QUANTIFICATION OF POLYETHYLENE MICROPLASTICS IN LIQUID SAMPLES: PRELIMINARY RESULTS USING NILE RED AND IMAGEJ ANALYSIS

CUANTIFICACIÓN DE MICROPLÁSTICOS DE POLIETILENO EN MUESTRAS LÍQUIDAS: RESULTADOS PRELIMINARES UTILIZANDO ROJO DE NILO Y ANÁLISIS CON IMAGEJ

María T. López-Martínez[1], Rosario González-Mota[2*] Juan Reyes-Luévano[1], J. Ascención Guerrero-Viramontes[1], Juan J. Soto-Bernal[3], Iliana Rosales-Candelas[3]

Tecnológico Nacional de México/Instituto Tecnológico de Aguascalientes, (1) División de Estudios de Postgrado e Investigación, (2) Departamento de Ingeniería Química y Bioquímica, (3) Departamento de Ingeniería Eléctrica Electrónica, Av. Adolfo López Mateos 1801 Ote., C. P. 20256, Aguascalientes, México

(*e-mail: rosario.gm@aguascalientes.tecnm.mx)

Recibido: 15/10/2023 - Evaluado: 08/11/2023 - Aceptado: 20/12/2023

ABSTRACT

Microplastics have become a global pollution issue due to their presence in several ecosystems such as oceans, coasts, rivers, and lakes, among others. However, there is no standardized protocol for detecting and quantifying microplastics in liquid samples; but also, most quantification methods rely on visual counting of microplastics suspended in a solid medium, typically filter paper. This study presents preliminary results of microplastic quantification in liquid samples through image analysis using ImageJ software and different threshold values. Polyethylene microplastic in liquid samples were prepared in the laboratory using virgin polyethylene microplastics dyed with Nile red, 1 µg mL^{-1}, and HPLC-grade water. The statistical analysis of the results performed using the Anderson-Darling normality test in Minitab® software, version 18.1, shows that this protocol is reliable for concentrations of up to 16 microplastics, with a 95% confidence interval. These results demonstrate the reliability of the proposed method for the quantification of microplastics in liquid samples.

RESUMEN

Los microplásticos se han convertido en un problema de contaminación mundial debido a su presencia en diversos ecosistemas como océanos, costas, ríos y lagos, entre otros. Sin embargo, no existe un protocolo estandarizado para detectar y cuantificar los microplásticos en muestras líquidas; además, la mayoría de los métodos de cuantificación se basan en el recuento visual de microplásticos suspendidos en un medio sólido, normalmente papel de filtro. Este estudio presenta resultados preliminares de la cuantificación de microplásticos en muestras líquidas mediante el análisis de imágenes utilizando el software ImageJ y diferentes valores umbral. Las soluciones de microplásticos en agua fueron preparadas en el laboratorio, utilizando microplásticos de polietileno virgen teñidos con rojo Nilo, 1mg mL^{-1}, y usando agua grado HPLC. El análisis estadístico de los resultados, realizado mediante la prueba de normalidad Anderson-Darling usando el software Minitab®, versión 18.1, muestra que este protocolo es fiable para concentraciones de hasta 16 microplásticos, con un intervalo de confianza del 95%. Estos resultados demuestran la confiabilidad del método propuesto para la cuantificación de microplásticos de polietileno en muestras líquidas.

Keywords: images, fluorescence staining, threshold, reliability
Palabras clave: imágenes, tinción fluorescente, umbral, confiable

INTRODUCTION

Microplastics (MPs), tiny plastic particles equal or less than 5 mm, have gained significant attention in recent years due to their widespread presence and persistence in different environments like oceans, lakes, rivers, freshwater, soil, and even air and food (Pastor & Agulló, 2019; Periyasamy, 2023; Picó & Barceló, 2019). MPs founded in the environment can be primary MPs, which are intentionally produced at that size to be used as additives in cosmetics, personal care products, marine coatings, plastic pellets, beads, and secondary MPs, which result from the partial degradation of larger plastics during weathering processes, by physical, biological and chemical factors, like tyre wear and tear (EFSA CONTAM, 2016; Martínez-Francés *et al*., 2023; Munoz-Pineiro, 2018; Pastor & Agulló, 2019; Picó & Barceló, 2019; Rezania *et al*., 2018).

