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## How suitable is the gold-labelling method for the quantification of nanoplastics in natural water?

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### ABSTRACT

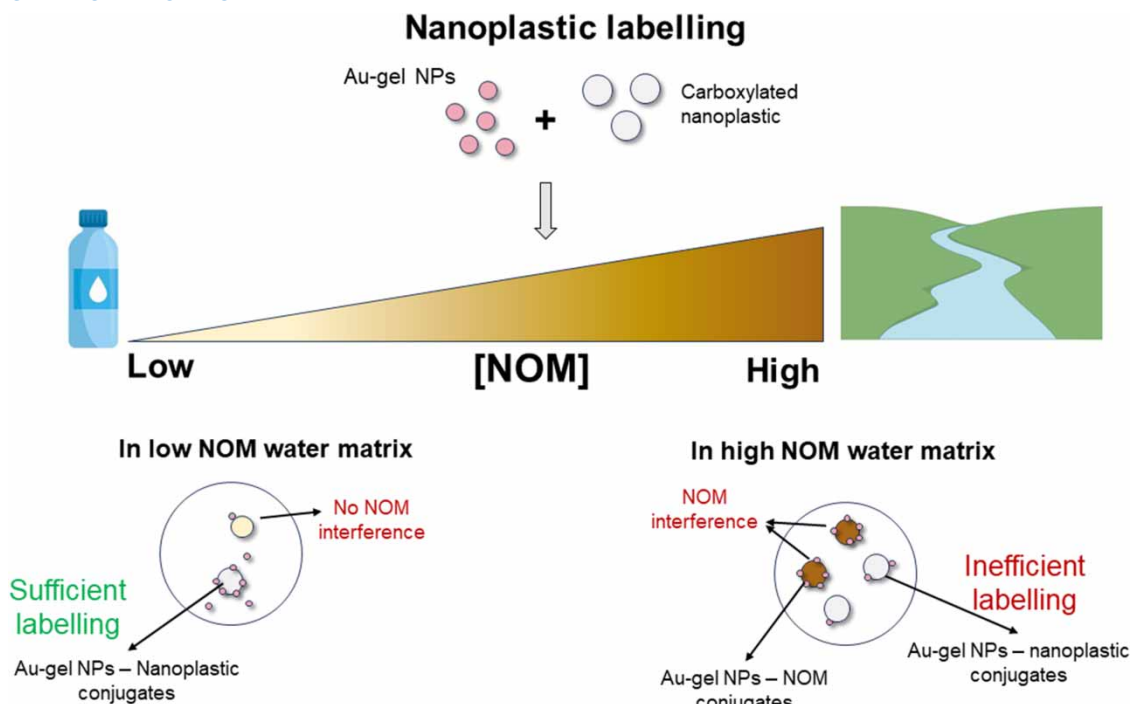
Nanoplastics are detected in surface water, yet accurately quantifying their particle number concentrations remains a significant challenge. In this study, we tested the applicability of a gold-labelling method to quantify nanoplastics in natural organic matter (NOM) containing water matrices. Gelatin-coated gold nanoparticles (Au-gel NPs) form conjugates with nanoplastics via electrostatic interaction which produces peak signals which can be translated into particle number concentration using single-particle inductively coupled plasma–mass spectrometry (SP-ICP-MS). We used water samples with various NOM concentrations, with and without the addition of  $1 \times 10^7$  particle<sup>-1</sup> nanoplastics. Our results indicate that nanoplastics in low NOM samples ( $<1$  mg-C L<sup>-1</sup>) could be successfully quantified. However, in high NOM samples ( $>15$  mg-C L<sup>-1</sup>), only 13–19% of added nanoplastics were successfully quantified. Further digestion to remove NOM yielded only 10% of spiked nanoplastics. This discrepancy in high NOM samples could likely be attributed to the competition between nanoplastics and NOM existing in the water sample to bind with Au-gel NPs. Our study highlights the suitability of the Au-gel labelling method for quantifying nanoplastics in low NOM water samples. Nevertheless, further optimization, including pre-digestion steps, is essential to apply this method for high NOM water samples effectively.

**Key words:** ICP-MS, metal labelling, nanoplastics quantification, NOM interference

### HIGHLIGHTS

- Quantification of nanoplastic with gold-labelling using single-particle ICP-MS.
- Application of nanoplastic labelling in natural waters.
- Underestimation of nanoplastic due to competing negatively charged NOM.
- Inefficient digestion to remove NOM.

