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Determination of dissolved trace metals in seawater with EDTA: Reassessment and optimization

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ABSTRACT

Trace metal determination in seawater is crucial for understanding marine biogeochemical cycles, but organic complexing agents such as ethylenediaminetetraacetic acid (EDTA) and natural organic ligands pose analytical challenges. EDTA, a widely used ligand in phytoplankton cultures, was selected as a model compound in this study. We systematically evaluated two pretreatment methods—HNO_3/H_2O_2 digestion and UV/H_2O_2 irradiation— for improving trace metal recovery in EDTA-rich seawater using an automated preconcentration system (*seaFAST*). Our results demonstrate that both methods achieve nearly 100 % metal recovery. For HNO_3/H_2O_2 digestion, we recommend heating seawater samples to 80–90°C for at least 3 hours with 0.5 M HNO_3 and 26 mM H_2O_2, followed by pH adjustment to 1.6–1.8 before *seaFAST* preconcentration. For UV/H_2O_2 treatment, optimal conditions involve 18 mM H_2O_2 and 2 hours of UV irradiation using cost-effective UV-C lamps for acidified seawater samples stored in high-transmissibility fluorinated ethylene propylene or perfluoroalkoxy alkane bottles. Metals with low EDTA stability constants (e.g., Mn, Zn, and Cd) require shorter pretreatment time, whereas EDTA concentrations exceeding 200 μM necessitate extended digestion for complete degradation. In summary, the UV/H_2O_2 method provides a simple, energy-efficient, and scalable solution for trace metal analysis in seawater with high EDTA. These findings support reliable trace metal quantification in complex seawater matrices and improve methodological foundations for studying trace metal cycling and phytoplankton-metal interactions.

1. Introduction

Some transition metals, including iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co), and nickel (Ni), are biologically essential micronutrients in the ocean. These metals play critical roles as cofactors in various enzymatic processes that support essential biological functions such as photosynthesis, respiration, and the acquisition and assimilation of carbon, nitrogen, and phosphorus in marine phytoplankton (Morel and Price, 2003). Understanding the concentrations and cycling mechanisms of these metals is crucial for elucidating the biogeochemical cycling of key elements, such as carbon, in marine ecosystems. In contrast, other trace elements, such as cadmium (Cd) and lead (Pb), pose environmental concerns due to their toxic effects on marine organisms through bio-accumulation (Brand et al., 1986; Morel et al., 2020). Accurately determining the dissolved concentrations of these trace metals in seawater is essential for both advancing scientific research and monitoring marine environmental pollution.

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Determining the dissolved concentrations of trace metals in the ocean presents a significant challenge due to their extremely low concentrations and the complex matrix of seawater. Trace metal concentrations in surface seawater typically range from picomolar to nanomolar levels. Accurate measurement requires both preconcentration of the target elements and the removal of major seawater ions (e.g., Na, K, Mg, and Ca) to minimize interferences during ICPMS (Inductively Coupled Plasma Mass Spectrometry) analysis. The most commonly employed preconcentration technique is solid-phase extraction using chelating resins (Ho et al., 2010; Sohrin et al., 2008). Among the functional groups incorporated into commercially available chelating resins, aminocarboxylic acids are the most widely used due to their high selectivity for binding trace metals in seawater. Notable examples of such resins include Bio-Rad's Chelex-100, Hitachi's NOBIAS Chelate-PA1 (PA1), and Fujifilm's Presep PolyChelate (Biller and Bruland, 2012; Kagaya et al., 2013; Sohrin et al., 2008).

It is important to note that certain transition metals are strongly complexed by natural organic ligands in natural seawater (Coale and Bruland, 2003; Rue and Bruland, 1995; Saito and Moffett, 2002) so that the commonly used chelating resins may not fully outcompete these natural ligands for some trace metals through solid phase extraction. For example, Co and Cu require UV irradiation prior to the preconcentration step to break down natural organic complexes (Achterberg and van den Berg, 1994; Milne et al., 2010; Noble et al., 2017). The required irradiation time depends on the type and strength of the UV source as well as the material of the sample bottles. Milne et al. (2010) reported that, after 1 h of UV treatment, the concentrations of Co and Cu in deep seawater samples increased by approximately 50 and 10 %, respectively. Without appropriate pretreatment, the measurement of Co and Cu would be significantly underestimated due to incomplete extraction during solid phase extraction.

In laboratory culture studies of marine phytoplankton, ethylenediaminetetraacetic acid (EDTA) is widely used to simulate natural conditions by buffering trace metal concentrations at appropriately low and stable levels. The inorganic trace metal concentrations may be calculated (Anderson and Morel, 1982; Ho et al., 2003; Sunda and Huntsman, 1985) as the complexation affinities of EDTA with transition metals are well characterized. For instance, the constants are 25.2, 25.1, 41.4, 18.4, 18.8, 16.5, 16.5, and 18.0 for Mn^{2+}, Fe^{3+}, Co^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}, respectively (Harris and Lucy, 2016). Adding 100 μM EDTA, the inorganic metal concentrations of Mn, Fe, Zn, Cu, Co, and Cd were 10,000, 500, 20, 0.2, 20, and 20 pM, respectively (Ho et al., 2003). However, EDTA can also compete with chelating-resins used in solid phase extraction and thus influence metal recovery during the preconcentration. Fujimori et al. (2021) demonstrated that in freshwater samples with 5 μM EDTA, the recovery for Fe, Co, Ni, Cu, Zn, Cd, and Pb were all below 10 % when using various chelating resins. Thus, there is a critical need to develop simple and effective pretreatment methods to recover trace metals from seawater samples containing either natural organic ligands or EDTA. These methods would enhance the accuracy and reliability of trace metal analyses in marine environments.