MPs have become a global concern, prompting governments, consortiums, scientists, environmental groups, and consumers to establish strategies to reduce their consumption, eliminate their widespread presence in the environment, and support related research (Erni-Cassola *et al*., 2017; Schymanski *et al*., 2018; Silva *et al*., 2018). The growing interest in MPs is reflected in the rapid increase in publications focused on sampling, detection, quantification, characterization, and environmental impact assessment (Environment and Climate Change Canada, 2019; Jenkins *et al*., 2022; Maes *et al*., 2017).

The characterization of microplastics using a single analytical technique is still a challenge; therefore, many studies employ a combination of microscopic and analytical methods (De Frond *et al*., 2023). MPs analysis involves multiple steps, including extraction, sieving, and/or vacuum filtration, visual quantification or microscopy/stereomicroscopy, and identification using spectroscopic techniques (De Frond *et al*., 2023). Although numerous analytical methods are available to detect, identify, and quantify MPs in different environmental settings (Jenkins *et al*., 2022; Kaile *et al*., 2020), most are time-consuming, and the results are often not intercomparable (Kaile *et al*., 2020; Martínez-Francés *et al*., 2023).

Nowadays, to the best of the author's knowledge, most published protocols for MPs detection and quantification are time-consuming and unreliable. This study adds to the growing literature on MPs detection and quantification; but also presents preliminary results from the detection and quantification of MPs in liquid samples, using ImageJ software for quantification, and without vacuum filtration. This protocol is carried out in two stages. The first stage consists of preparing liquid samples of different concentrations of virgin polyethylene (PE) MPs dyed with Nile Red (NR) in the laboratory, while the second stage involves the detection and quantification of MPs using ImageJ software. PE MPs were used because polyethylene is the most widely consumed plastic material worldwide (Liang *et al*., 2025). MPs were dyed with NR due to PE has high affinity for this dye, similar to other types of MPs (Anger *et al*., 2018; Chen *et al*., 2021; Erni-Cassola *et al*., 2017; Huppertsberg & Knepper, 2018; Leonard *et al*., 2022; Li *et al*., 2018; Maes *et al*., 2017; Mason *et al*., 2018; Mintenig *et al*., 2019; Mukhanov *et al*., 2019; Prata *et al*., 2019; ; Prata *et al*., 2020; Rocha-Santos & Duarte, 2015; Schymanski *et al*., 2018; Shim *et al*., 2016; Stanton *et al*., 2019; Wang & Wang, 2018).

ImageJ software is usually used for detecting and quantifying MPs; but also, this software allows to determine another MPs characteristic like morphology and size, allowing detailed analysis and automatic quantification of MPs across large datasets (Bankhead, 2014; Chen *et al*., 2021; Mukhanov *et al*., 2019; Prata *et al*., 2019; Prata *et al*., 2020). Nevertheless, the threshold value is an important parameter in MPs quantification because it significantly influences the results. The threshold value separates pixel intensities in an image to create a binary representation, provides an optimal balance between reducing false positives from background noise and enhancing the recognition of fluorescent MPs (Nichele *et al*., 2020). Furthermore, it provides a better signal-to-noise ratio and allows the identification of pixels above the threshold value, foreground, and below background. In ImageJ, pixels that do not meet the threshold value are shown as white, while those that do are shown as black (Chen *et al*., 2021).

This study aims to report preliminary results on the quantification of PE MPs, dyed with NR in liquid samples using ImageJ software; this procedure addresses a new, less time-consuming, and reliable method for quantifying PE MPs in aqueous solutions.

MATERIALS AND METHODS

Preparation of MPs samples

MPs were obtained from single-use commercial virgin PE bags from Reyma®, obtained in Plásticos Comte©, a plastic products store in Aguascalientes, Ags. México, through a process of cutting, grinding, and sieving, the particle size was between 250-500 µm.