## GRAPHICAL ABSTRACT



## INTRODUCTION

It is predicted that around 11% (~ 19–23 Mt) of the total global plastic waste emission has entered aquatic ecosystems (Borrelle *et al.* 2020) and this number will potentially increase up to 710 Mt by the year 2040 (Lau *et al.* 2020). As plastics are hard to degrade, they will eventually accumulate in the environment (da Costa 2018; Mitrano *et al.* 2021) where it undergoes chemical, physical, or biological weathering and degradation (Lehner *et al.* 2019). Such processes result in smaller plastic particles, including nanoplastics (Song *et al.* 2020; Sorasan *et al.* 2021). Most literature agreed to classify nanoplastics as plastics between 1 nm and 1  $\mu\text{m}$  (da Costa *et al.* 2016; Gigault *et al.* 2018; Hartmann *et al.* 2019; Jakubowicz *et al.* 2021; Valsesia *et al.* 2021). Since nanoplastics can be generated from microplastics degradation, the number of particles might significantly increase upon breaking down to nanoscale. Nanoplastics pose a more significant threat to various aspects of ecosystems due to their higher specific surface area, higher particle number concentration, and enhanced reactivity compared to microplastics (Mattsson *et al.* 2018; Tallec *et al.* 2019). Detecting nanoplastics in water is thus a crucial tool for establishing baseline data as well as guiding policy decisions to combat nano and/or microplastic pollution. Recently, nanoplastics have been detected in multiple environmental matrices including surface water (Materić *et al.* 2022c; Xu *et al.* 2022), wastewater (Xu *et al.* 2023), soil (Wahl *et al.* 2021), and wastewater treatment sludge (Ho 2022). To date, methods to quantitatively indicate their size and particle number concentration are still developing (Cai *et al.* 2021). These methods include microscopic and light scattering techniques such as transmission electron microscopy (TEM) (Chen *et al.* 2017), scanning electron microscopy (SEM), dynamic light scattering (DLS) (Hernandez *et al.* 2017), and nanoparticle tracking analysis (NTA) (Hernandez *et al.* 2019). The disadvantage of all these techniques, however, is that the material composition of the particle has to be determined with additional methods. Techniques based on mass spectrometry have also been developed in recent years and allow us to detect the plastic type and mass. For instance, pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) was sensitive and reliable for identifying nanoplastics with additional preconcentration before analysis (Halle *et al.* 2017; Zhou *et al.* 2019; Xu *et al.* 2023). Nonetheless, the method requires laborious sample preparation (Nguyen *et al.* 2019). A promising quantification method to differentiate nanoplastics types was successfully tested by thermal desorption-proton transfer reaction-mass spectrometry (TD-PTR-MS) in different environmental samples such as high-altitude snow, surface waters, and seawater without preconcentration, digestion, or separation (Materić *et al.* 2020, 2022a, 2022b, 2022c). With

TD-PTR-MS, plastic type and (low) mass could be accurately determined, but the device could not provide information on particle number concentration. Lastly, a total organic carbon (TOC)-based method was developed to estimate TOC originating from microplastics in sewage (Hong *et al.* 2021; Li *et al.* 2022). However, this procedure cannot characterize or quantify individual microplastics. Recently, inductively coupled plasma-mass spectrometry was used to detect microplastics directly by measuring the signal intensity of the carbon-13 isotope ( $^{13}\text{C}^+$ ) (Bolea-Fernandez *et al.* 2020; Laborda *et al.* 2021). However, background noise from other C-species present in the samples can interfere with the measurement. Another method was developed to aid this issue by indirect measurement of nanoplastics using metallic tagging. The particle number concentration of nanoplastics was measured by binding nanoplastics to gold nanoparticles coated with gelatin (Au-gel NPs), which was then analysed using inductively coupled plasma-mass spectrometry in single-particle mode (SP-ICP-MS) (Jiménez-Lamana *et al.* 2020). Due to surface oxidation, nanoplastics are expected to have different chemical groups on their surface, such as carboxyl, hydroxyl, and carbonyl (Liu *et al.* 2019). In this gold-labelling method, carboxylated nanoplastics were used as a nanoplastics model to mimic the oxidized surface of environmental nanoplastics. The method relies on the electrostatic interaction of positively charged Au-gel NPs and negatively charged carboxyl groups on the surface of the nanoplastics. The binding is dictated by the number of carboxyl groups on the surface of nanoplastics (Marigliano *et al.* 2021). Using 17 nm AuNPs and model polystyrene (PS) with carboxyl groups at the surface, the method successfully detected and determined nanoplastics particle number concentration for a range of spherical model nanoplastics ( $<1\ \mu\text{m}$ ).