Two commonly used pretreatment methods for EDTA digestion or degradation are employed in trace metal analysis for aqueous samples. The first method involves the use of HNO_3/H_2O_2 digestion under high-temperature conditions for a specific period, typically ranging from 2 to 12 hours. Fujimori et al. (2019) demonstrated that adding HNO_3 and H_2O_2 and heating the mixture to 170–200°C on a hot plate for 4 hours effectively degrades EDTA, achieving 100 % recovery for all metals except Co. This approach has been primarily applied to sewage and freshwater samples with EDTA concentrations ranging from 0.01 to 5 μM. However, the significantly higher EDTA concentrations used in marine phytoplankton culture media, which typically range from 20 to 100 μM (Chen, 2022; Ho et al., 2003; Sunda and Huntsman, 1985), may require modified conditions, including adjustments to HNO_3, H_2O_2 concentrations, temperature, and digestion time. These specific conditions are not yet well-documented for seawater applications, highlighting the need for further optimization of the digestion method.

UV irradiation, coupled with various photochemical processes or photocatalysis, represents the second most common method for EDTA or organic compound degradation. These techniques include UV treatment alone, UV/H_2O_2, $UV/Fe^{2+}/H_2O_2$, O_3/UV, and TiO_2/UV (Achterberg and van den Berg, 1994; Kagaya et al., 1997; Katsoyiannis et al., 2011; Legrini et al., 1993; Rekab et al., 2014). Among these approaches, UV alone or UV/H_2O_2 are the most frequently employed methods for contamination control during analysis. While extensive research has explored the degradation of EDTA using these methods, studies on the recovery of metals through solid phase extraction with chelating resins remain limited. The degradation process often generates intermediate byproducts such as nitrilotriacetic acid (NTA), iminodiacetic acid (IDA), and oxalic acid (Jiraroj et al., 2006). These compounds can continue to form complexes with trace metals, potentially interfering with their extraction by chelating resins. Systematic investigations are needed to determine optimal conditions for combining UV digestion and/or HNO_3/H_2O_2 digestion with solid phase extraction. Such studies would enhance the recovery efficiency and obtain accurate concentrations of dissolved trace metals in seawater.

In this study, we have systematically investigated the effects of the two pretreatment approaches- HNO_3/H_2O_2 digestion and UV irradiation digestions on the recovery of trace metals from seawater with high levels of EDTA, using solid phase extraction with PA1 resin. The findings provide an effective and simple method for determining trace metal concentrations or isotopic composition in seawater containing high level organic ligands such as EDTA and EDTA-like ligands. Moreover, these results may enable a reevaluation of trace metal concentrations and cycling in some oceanic regions characterized by high and complex organic ligand levels, such as estuarine and coastal areas. These insights would significantly advance analytical methodologies for investigating trace metal-phytoplankton interactions and trace metal biogeochemistry in marine environments.

2. Material and methods

2.1. Preconcentration and analysis

Solid phase extraction approach using column packed with chelating resins has become the most widely adopted preconcentration approach for trace metal analysis in seawater. Various laboratory-specific designs have been developed to automate the removal of major ions and the preconcentration of the target trace metals (Ho et al., 2010; Lagerström et al., 2013; Minami et al., 2015). Over the past two decades, large-scale oceanographic trace metal studies and programs, such as GEOTRACES, have further driven advancements in automated preconcentration systems for seawater trace metal analysis. One reliable and widely used system is the commercially available *seaFAST* system (Elemental Scientific, Inc., USA), specifically engineered for the high-efficiency, low--contamination, and fully automated preconcentration of dissolved trace metals in seawater (Lagerström et al., 2013; Wuttig et al., 2019). The pretreatment system has been widely utilized by most of the researchers in the global program, GEOTRACES (e.g., Bown et al., 2017). The chelating resins used in the *seaFAST*, known as PA1 (NOBIAS Chelate-PA1, Hitachi, Japan), are functionalized with ethylenediaminetriacetic acid (EDTriA) and iminodiacetic acid (IDA). These resins exhibit exceptionally high stability constants with trace metals while maintaining extremely low stability constants with Group IA and IIA metals (e.g., Na, K, Mg, Ca)(Sohrin et al., 2008; Wang et al., 2014). As a result, PA1 effectively binds trace metals from the sample while allowing major matrix ions to pass through freely. For example, the retention of Group IA elements (e.g., Na) is less than 0.0005 %, whereas the retention of the Group IIA metals (e.g., Mg or Ca) is equal to or below 0.002 % (Wang et al., 2014). Compared to IDA resins such as Chelex-100 resin, PA1 resins show 100-fold lower recovery for Mg and Ca, significantly reducing interference during trace metal analysis by ICPMS, particularly in samples with varying salinity (Wang et al., 2014).

In this study, all samples were thus processed using the automated preconcentration system (*seaFAST*, Elemental Scientific Inc.) and subsequently analyzed with a sector field high resolution ICPMS (Element XR, Thermo Fisher Scientific). The preconcentration factor was set at 10-fold. During preconcentration process, seawater samples were mixed with 4.8 M ultrapure acetic ammonia buffer (pH=7.2) at a sample to buffer ratio of 3.7, resulting in an adjusted loading pH of approximately 6.1 ± 0.1 in the *seaFAST* system. For metal measurements, the isotopes of ^{115}In, ^{111}Cd, ^{114}Cd, ^{207}Pb and ^{208}Pb were analyzed at low resolution ($M/\Delta M \sim 300$), ^{55}Mn, ^{54}Fe, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{61}Ni, ^{63}Cu, ^{65}Cu, ^{64}Zn, ^{66}Zn and ^{115}In were analyzed at medium resolution ($M/\Delta M \sim 4000$). Detailed information regarding the precision, accuracy validation, and detection limits of the ICPMS method can be found in our previous studies (Ho et al., 2010; Wang et al., 2014).