MPs were stained adding 1 mL of RN stock solution, prepared with methanol, 10 µg mL^{-1}, and the MPs to a 10 mL volumetric flask, filling up with HPLC grade water to obtain an RN concentration of 1 µg mL-1. Once MPs were stained, they were added to the glass cells. According to other studies, this concentration caused the least background fluorescence and is enough to stain different plastic types, providing an acceptable balance between speed, visibility, and background signal (Maes *et al*., 2017; Wiggin & Holland, 2019). The staining solution was stirred at 100 rpm for 15 minutes and allowed to incubate in darkness at room temperature for 24 hours (Erni-Cassola *et al*., 2017).

All the liquid samples, containing 5, 10, 16, and 25 stained MPs, were prepared into glass cells of 3.5 mL, using HPLC grade water; five replicates per concentration were prepared. MPs were individually counted and placed into glass cells. To minimize cross-contamination, cotton clothing and nitrile gloves were worn, and lab surfaces were cleaned with a 30% deionized water/ethanol solution before each use. Additionally, all glassware was pre-washed and rinsed three times with a 50% deionized water/ethanol solution (Cruz-Salas *et al*., 2023; Leonard *et al*., 2022; Maes *et al*., 2017; Schymanski *et al*., 2018).

Images acquisition

Images of stained MPs in liquid samples were captured using a Logitech C922 Pro Stream webcam with a resolution of 3 megapixels, 1920x1080, while being exposed to a 450 nm blue laser. The setup included a circular lens and an orange filter, $\lambda\sim$570 nm Edmund Optics OG. The blue laser was used to induce fluorescence in the MPs (Erni-Cassola *et al.,* 2017*;* Leonard *et al*., 2022; Mason *et al*., 2018; Prata *et al*., 2019), while the orange filter enhanced image quality by blocking the blue light. A total of 250 images for each concentration were captured.

Quantification of MPs

Quantification of MPs was performed using ImageJ software, 1.52v developed by the National Institutes of Health (NIH), in three steps: a) Conversion into 8-bit grayscale: since this format is required for MPs quantification process (Bankhead, 2014). b) Image thresholding: is a technique that simplifies a grayscale image into a binary image by classifying each pixel value as either black or white based on its intensity level or gray-level compared to the threshold value. So, the threshold value is a critical parameter in the quantification of MPs to detect the MPs from the background, below the threshold value (Nichele *et al*., 2020). Although ImageJ provides various binarization tools, the default tool was used because the auto-thresholding is a variation of the IsoData algorithm. The threshold values used in this work were 60 to 100 in increments of 10 to achieve an optimal balance between reducing the false positives from background noise and enhancing detection of fluorescent MPs, which also improves the signal-to-noise ratio. In ImageJ software, after applying the threshold, any MPs that do not meet the level of fluorescence are displayed as white, while any MPs that meet the requirement are displayed as black (Chen *et al*., 2021). c) MPs quantification: the particle size detection limit was set to 25 px^2 to ensure that image noise did not interfere with the quantification results (Mukhanov *et al*., 2019).

Statistical analysis

Statistical analysis of MPs quantification results was performed using Minitab® 18.1v software, applying the Anderson-Darling normality test with a significance level of 0.05 to assess the reliability of method proposed in this study (Minitab, 2019).

RESULTS AND DISCUSSION

In this work, laboratory-prepared solutions with known concentrations, 5, 10, 16, and 25, of MPs stained with NR, a widely used dye due to its fast-staining capability, low cost, high fluorescence intensity, and effectiveness in dyeing and fluorescing almost all kinds of polymers and textile fibers, except nylon, were used (Prata et al., 2019). This method provides reliable results under blue or UV light (Chen *et al*., 2021; Erni-Cassola *et al*., 2017; Leonard *et al*., 2022; Maes *et al*., 2017; Mason *et al*., 2018; Prata *et al*., 2019; Shim *et al*., 2016; Stanton *et al*., 2019).