With ICP-MS widely available in most analytical laboratories, the gold-gelatin labelling method offers a promising alternative for streamlining nanoplastics monitoring routines in surface water samples, reducing laborious extraction procedures. However, besides environmental nanoplastics, surface water also contains natural organic matter (NOM) (Oriekhova & Stoll 2018). NOM concentration in surface water, especially in the Netherlands, ranges from 2 to  $>10\ \text{mgC L}^{-1}$  (Beckett & Ranville 2006). Meanwhile, groundwater has a relatively lower NOM concentration at around  $0.1\text{--}4\ \text{mgC L}^{-1}$  (Regan *et al.* 2017). Similar to environmental nanoplastics, NOM also has various functional groups on its surface including carboxyl groups (Adusei-Gyamfi *et al.* 2019). NOM might therefore also bind with gold-gelatin particles when the gold-labelling method is applied to water with high NOM concentration, thereby obscuring binding sites for nanoplastics. It is, therefore, necessary to investigate the method's sensitivity in the presence of NOM. This study aimed to fill that gap and to apply the nanoplastics labelling method to real environmental samples with different NOM concentrations and explore the possibilities and/or limitations of this method for routine measurement of nanoplastics. Tap and bottled water were chosen to represent low NOM concentration, while surface water samples obtained from an urban environment in the western part of the Netherlands were used as high NOM concentration samples.

## MATERIALS AND METHODS

### Reagents and chemicals

Carboxyl-functionalized polystyrene (PS) nanoplastics and gold nanoparticles coated with gelatin (Au-gel NPs) used for this experiment were synthesized as described earlier (Jiménez-Lamana *et al.* 2020). These nanoparticles were prepared by the nanoparticles Lab of NTNU Norway. The particle number concentrations of nanoplastics and Au-gel NPs, obtained with ZetaView (Particle Metrix), were  $3.9 \times 10^{15}$  particles  $\text{L}^{-1}$  and  $3.0 \times 10^{13}$  particles  $\text{L}^{-1}$ , respectively. The hydrodynamic size of nanoplastics in suspension was measured at 390 nm with a poly dispersivity index (PDI) of 0.06 and Au-gel NPs particles at 56 nm with a PDI of 0.52. Both values were measured with ZetaSizer (Malvern). Furthermore, 50 and 80 nm gold nanoparticles (BBI Solution Crumlin, UK) were used to determine the transport efficiency of SP-ICP-MS. Ultrapure water, obtained with a MilliQ system, was used to dilute all chemicals.

### Instrument

A Thermofisher X Series II ICP-MS operated in single-particle mode with gold as the targeted analyte was used throughout the experiment. The dwell time was set at 5 ms and the total acquisition time was 60 s. Thus, a total of 12,000 data signals were obtained per replicate. The transport efficiency ( $\eta$ ) in this study was determined using the particle frequency method (Pace *et al.* 2011) after separating the background noise and particle signals using the criteria of three standard deviations ( $3\sigma$ ) above the mean signal as the baseline. Details about  $\eta$  calculation and their potential error are summarized in the Supplementary Information.

## Water samples

We used three types of water samples: commercially bottled water from a plastic bottle, tap water of Delft, and environmental water from the Schie canal near Delft, the Netherlands (52.019607, 4.352881). There to, 500 mL water was collected from five sampling locations along the canal, approximately 1 km between each sampling point. All samples were collected using an amber glass reagent bottle, previously washed with hydrochloric acid (1:5, HCl: water ratio), and rinsed thoroughly with demineralized water. The DOC of all water samples was measured using a Shimadzu TOC-L Series after filtering the samples (Whatman Spartan 30/0.45  $\mu\text{m}$  RC).

## Determination of carboxyl groups

The carboxyl group density at nanoplastics surface was determined using a potentiometric titration method with a Titrino 848, according to [Pessoni \*et al.\* \(2019\)](#). 1 mL of nanoplastics stock solution was diluted into 100 mL of ultrapure water, and 60 mL was used for the experiment. The solution was titrated with 50  $\mu\text{L}$  0.01 M NaOH every 5 min under a constant nitrogen gas supply. Throughout the titration, the pH and conductivity of the solution were measured. The measurement was done until the pH of the sample reached 12.0.