2.2. Effect of EDTA concentrations on metal recovery by seaFAST

Seawater samples used for this study were collected from the Surface water in the Northwestern Pacific Ocean (19°30.02'N, 143°16.59'E) during cruise TN-417 aboard the RV *Thompson* (US). EDTA was added to achieve final concentrations ranging from 0.1 to 500 μM, at intervals of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, and 500 μM. A 0.5 ppb multi-element standard was then introduced, resulting in final metal concentrations of 6.1, 10.8, 5.3, 9.3, 9.0, 13.6, 4.6, and 2.4 nM for Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb, respectively. The seawater was then acidified to a pH of 1.6–1.8 before the preconcentration process, as suggested by the GEOTRACES cookbook (Cutter et al., 2017). For metal recovery analysis, 20 ml of each prepared sample was processed using the *seaFAST* automated preconcentration system. Metal recovery efficiency was determined by comparing the concentrations of metals in the EDTA-treated samples after preconcentration to those in untreated samples.

2.3. HNO_3/H_2O_2 digestion: Effect of HNO_3/H_2O_2 Concentration, Temperature, and Time

For HNO_3/H_2O_2 treatment experiments, we adjusted the concentrations of HNO_3 and H_2O_2, as well as the heating temperature, and monitored the recovery over 8 hours. In all experiments, seawater samples subjected to various conditions were transferred into 22 ml PFA vials and heated on a hotplate. Samples were removed at scheduled intervals of 1, 2, 3, 4, 6, and 8 hours, then cool to room temperature. Afterward, the pH of each sample was adjusted to 1.6–1.8 before proceeding *seaFAST* process. It is important to emphasize that in treatments involving HNO_3/H_2O_2, where the HNO_3 concentration can reach 0.8 M, precise pH adjustment back to pH 1.6–1.8 by using ultrapure base (e.g., NaOH) is critical for high recovery through *seaFAST*. Insufficient pH adjustment may compromise the effectiveness of acetic ammonia buffer for pH control during the metal preconcentration process in *seaFAST*, potentially affecting recovery efficiency.

In the HNO_3 concentration experiments, the hotplate was set to 190°C, with H_2O_2 maintained at 176 mM, and EDTA at 20 μM. HNO_3 concentrations were varied at 0, 0.1, 0.2, 0.3, 0.4, 0.6, and 0.8 M. For temperature variation experiments, the HNO_3 concentration held constant at 0.8 M, along with H_2O_2 concentration at 176 mM and EDTA at 20 μM, hotplate temperatures were adjusted to 50, 120, 155, and 190°C, corresponding to internal PFA vial temperature of 33 ± 2, 52 ± 2, 68 ± 2, and 87 ± 2°C, respectively. In the H_2O_2 concentration experiments, the hotplate temperature was maintained at 190°C, with HNO_3 at 0.8 M and EDTA at 20 μM. H_2O_2 concentrations were varied at 0, 8.8, 26, 44, 88, 132, and 176 mM.

2.4. UV digestion: Effect of H_2O_2 concentration and time

In the UV treatment process, acidified seawater samples were digested under UV irradiation with final H_2O_2 concentrations adjusted to 0, 18, 44, and 88 mM. The UV digestion system utilized an in-house developed low-power UV system equipped with two 15 W UV-C lamps (20 W m^{-2}, 254 nm), positioned 2 cm from either side of 20 ml PFA Teflon sample bottles (AS ONE Corporation, >95 % transmittance) (Figure S1). Following UV exposure, the seawater samples were directly processed using the *seaFAST* system without any additional treatment. Notably, treated samples with higher H_2O_2 concentrations (44 and 88 mM) under UV irradiation can lead to rapid gas production, primarily CO_2 and O_2. This gas generation may deform sample bottles, particularly those made from UV-transmissive materials like fluorinated ethylene propylene (FEP) or perfluoroalkoxy alkane (PFA).

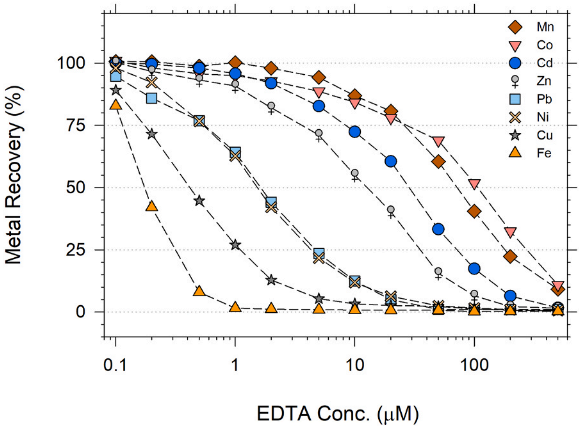

Fig. 1. Metal recovery of untreated EDTA seawater by an automated pre-concentration system (*seaFAST*) with Nobias PA1 resins under various EDTA concentrations ranging from 0.1 to 500 μM.