The images were captured while MPs were suspended in water, providing an advantage over other reported methods, since, to the best of the author's knowledge, most reported methods rely on vacuum filtration, which can lead to significant MPs loss and introduce a significant error during quantification. Additionally, it is worth mentioning that quantification is usually performed while MPs are in a solid medium, such as filter paper.

A total of 1000 images of all different concentrations of MPs in liquid samples were processed and analyzed using ImageJ software (Abràmoff *et al*., 2004; Forero *et al*., 2009; Mahadevan *et al*., 2013). MPs were counted by converting the images into 8-bit-grayscale and using different threshold values, see Figure 1. Due to the threshold value is a critical parameter in the quantification results of MPs, we use different threshold values, 60-100.

The results obtained with a threshold value of 60 are higher than the real concentrations for all the samples analyzed. In contrast, threshold values of 80, 90, and 100 yield results lower than the actual concentrations because of reducing the visualization of MPs. A threshold value of 70 gives average results closest to the real concentration of the samples prepared in the laboratory, except for the 25 MPs sample, therefore, this threshold value was used to quantify the MPs. It is important to highlight that for the 25 MPs concentration, all threshold values give results below the real concentration. Table 1 shows the statistical analysis of mean values and standard deviations for 5, 10, 16, and 25 MPs, highlighting the significant influence of threshold values on MPs quantification results. It is important to select the appropriate threshold value according to image quality to achieve optimal results in MPs quantification.

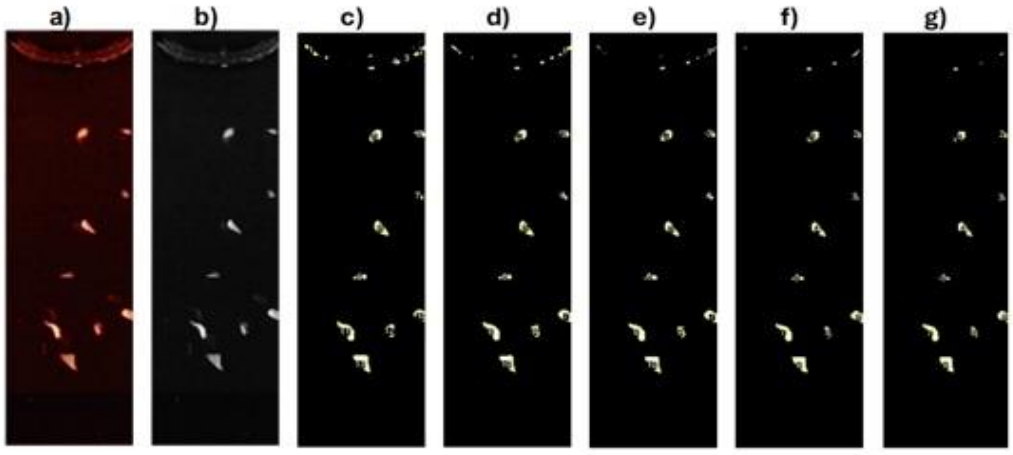

Fig. 1: Images for the 10 MPs concentration processed with ImageJ software a) Original image of MPs in liquid samples; b) Image converted into 8 bits-grayscale; c), d), e), f), and g) Thresholded image using a threshold value of 60, 70, 80, 90, and 100, respectively.

Table 1: Mean and standard deviation of MPs quantification across different threshold values.

MPs	Threshold	Mean	StDev		MPs	Threshold	Mean	StDev
5	60	7.244	3.754		16	60	16.392	3.479
	70	5.772	3.119			70	14.492	3.468
	80	4.776	2.664			80	12.620	3.156
	90	4.296	2.417			90	11.356	3.177
	100	3.772	2.115			100	10.116	3.242
10	60	11.368	4.053		25	60	23.820	5.788
	70	9.756	3.877			70	21.332	6.931
	80	8.272	3.378			80	19.040	7.703
	90	7.212	3.285			90	16.876	8.359
	100	6.356	3.173			100	15.288	8.457