## Determination of Au-gel NPs and nanoplastics conjugates particle concentration

Nanoplastics were added to ultrapure water in a concentration range from  $7 \times 10^6$  to  $9 \times 10^7$  particles  $\text{L}^{-1}$ . This range was selected according to the recommended range of particle detection with SP-ICP-MS to ensure the number of peaks in the time scan will not exceed 10% of the maximum number of peaks based on the dwell time, which is between  $1 \times 10^6$  and  $1 \times 10^8$  particles  $\text{L}^{-1}$  ([Pace \*et al.\* 2011](#)). Au-gel NPs were added to nanoplastics suspension at the recommended number ratio of 500:1 for labelling translating into sufficiently high ICP-MS signals ([Jiménez-Lamana \*et al.\* 2020](#)). All suspensions were immediately analysed on the SP-ICP-MS. From the tested nanoplastics concentration range, the ideal nanoplastics-Au-gel NP conjugate concentration was selected for all subsequent tests in other water matrices.

Au-gel NPs and the PS nanoplastics stock were diluted to 50 mL of water samples (bottled, tap, and canal) to obtain the desired particle number concentration based on the previous test. To determine the presence of nanoparticles with a negative charge (e.g. due to existing nanoplastics or NOM), Au-gel NPs with the same concentration were added to the raw sample without adding nanoplastics.

Oxidation nanoplastics in the environment have O-containing functional groups on their surface, such as carboxyl groups (COOH) ([Blanco \*et al.\* 2021a](#)). The determination of nanoplastics particle number concentration in this study relies on the electrostatic binding between the carboxyl groups present at the surface of nanoplastics and positively charged Au-gel NPs ([Jiménez-Lamana \*et al.\* 2020](#); [Marigliano \*et al.\* 2021](#)). Therefore, when a sample spiked with Au-gel NPs is analysed with SP-ICP-MS, signals are correlated to two different Au sources, namely signals from ‘unbound’ Au-gel NPs and signals from conjugates between nanoplastics and Au-gel NPs. All signals which exceeded the baseline of  $3\sigma$  above the mean signal were counted as nanoplastics – Au-gel NPs conjugates. Eventually, this concentration is measured as a proxy for nanoplastics concentration. The calculation of particle number concentrations from nanoplastics-Au-gel NPs conjugates is summarized in the Supplementary Information.

## Pre-digestion for high NOM water sample

To reduce interference from other negatively charged nanoparticles such as NOM, some of the canal water samples were subjected to Fenton digestion proposed by [Cunsolo \*et al.\* \(2021\)](#). Briefly, 100 mL of 0.05 M  $\text{FeSO}_4$  followed by 100 mL of  $\text{H}_2\text{O}_2$  were added to 50 mL of sample in a 500-mL glass beaker. An ice bath was used to regulate the temperature below 50 °C to preserve environmental nanoplastics possibly present in the sample. Then, 2 M NaOH was added drop-by-drop to maintain the pH of the solution around 3.0–4.0 as the optimum pH condition and to avoid the reaction of soluble iron species with  $\text{H}_2\text{O}_2$ . The digestion process was carried out until no more bubbles were observed. After digestion, the pH of the solution was adjusted to around 7.0 which is the natural pH of the water sample by adding 2 M NaOH drop-by-drop. Also, in this pH range, nanoplastics and Au-gel NPs conjugate were stable according to the previous study ([Jiménez-Lamana \*et al.\* 2020](#)). Au-gel NPs and nanoplastics were then added to the sample. Results were compared between non-digested and digested canal water samples with and without added nanoplastics.

## RESULTS AND DISCUSSION

### Determination of nanoplastics in ultrapure water

In ultrapure water, the Au-gel labelling method performed well in the range of  $7 \times 10^6$  up to  $1 \times 10^7$  nanoplastics particles  $L^{-1}$ . Within this concentration range, the measured concentrations of nanoplastics – Au-gel NPs conjugates (experimental concentrations) were comparable to the added concentration of nanoplastics (theoretical concentration). However, at higher added nanoplastics concentrations, the method underestimated the concentration (Figure S1). The observed trend might be related to the selection of dwell time intervals used in this study. With a dwell time of 5 ms and a total acquisition time of 60 s, the optimum particle number concentration is in the order of  $10^7$  particles  $L^{-1}$  (Abad-Álvarez *et al.* 2016).