2.5. CRM validation and EDTA degradation limitation

For CRM validation, EDTA was added to Certified Reference Material (CRM, NIST CASS-6 and NASS-7) at a concentration of 20 μM as well. The samples were then treated under the recommended conditions for either HNO_3/H_2O_2 or UV/H_2O_2 digestion to evaluate whether metal recoveries after the preconcentration process align with the certified values. In a separate test to assess EDTA degradation limitations, multi-element seawater samples were prepared by adding EDTA to acidified seawater at concentrations ranging from 5 to 500 μM, specifically at intervals of 5, 10, 20, 50, 100, 200, and 500 μM. Metal recoveries were analyzed following two different digestion treatments and subsequent *seaFAST* preconcentration processes to compare the effectiveness of each treatment approach.

3. Results and discussion

3.1. Effects of EDTA concentration on preconcentration recovery

Using *seaFAST* as preconcentration method, Fig. 1 shows the recovery of eight trace metals studied under various EDTA concentrations ranging from 0.1 to 500 μM in the natural seawater matrix mentioned in the Method section. The recoveries are inversely correlated with EDTA concentrations, decreasing as EDTA concentrations increase. At 0.1 μM EDTA, recoveries for all metals exceed 80 %, whereas at 500 μM EDTA, recoveries drop to extremely low levels (<2 %) for most metals. The stability constants (log K) of EDTA–metal complexes in their common aqueous oxidation states play a decisive role in determining metal competition between EDTA and PA1. According to Harris and Lucy (2016), the log K values are as follows: Mn^{2+} (13.9), Co^{2+} (16.5), Cd^{2+} (16.5), Zn^{2+} (16.5), Pb^{2+} (18.0), Ni^{2+} (18.4), Cu^{2+} (18.8), and Fe^{3+} (25.1), respectively (Harris and Lucy, 2016). As shown, metals with higher stability constants, such as Fe, Pb, Ni, and Cu, exhibit convex recovery patterns (Fig. 1). Using Fe (with the highest constant among the 8 metals studied) as an example, its recovery shows a sharp decline with increasing EDTA concentrations. Relatively to the elements with high stability constants, metals with lower stability constants, including Mn, Co, Cd, and Zn, display concave recovery patterns. For example, Mn, with the lowest stability constant, exhibits a gradual decline in recovery between EDTA concentrations of 0.1–10 μM, followed by a significant drop beyond 10 μM. For the EDTA concentration range commonly used in marine microalgal cultures (20–100 μM), most metal recoveries would fall below 50 %. For instance, at 100 μM EDTA, the recovery percentages are as follows: Mn (40 %), Fe (0.2 %), Co (52 %), Ni (1.6 %), Cu (1.2 %), Zn (6.5 %), Cd (17 %), and Pb (1.1 %). These relatively low recoveries underscore the necessity of decomposing EDTA to achieve higher recoveries in dissolved trace metal analysis for seawater samples containing added EDTA. EDTA is a widely used artificial organic ligand in industry so that its concentrations can reach μM levels in industrial wastewater (Bedsworth and Sedlak, 1999; Stockdale et al., 2015). Its concentrations in estuarine regions typically range from 0.1 to a few hundred nM levels, depending on the degree of pollution (e.g., Kemmei et al., 2012; Stockdale et al., 2015). In comparison, strong natural metal-binding organic ligands are typically present at much lower concentrations, ranging from picomolar levels for individual compounds to nanomolar levels at ligand class level in open ocean (Gledhill and Buck, 2012). With the increased presence of these artificial and natural organic ligands, their potential effect on metal recovery during PA1 resin pretreatment needs careful consideration.

3.2. Effects of HNO_3 concentration and temperature on HNO_3/H_2O_2 pretreatment

The combination of HNO_3 and H_2O_2 is a traditional digestion method for decomposing organic matter in various marine organic samples, including materials such as particulate organic matter, microalgae, and fish muscle, to facilitate trace metal analysis

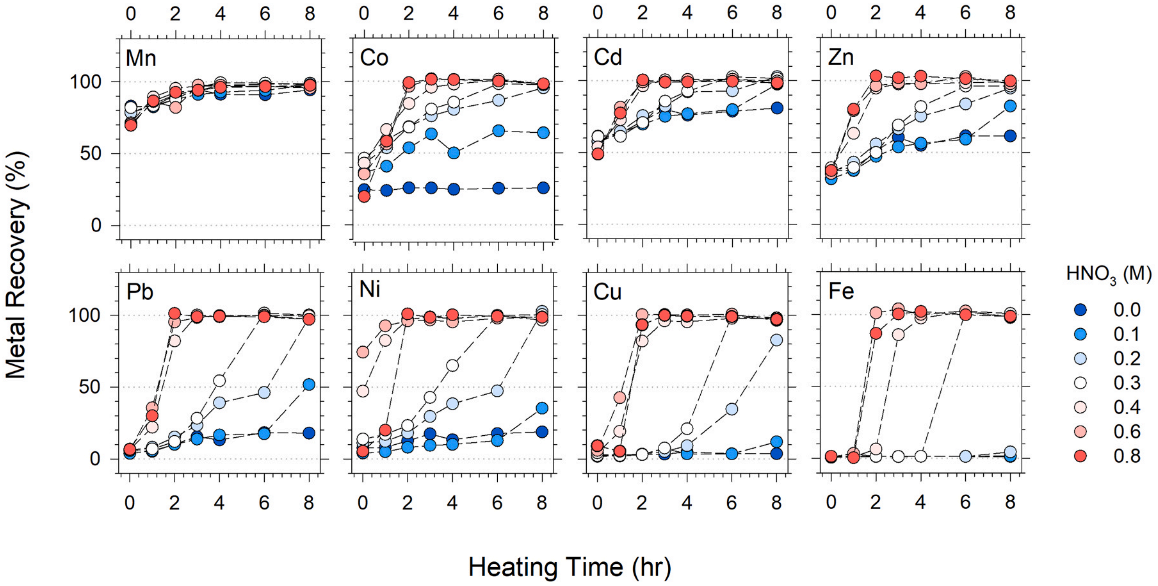

Fig. 2. Effect of nitric acid concentrations on metal recovery over time by *seaFAST* and ICPMS analysis. The hotplate temperature was set at 190°C, along with H_2O_2 concentration at 176 mM, and EDTA concentration at 20 μM.