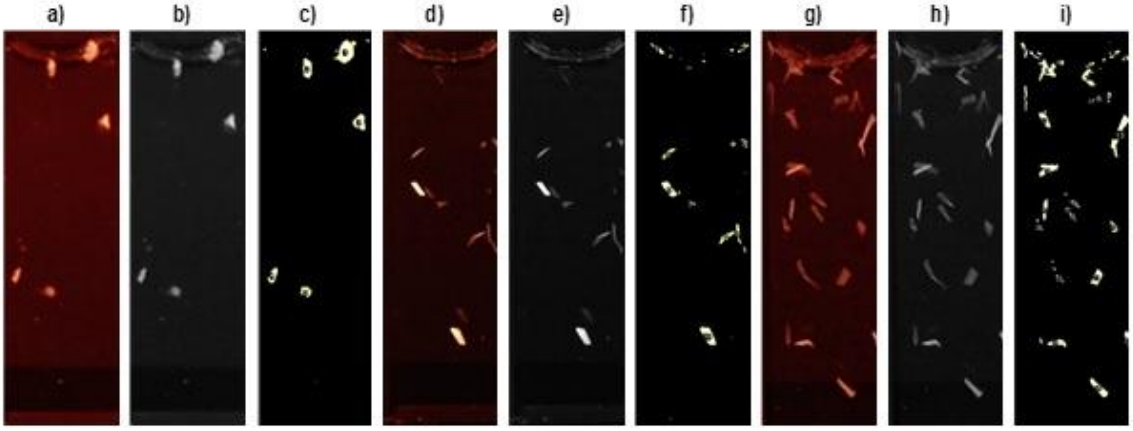

Fig. 2: Images of MPs in liquid sample during quantification process using ImageJ; a), d), and g) original images; b), e), and h) images converted into 8-bit grayscale; c), f), and i) thresholded images.

In some images of 25 MPs concentration, overlapping and agglomeration of MPs can be observed (see figure 2), which could be caused by overconcentration or the buoyancy of MPs. The buoyancy of MPs is a factor that does not allow obtaining a solution with homogeneously distributed suspended particles (Leonard *et al*., 2022). Once the images are thresholded, these characteristics could not affect the quantification results of MPs using ImageJ software. The results closest to the real concentration of MPs in liquid samples were obtained using a threshold value of 70; therefore, this threshold value was used to threshold the images for quantifying MPs.

Statistical analysis of MPs quantification

The box plot diagram of the results of MPs quantification is shown in Figure 3. Each concentration has its own box plot, allowing comparison of data spread, central tendency, and variability across all the concentrations of MPs. The results of 5 MPs have less variability, with the box and whiskers covering values from approximately 2 to 12, but also, outliers are present above the upper whisker. For 10 MPs, the median is higher than that of the 5 MPs concentration, and the interquartile range, IQR, is slightly higher, suggesting more spread; there are no noticeable outliers, but the data range extends further than in 5 MPs. For 16 MPs, the median is higher than that of 5 and 10 MPs and the data distribution shows a slight increase in variability, but also, there are a few outliers above the upper

whisker. The results for 25 MPs concentration show the highest median and the largest IQR, indicating a wider spread of data. The whiskers extend significantly, implying more variability in the data and no outliers are visible.

The box plots analysis shows a progressive increase in the medians and the overall dispersion as increase the MPs concentration; this trend suggests that the variation in quantification results increases as the concentration of MPs increases.