The number of Au-gel NPs per nanoplastics can be calculated from the measured data by dividing the mass of Au-gel NP and nanoplastics conjugates measured via signal peaks in SP-ICP-MS by the mass of a single Au-gel NP (Supplementary Information). With a nanoplastics concentration of  $1 \times 10^7$  particles  $L^{-1}$ , our study obtained 230 Au-gel NPs attached per nanoplastics. This number is almost half of the measured number in the previous study, although similar concentrations were used (Jiménez-Lamana *et al.* 2020). We argue that differences between the two studies are resulting from the lower amount of COOH groups on the surface of our nanoplastics (27 COOH groups per  $nm^2$ ) compared to the previous study (41 COOH groups per  $nm^2$ ). Given that the interaction between carboxyl-modified nanoplastics with positively charged metallic nanoparticles is determined by the amount of COOH groups on the surface of nanoplastics, a lower COOH group availability leads to measuring lower Au-gel NPs attached per nanoplastics. Indeed, a study by Marigliano *et al.* (2021) also observed that the gold-labelling method was dependent on the surface functionalization of nanoplastics. This result indicated that nanoplastics labelling with Au-gel NPs will also depend on the degree of aging of environmental nanoplastics which may determine the carboxyl group density on the surface (Zhang *et al.* 2022).

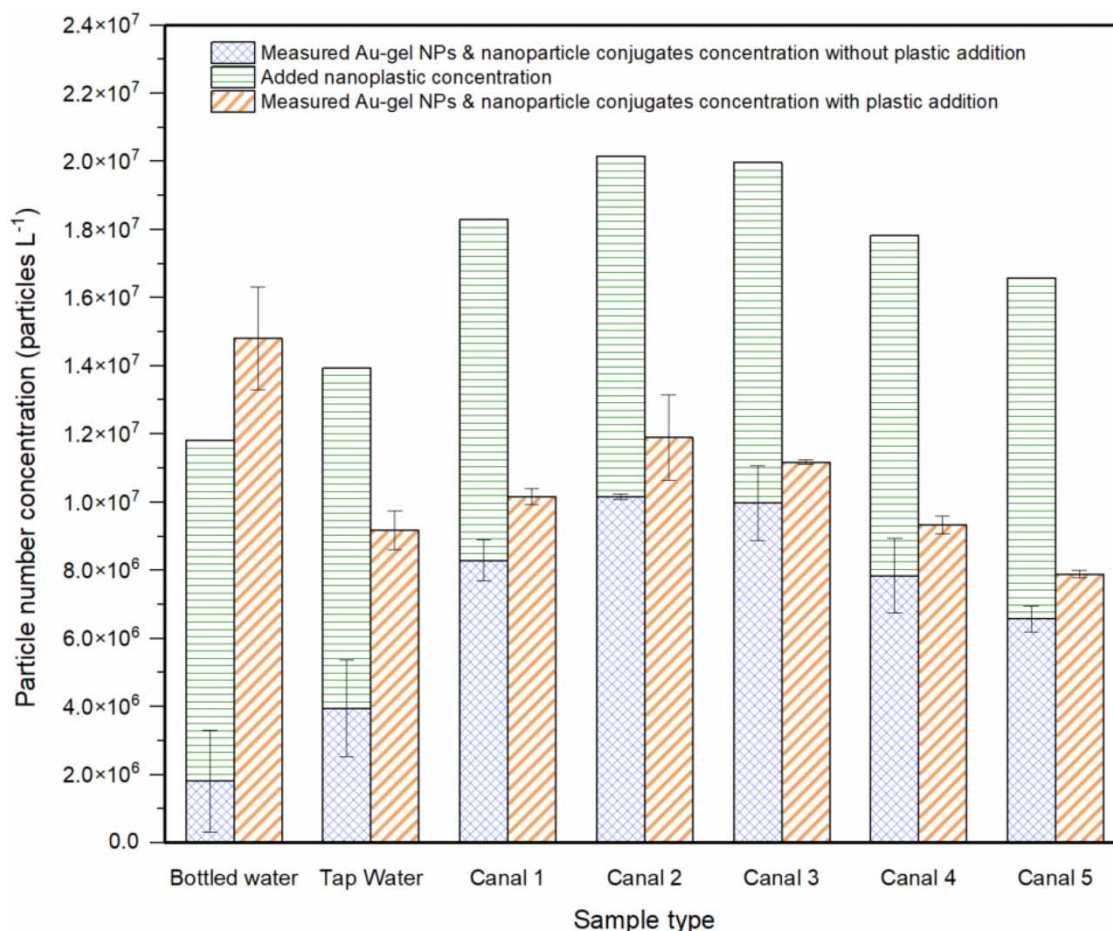
### Determination of nanoplastics in water with various NOM concentrations