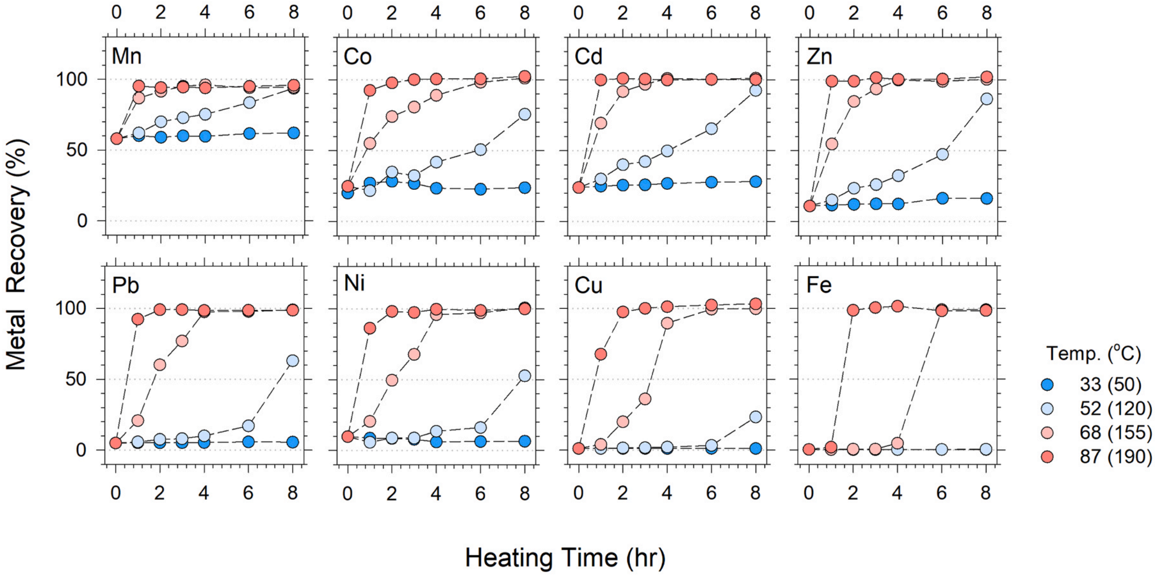

Fig. 3. Effect of temperature on metal recovery over time by *seaFAST* and ICPMS analysis. The HNO_3 concentration was set at 0.8 M, along with H_2O_2 concentration at 176 mM and EDTA at 20 μM.

(Mohammed et al., 2017; Pettersson and Olsson, 1998). The efficiency of organic compound degradation depends on the following factors, including temperature, reaction time, and the concentrations of HNO_3 and H_2O_2. For aqueous samples with EDTA, Fujimori et al. (2019) used a relatively dilute HNO_3 and H_2O_2 mixture for EDTA degradation, which consists of 0.8 M HNO_3 and 2 % H_2O_2. It should be noted that maintaining an appropriate pH range is critical for achieving effective metal complexation by PA1 chelating resins (Sohrin et al., 2008). For optimal long-term preservation and pH stabilization during pretreatment, it is recommended that the sample pH be adjusted to a dilute acidic range (pH 1.6–1.8) before processing with the *seaFAST* system (Cutter et al., 2017). The concentration of HNO_3 used for EDTA degradation would affect the amount of ultrapure NaOH used for pH adjustment, which is essential for high metal recovery (Table S1). We thus used limited HNO_3, from 0.0 to 0.8, for experimental testing to minimize the need for pH adjustment during the degradation process.

Fig. 2 exhibits the effects of different HNO_3 concentration levels on metal recovery and EDTA degradation efficiency over different heating times. During this study, HNO_3 concentrations ranging from 0 to 0.8 M were applied in the degradation process, alongside

