Among the nine targeted nanoplastics (PE, PP, PET, PS, PMMA, PC, Nylon 6, PVC, and Nylon 66), eight were successfully identified and quantified, with PE, PET, PP, and PS being the most prevalent. This research provided valuable insights into the actual pollution levels of these targeted nanoplastics in environmental and potable water samples, offering crucial reference data for future studies, and demonstrating the effectiveness of the proposed analytical method.

Table 4
Concentrations of nanoplastics from the current study compared with previous studies (μg/L).

Sample type	Country	PE	PP	PS	PET	PMMA	Nylon 6	Reference
Surface and ground waters	China	<LOQ–0.242	0.014–0.389	<LOQ–0.058	<LOQ–0.079	<LOQ–0.046	-	[11]
Wastewater	China	<LOQ–1.752	0.084–1.463	<LOQ–0.038	0.008–0.533	<LOQ–0.248	<LOQ –0.049	[54]
River water and influent	China	-	-	1.92–2.82	-	nd	-	[12]
Potable, tap, lake, river, and seawater water samples	China	-	-	< 0.07–0.73	-	nd	-	[20]
Snow pit and surface snow samples	-	-	-	-	5.4–27.4	-	-	[45]
Environmental and potable waters (present study)	Australia	<LOD–1.17	<LOD–0.79	<LOD–0.53	<LOD–0.91	<LOD–0.39	<LOD–0.27	

nd: not detected in a sample, -: not analyzed in a sample, Poly(methyl methacrylate) (PMMA), Polypropylene (PP), Polyethylene (PE), Polyethylene terephthalate (PET) and Polystyrene (PS). LOD: below method detection limits in a sample, LOQ: below method quantification limits in a sample

CRediT authorship contribution statement

Elvis Dartey Okoffo: Conceptualisation, Methodology, Investigation, Validation, Visualization, Formal analysis, Resources, Writing, Project administration. **Kevin V. Thomas**: Conceptualisation, Methodology, Resources, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Supporting information

Additional information on instrumental parameters of the Pyr-GC/MS; and method validation can be found in the supplementary document.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.133013.

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