Based on the concentration range test in ultrapure water (Figure S1), a nanoplastics concentration of  $1 \times 10^7$  particles  $L^{-1}$  was chosen and added to samples with varying NOM concentrations. DOC concentrations in low NOM samples such as bottled water and tap water were  $0.32 \pm 0.06$  and  $1.76 \pm 0.03$  mg-C  $L^{-1}$ , respectively. DOC concentrations in samples with higher NOM content from the Schie canal in Delft ranged between  $15.47 \pm 0.00$  and  $19.64 \pm 0.14$  mg-C  $L^{-1}$  (Figure S2). Particles were detected in all samples even without the addition of nanoplastics, which indicated the presence of negatively charged nano-sized material that binds with Au-gel NPs (Figure 1). Since the method we tested cannot identify the chemical characteristic of the measured particle (i.e. the polymer type) without a combination of other spectroscopic techniques such as FT-IR, Py-GC-MS, or TD-PTR-MS, we identified these particles as Au-gel NPs conjugates throughout the text.

The measured number concentrations of Au-gel NPs conjugates (blue bars in Figure 1) were proportional to DOC concentrations. For instance, low particle number concentrations were measured in low NOM, bottled water, and tap water samples, with  $1.8 \pm 1.5 \times 10^6$  and  $3.9 \pm 1.4 \times 10^6$  particles  $L^{-1}$ , respectively.

Particles detected in bottled water are unlikely to be related to the presence of NOM in the sample. Another potential source might have been the plastic of the bottle itself. Bigger microplastics have been previously detected in bottled water with particle number concentrations between 14 and  $5.42 \times 10^7$  particles  $L^{-1}$  (Ormann *et al.* 2018; Schymanski *et al.* 2018; Zuccarello *et al.* 2019). However, these results should be carefully interpreted due to the sensitivity and size detection of the different analytical techniques used. Nonetheless, particles detected in the bottled water sample in this study should not be neglected, and a dedicated study for chemical identification and quantification in bottled water is required.

Meanwhile, higher particle number concentrations of Au-gel NPs conjugates ( $6.5 \pm 0.4 \times 10^6$  particles  $L^{-1}$  up to  $1.0 \pm 0.1 \times 10^7$  particles  $L^{-1}$ ) were measured in canal water samples (high NOM samples). Negatively charged nano-sized NOM is a ubiquitous constituent of surface water, with carboxyl and phenolic groups as the predominant functional groups (Lodeiro *et al.* 2020). For instance, carboxyl groups account for 78–90% and 69–82% of all functional groups on the surface of nano-sized NOM, such as humic acid and fulvic acid (Ritchie & Michael Perdue 2003). Having the same charge, it is likely for NOM to adsorb and bind with Au-gel NPs. Unfortunately, SP-ICP-MS cannot distinguish the origin of particles bound to Au-gel NPs, which produced signals above the background and are counted as particle number concentration. Thus, due to interference from any other negatively charged particles in surface water, the measured particle number concentration of Au-gel NPs conjugates might not be an accurate representation of nanoplastics.