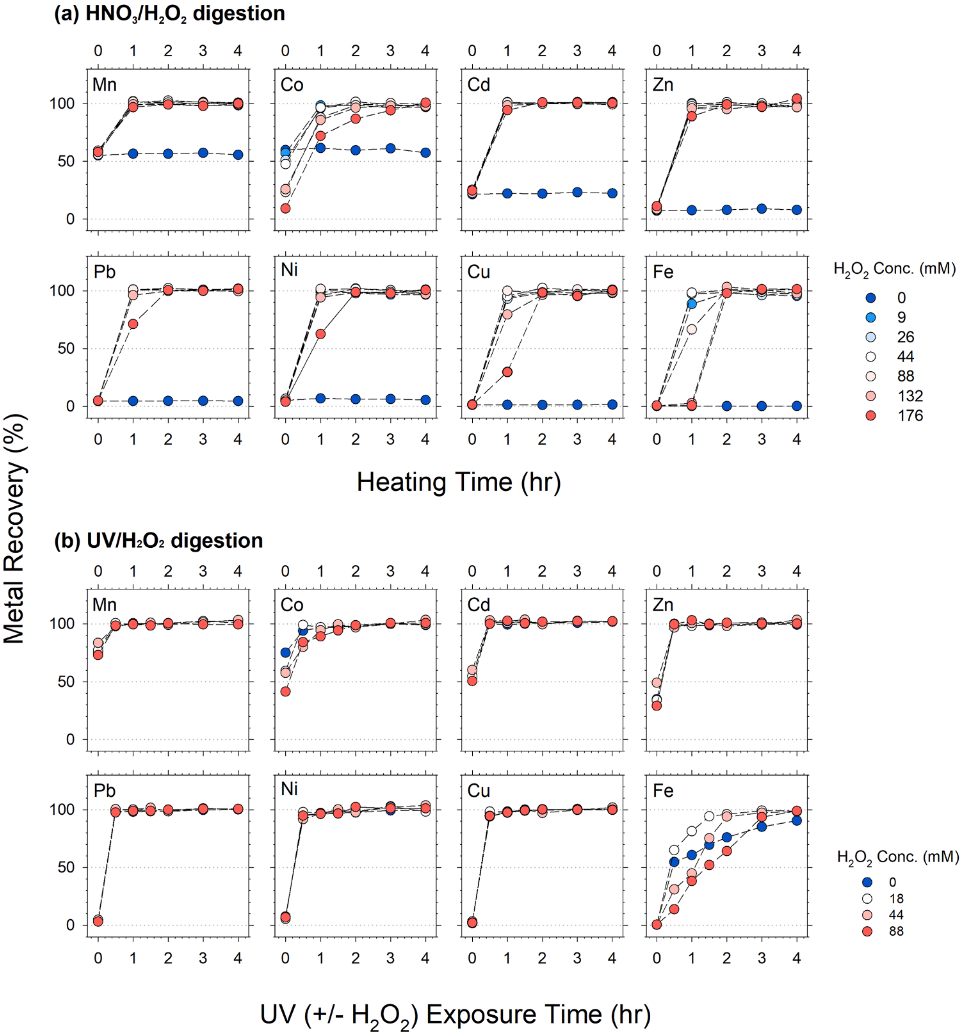

Fig. 4. Effect of heating time or UV exposure time with various H_2O_2 concentrations on metal recovery and EDTA degradation , using (a) 0.8 M HNO_3 and heating temperature at 87°C with 20 μM EDTA, and (b) UV irradiation at room temperature.

176 mM H_2O_2 and a heating solution temperature of 87°C suggested by Fujimori et al. (2019). The subfigures are arranged according to the stability constants of the respective EDTA-metal complexes. We observed that metals with lower stability constants show lower HNO_3 concentrations (~0.2 M) and shorter heating times to achieve high recovery (> 95 %). The results indicate that Mn recovery can reach nearly 100 % within 3 hours, even without the addition of HNO_3. In contrast, metals with higher stability constants, such as Fe and Cu, require HNO_3 higher concentrations and/or longer heating time to achieve higher recovery. As shown in Fig. 1, even 0.5 μM of residual EDTA can significantly reduce the metal recovery by *seaFAST*, with Fe and Cu recoveries dropping to 8.0 % and 45 %, respectively. At 20 μM EDTA, HNO_3 concentrations below 0.3 M were insufficient to effectively degrade EDTA within 8 hours for metals with high stability constants. However, at HNO_3 concentrations exceeding 0.4 M, metal recovery surpassed 90 % within 2 hours. Specifically, at 0.4 M HNO_3, the metal recoveries were 98 % for Mn, 99 % for Co, 101 % for Cd, 91 % for Zn, 101 % for Pb, 99 % for Ni, 101 % for Cu, and 94 % for Fe. To balance efficiency and minimize the amount of ultrapure NaOH required for pH adjustment prior to the preconcentration procedure, we recommend using HNO_3 concentration between 0.4 and 0.5 M. For samples with EDTA concentration exceeding 20 μM, we recommend extending the heating time or using higher temperature to ensure effective EDTA breakdown.

Higher temperatures provide higher kinetic energy for reactant molecules, increasing the frequency and energy of collisions, thereby accelerating the reaction. Fig. 3 shows the effects of heating temperature on metal recovery and EDTA degradation efficiency over time. The subfigures are arranged sequentially according to the stability constants of their respective EDTA-metal complexes, with Mn in the upper-left subfigure and stability constants increasing progressively to the right, culminating with Fe, which has the highest stability constant. The metal recovery patterns are also closely correlated with the stability constants as well. At a sample solution temperature of 33°C, metal recovery remained largely unchanged over time. However, the heating condition at 52°C significantly improved the recovery for metals with lower stability constants (Mn, Co, Cd, and Zn) over 8 h period, achieving recovery of 94, 76, 92, and 86 %, respectively. In contrast, the recoveries for metals with higher stability constants, Pb, Ni, Cu, and Fe, remained relatively low, which are 63, 52, 23, and 0.6 %, respectively.

As expected, increasing sample temperature significantly enhances the efficiency of EDTA degradation by HNO_3/H_2O_2 treatment. At 68°C, both metal recovery and EDTA degradation efficiency improved markedly, with recoveries exceeding 95 % for all metals within 6 hours. At 87°C, the recovery and degradation efficiency were further enhanced, probably achieving complete EDTA degradation within 4 hours and metal recoveries approaching 100 %. These findings underscore the critical role of sample temperature in optimizing EDTA degradation and metal recovery by using HNO_3/H_2O_2 treatment. It should be noted that the actual heating temperature of sample liquid can be affected by factors such as the heating device or the material of the material of sample bottles. It is thus crucial to directly measure the temperature of the sample liquid and maintain it at 80–90°C, especially for the analysis of the metals with high stability constants, such as Pb, Ni, Cu, and Fe.

3.3. The effect of H_2O_2 on HNO_3/H_2O_2 or UV/H_2O_2 treatments

Both UV treatment and H_2O_2 addition are well known to be able to generate reactive oxygen species and thus highly enhance the efficiency of organic compound degradation (Legrini et al., 1993). By coupling UV treatment and H_2O_2 addition, hydroxyl radicals (•OH) are generated through the direct photolysis of H_2O_2 by ultraviolet light (Legrini et al., 1993). In the HNO_3/H_2O_2 coupling process, the combination produces reactive intermediates, such as peroxynitric acid ($HOONO_2$) and other oxygen-rich species (Raschig, 1904). These highly reactive radicals effectively degrade and mineralize dissolved organic matter into simpler, inorganic compounds (Jiraroj et al., 2006).

Fig. 4 shows the effects of H_2O_2 concentrations on EDTA degradation efficiency for both HNO_3/H_2O_2 and UV/H_2O_2 treatments. Besides temperature, the experimental conditions were based on Fujimori et al. (2019), who recommended 0.8 M HNO_3 and heating temperature of 87°C for samples with 20 μM EDTA. Fig. 4a indicates that without the addition of H_2O_2, even in the presence of 0.8 M HNO_3 and heating to 87°C, metal recovery showed no significant improvement within 8 hours. However, with the addition of H_2O_2 at 9 mM, EDTA was effectively degraded, and most metal recovery exceeded 95 % within 2 h. However, higher levels of H_2O_2 would reduce metal recovery within the first 2 h for most metals. For example, in the case of Co, higher concentrations of H_2O_2 (e.g., 176 mM) required a longer processing time to achieve complete recovery compared to other metals. The decrease of the recovery is likely to be attributed to the oxidation of Co^{2+} to Co^{3+}, in which possesses extremely high stability constant with EDTA (41.4) (Harris and Lucy, 2016).

Fig. 4b exhibits the influence of varying H_2O_2 concentrations on metal recovery over time under fixed UV irradiation at room temperature. The data indicate that UV irradiation alone can effectively degrade EDTA, achieving complete recovery for most of the metals within 0.5 h, except for Fe. Without H_2O_2, Fe recovery reached only 90 % after 4 hours of UV treatment. However, the addition of H_2O_2 significantly improved Fe recovery, approaching 100 % within 1.5, 2, and 3 h at H_2O_2 concentrations of 18, 44, and 88 mM, respectively. Once again, the addition of higher H_2O_2 concentrations showed negative effects on Fe recovery, similar to observations in the HNO_3/H_2O_2 digestion process. Jiraroj et al. (2006) reported that UV treatment for EDTA degradation would produce byproducts like nitrilotriacetic acid (NTA) and iminodiacetic acid (IDA), with higher H_2O_2 concentrations promoting greater NTA formation. The stability constant of Fe^{3+} for NTA (15.9) is significantly higher compared to IDA (10.7) (Burgess, 2004), which can hinder Fe recovery during the preconcentration process. These byproducts, particularly NTA, can form relatively strong complexation with Fe and result in lower recovery. In this study, although we did not analyze the concentrations of the byproducts during the EDTA degradation process, we speculate that the lower Fe recovery observed under higher H_2O_2 concentrations might be attributed to increased production of NTA. To minimize their interference, a lower concentration of H_2O_2 or extended degradation time may be required.

Based on the above results and the previous section, we recommend heating temperature to be 80–90°C for at least 3 h, using a final concentration of 0.5 M HNO_3 and 26 mM H_2O_2. Additionally, adjust the pH to 1.7–1.8 prior to the preconcentration process. For the UV/H_2O_2 treatment, we recommend 18 mM H_2O_2 with UV irradiation for 2 h to optimize both EDTA degradation and trace metal recovery efficiency in seawater samples with high EDTA levels. In terms of EDTA degradation limitations, our results demonstrated that both HNO_3/H_2O_2 and UV/H_2O_2 digestion treatments achieved high metal recovery for EDTA concentrations up to 200 μM (Table S2), covering typical levels found in natural seawater and laboratory culture media. For EDTA concentrations exceeding 200 μM, we recommend extending the processing time to ensure complete metal recovery, particularly for Fe.