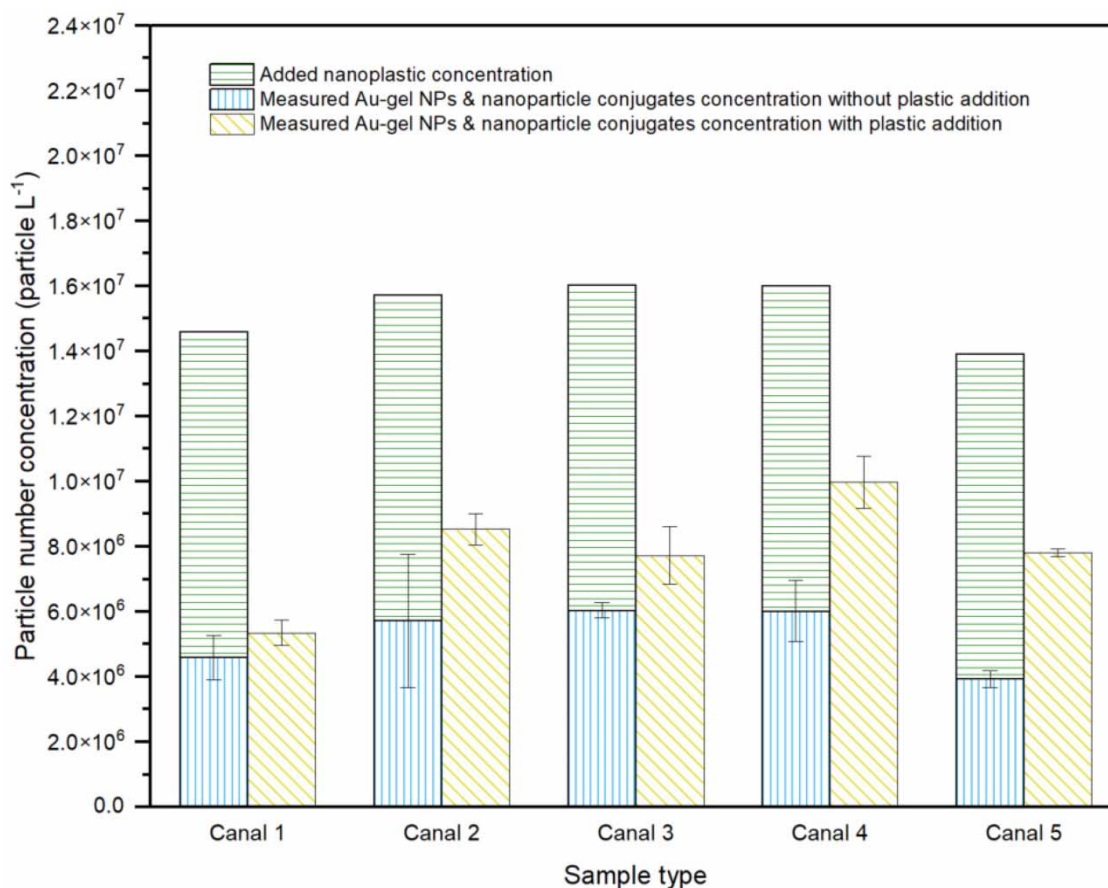
**Figure 1** | Detected Au-gel NPs-nanoparticles conjugates with (orange) and without (blue) the addition of  $1.0 \times 10^7$  nanoplastics (green) particles in water with low NOM concentrations (bottled water:  $0.32 \pm 0.06 \text{ mg-C L}^{-1}$ , tap water:  $1.76 \pm 0.03 \text{ mg-C L}^{-1}$ ) and high NOM concentrations (Canal water 1–5:  $15.47 \pm 0.00 \text{ mg-C L}^{-1}$  to  $19.64 \pm 0.14 \text{ mg-C L}^{-1}$ ). The stacked bar is the expected conjugate concentration after the addition of nanoplastics. The difference between the stacked bar and orange bar indicates the over/underestimation of nanoplastics content due to the presence of NOM or other nanoparticles. Error bar: standard deviation,  $n = 3$  replicates).

We further confirmed this interference by spiking the same water samples with  $1 \times 10^7$  nanoplastics particles  $\text{L}^{-1}$  (green bars), upon which higher particle number concentrations of Au-gel NPs conjugates were measured in all samples (orange bars in Figure 1). In bottled water (DOC of  $0.32 \pm 0.06 \text{ mg-C L}^{-1}$ ), the measured concentration of Au-gel NPs conjugates was  $1.5 \pm 1.5 \times 10^7$  particles  $\text{L}^{-1}$ , with an expected particle number concentration of  $1.18 \pm 0.1 \times 10^7$  particles  $\text{L}^{-1}$ . The measured concentration of conjugates after nanoplastics spiked (orange bar in Figure 1) was slightly higher than the expected concentration after the addition of nanoplastics; however, this result is still within the acceptable range which was also observed in the study by Marigliano *et al.* (2021). In bottled water, Au-gel NPs were effectively bound with nanoplastics added to the sample indicating no interference from other negatively charged nanoparticles. However, in tap water (DOC  $1.76 \pm 0.03 \text{ mg-C L}^{-1}$ ), the measured concentration after spiking was  $9.17 \pm 0.56 \times 10^6$  particles  $\text{L}^{-1}$  with an increase of only  $5.23 \pm 0.84 \times 10^6$  particles  $\text{L}^{-1}$  (equal to 52.3%) compared to the samples without the addition of nanoplastics. These results indicate that NOM – even if present only at  $1.76 \pm 0.03 \text{ mg-C L}^{-1}$  – competed for the labelling agent. As such, only water samples with minimum interference from other negatively charged nanoparticles, i.e.  $\text{DOC} < 1 \text{ mg-C L}^{-1}$  allow successful quantification of nanoplastics particles. In spiked canal water samples with high NOM concentrations, the measured increase in particle numbers was consistently lower than tap water. In those samples, measured concentrations after spiking ranged between  $7.89 \pm 0.11 \times 10^6$  and  $1.19 \pm 1.24 \times 10^7$  particles  $\text{L}^{-1}$ . The concentration differences before and after spiking nanoplastics resulted in a particle increase of less than  $2 \times 10^6$  particles  $\text{L}^{-1}$ . These results were equal to a roughly 13.10–18.72% of the actual spiked nanoplastics concentration of  $1 \times 10^7$  particles  $\text{L}^{-1}$ , indicating ineffective binding