3.4. CRM validation

We have added 20 μM EDTA to Certified Reference Material (CRM) NASS-7 and CASS-6 to validate the accuracy of the proposed recipe. The results show that a significant decrease in metal recovery without the appropriate pretreatment, particularly for Fe, Ni, Cu, and Pb, with recovery to be 3.6, 8.6, 3.6, 15 %, and 0.7, 5.9, 2.0, 5.5 % for NASS-7 and CASS-6 respectively, which are comparable to the results shown in Fig. 1. Processed with either HNO_3/H_2O_2 digestion or UV/H_2O_2 irradiation treatment, the metal recovery was comparable to the certified value. The metal recovery percentages for HNO_3/H_2O_2 digestion for NASS-7 and CASS-6 were as follows for the respective elements: Mn (88 ± 1 %, 83 ± 1 %), Fe (99 ± 3 %, 94 ± 1 %), Co (85 ± 3 %, 89 ± 2 %), Ni (96 ± 1 %, 93 ± 3 %), Cu (100 ± 3 %, 92 ± 5 %), Zn (93 ± 3 %, 87 ± 1 %), Cd (85 ± 1 %, 95 ± 2 %), and Pb (101 ± 5 %, 106 ± 4 %). Similarly, the metal recovery percentages for UV/H_2O_2 digestion for NASS-7 and CASS-6 were: Mn (95 ± 1 %, 87 ± 1 %), Fe (107 ± 2 %, 102 ± 1 %), Co (101 ± 5 %, 101 ± 1 %), Ni (96 ± 3 %, 102 ± 1 %), Cu (105 ± 5 %, 102 ± 2 %), Zn (91 ± 2 %, 91 ± 1 %), Cd (91 ± 2 %, 100 ± 2 %), and Pb (101 ± 3 %, 106 ± 2 %). The detection limits for the HNO_3/H_2O_2 digestion method were Mn (0.01 nM), Fe (0.2 nM), Co (0.01 nM), Ni (0.1 nM), Cu (0.05 nM), Zn (0.05 nM), Cd (0.001 nM), and Pb (0.003 nM), respectively. For the UV/H_2O_2 digestion

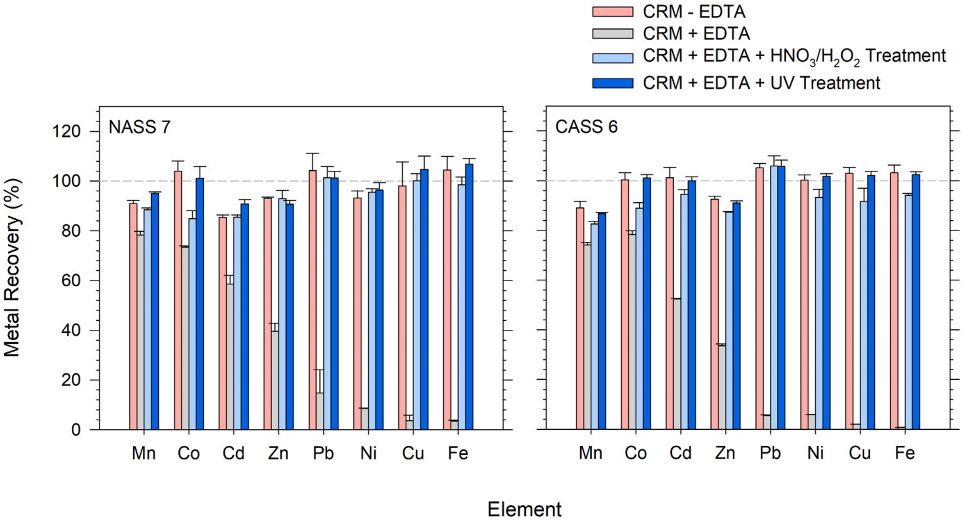

Fig. 5. The metal recovery of certified reference material NASS-7 and CASS-6 seawater with 20 μM EDTA addition. For the HNO_3/H_2O_2 treatment, the process involved heating seawater samples at 80–90°C for at 3 hours with a final concentration of 0.5 M HNO_3 and 26 mM H_2O_2. For the UV/H_2O_2 treatment, we used 18 mM H_2O_2 and 2 hours of UV irradiation.

method, the detection limits were Mn (0.01 nM), Fe (0.09 nM), Co (0.01 nM), Ni (0.06 nM), Cu (0.06 nM), Zn (0.08 nM), Cd (0.001 nM), and Pb (0.003 nM), respectively.

4. Conclusion

In this study, we demonstrated that both the HNO_3/H_2O_2 and UV/H_2O_2 digestion methods effectively achieve nearly 100 % metal recovery when using the *seaFAST* automated preconcentration system, making the digestion methods suitable for measuring low concentrations of trace metals in seawater with high EDTA or potentially high natural organic ligand concentrations. For HNO_3/H_2O_2 digestion, standard laboratory equipment such as hot plates and PFA vials, commonly available in marine trace metal studies, were utilized. We recommend an internal seawater sample heating temperature of 80–90°C for at least 3 h, with the final concentration of 0.5 M HNO_3 and 26 mM H_2O_2. The pH should then be adjusted to 1.7–1.8 before preconcentration. For UV/H_2O_2 treatment, we suggest using 18 mM H_2O_2 with 2 h of UV irradiation provided by commercially available, low-cost UV-C lamps, which are easy to install and have low electricity consumption. Samples for UV digestion were suggested to be put in UV-C high transmissible FEP or PFA bottles. We observed that for metals with low EDTA stability constants, such as Mn, Zn, and Cd, the required pretreatment time can be significantly reduced. However, if the EDTA concentration exceeds 200 μM, longer digestion time is needed to ensure complete EDTA degradation. The UV/H_2O_2 method offers an efficient and straightforward approach for degrading EDTA and determining trace metal concentrations or isotopic composition in seawater containing high organic ligands. It should be noted that the model organic ligand used in this study, EDTA, is not representative of naturally occurring organic ligands. Although the concentration level applied reflects that used in phytoplankton culture media, the findings may also be applicable to seawater and wastewater samples with high levels of EDTA contamination, as well as to certain natural organic ligands exhibiting similar complexation characteristics. Considering the complex and heterogeneous nature of organic ligands in seawater, extended exposure experiments are necessary before applying this method to environmental samples.

CRediT authorship contribution statement

Ho Tung-Yuan: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hsieh Chih-Chiang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2025.104235.

Data Availability

Data will be made available on request.

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