between Au-gel NP and nanoplastics. NOM has abundant functional groups and could adsorb easily to the surface of nanoparticles (Wang *et al.* 2022). We argue that the ineffective nanoplastics labelling in samples with high NOM concentration was due to the existing NOM in the samples that potentially contained a higher number of COOH groups. This NOM competed to bind with Au-gel NPs and hindered the interaction between nanoplastics and Au-gel NPs. Indeed, previous studies have observed that interaction between NOM and engineered gold nanoparticle are inevitable (Louie *et al.* 2013). Consequently, reduced attachment of Au-gel NPs to nanoplastics surface was observed in all samples with high NOM concentration.

In order to reduce the NOM interference, we conducted a Fenton digestion. Following this digestion, the dissolved organic carbon (DOC) in the canal water samples exhibited an 85% reduction, dropping to between  $2.76 \pm 0.02$  and  $3.05 \pm 0.24$  mg L<sup>-1</sup> (Figure S3). The addition of nanoplastics to the digested samples resulted in an increase in measured particle concentration compared to the non-digested sample. However, the observed differences in particle concentration remained below the anticipated concentration after nanoplastics spiking as depicted in Figure 2.

Although we observed an increase, a more optimum digestion procedure is required. The tests show the unsuitability of the proposed method in high NOM waters. While digestion could improve the adsorption of Au-gel NPs to nanoplastics, the remaining NOM after the digestion process still interfered with the conjugation process between the two particles. One possible alternative to aid this issue was demonstrated in a study by Lai *et al.* (2021) by combining acid digestion and cloud-point extraction. In the mentioned study, a different approach of gold-labelling was used by growing AuNPs directly on the surface of nanoplastics and subsequently quantifying them using SP-ICP-MS. To improve the method's sensitivity, acid digestion with



**Figure 2** | Particle number concentrations of conjugates in canal water samples after Fenton digestion with (yellow bar) and without (blue bar) addition of  $1 \times 10^7$  particles L<sup>-1</sup> nanoplastics (green bar). The stacked bar is the expected conjugate concentration after the addition of nanoplastics. The difference between the stacked bar and yellow bar indicates the over/underestimation of nanoplastics content due to the presence of NOM or other nanoparticles. Error bar: standard deviation,  $n = 3$  replicates.



a mixture of 5 mM HNO<sub>3</sub> and 40 mM HF was used to remove other environmental matrices, followed by cloud-point extraction to concentrate nanoplastics and improve the Au-labelling efficiency.

## CONCLUSION

We explored the possibility of applying an indirect measurement of nanoplastics via metallic tagging using gelatin-coated gold nanoparticles. Our research showed that both the density of carboxyl group on the surface of nanoplastics and the concentration of NOM in water samples were critical parameters that affected the sensitivity of the gold-labelling method. Based on our findings, the Au-gel labelling method was most effective for quantifying particle number concentrations in waters with very low levels of NOM, such as bottled water and possibly deep groundwater. Furthermore, our study showed the significance of employing a digestion protocol on water samples with high NOM concentration, prior to applying this method. The test results indicate that the proposed method required more extensive sample preparation with surface water that contains high levels of NOM. While Fenton digestion could reduce the interference of NOM, there was still a significant presence of NOM that continued to disrupt the adsorption process between Au-gel NPs and nanoplastics particles. It is essential to conduct a dedicated study aimed at developing a digestion method that effectively removes NOM while preserving the environmental nanoplastics present in the sample. Moreover, the comparison between our results and those of the initial study by Jiménez-Lamana *et al.* (2020) and Marigliano *et al.* (2021) highlights the crucial role of achieving an adequate density of COOH (carboxyl) groups on nanoplastics surface to ensure accurate nanoplastics labelling using Au-gel nanoparticles.

An important next step could be to investigate the lower limit of COOH group density at nanoplastics surface for which this method can successfully and accurately quantify nanoplastics in low NOM water samples. Furthermore, a recent study indicates nanoplastics in the environment might have a lower COOH group from weathering than COOH functionalized nanoplastics (Blanco *et al.* 2021b), hence optimizing this method for environmentally relevant COOH groups on the surface of nanoplastics is another avenue to explore. Lastly, only a combination of the gold-labelling and SP-ICP-MS method with spectroscopic techniques will allow the quantification nanoplastics particles and thus the distinction from other organic particles. In spite of current limitations, further optimization of digestion protocols might allow the use of this method as a screening tool for nanoplastics monitoring in suitable water matrices.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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