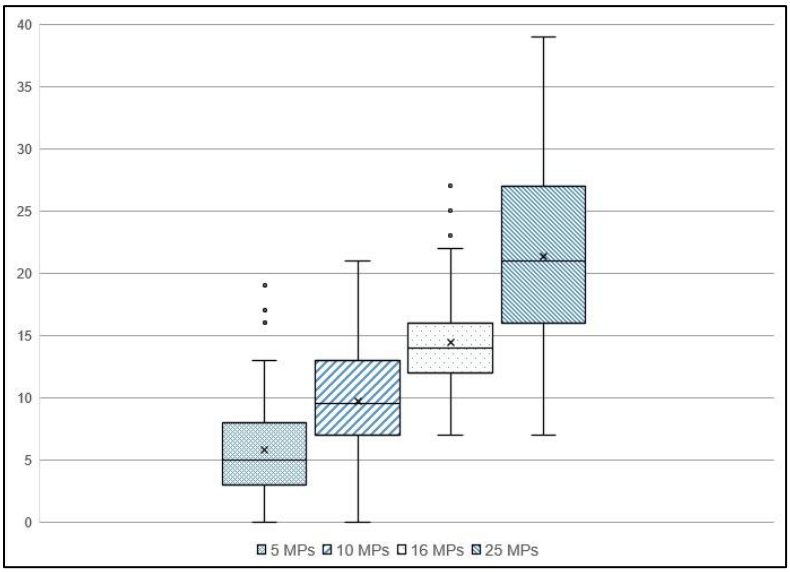

Fig. 3: Box plot diagram of MPs quantification results using ImageJ of a) 5, b) 10, c) 16, and d) 25 MPs.

The quantification results obtained using the ImageJ software with a threshold value of 70 were analyzed statistically to assess the reliability of the method. For 5 MPs, 39.2% of the data fall within the range of 4–6 MPs; For 10 MPs, 44.4% of the data fall within 8–12 MPs; similarly, 48.8% of the data for 16 MPs fall within 14–18 MPs and 45.6% of the data for 25 MPs fall within 20–30 MPs. The dispersion of the data suggests minor deviations from a normal distribution, but these are not significant, indicating that the reliability of the method is acceptable. It is important to highlight that the means were also analyzed, showing that the results for concentrations of 5, 10, and 16 MPs had average values ($\bar{X}$) close to the real values, with a standard deviation (σ) ranging between 3 and 4. On the other hand, the concentration of 25 MPs had an $\bar{X}$ of 21.34 and a σ of 6.9, indicating the greatest variability in the MPs quantification results, see Table 2 for details. These results are according to the box and plot diagram.

Table 2: Statistical analysis of MPs quantification using a threshold value of 70.

MPs	Mean	StDev	Variance	Median	Mode
5	5.77	3.12	9.73	5	3
10	9.76	3.88	15.03	9.5	9
16	14.49	3.47	12.03	14	15
25	21.33	6.93	48.04	21	15, 18

Based on the mean and standard deviation values, it could be stated that for concentrations up to 16 MPs, the results are accurate and reliable. However, for 25 MPs, the dispersion of the results increases significantly, which could be attributed to certain image characteristics, such as fluorescence intensity and particle overlap. Therefore, the statistical analysis shows that the accuracy of the results depends on the MPs concentration.

The protocol proposed in this work offers the advantages of non-visual counting, automated quantification, and avoiding the use of traditional spectroscopic methods like infrared and Raman microspectroscopy. Moreover, it is a cheaper protocol than the conventional methods used due to it using a low-power laser and the camera is a common webcam. This protocol would aid the development of a method alongside the equipment, significantly enhancing the studies on plastic pollution by providing more accurate data on MNPs in various ecosystems.

The protocol proposed in this work offers several advantages, including non-visual counting, semi-automated quantification, and the avoidance of traditional spectroscopic methods. Moreover, it is more cost-effective than conventional methods, as it uses a low-power laser and a standard webcam. This protocol could contribute to the development of both methods and equipment, significantly enhancing studies on plastic pollution by providing more accurate and reliable data.

CONCLUSIONS

The quantification of MPs using ImageJ software shows variations in results depending on the threshold value used. Thus, the threshold value is a critical parameter for accurately quantifying suspended MPs in liquid samples because some images characteristics may introduce uncertainties to the results. However, once the optimal threshold value is selected, reliable results could be obtained. In this work, a threshold value of 70 was used, as it provides an optimal balance between false positives and true MPs and allows obtain results closest to the real concentration of MPs in liquid samples. The mean and standard deviation values for each concentration highlight how the results vary with different threshold values.

Although several techniques are available for analyzing MPs, there is no universally accepted analytical method for their detection or quantification. Moreover, each reported study about MPs uses many different techniques and shows a wide range of results. The purpose of presenting these preliminary results on the detection and quantification of MPs using NR staining and ImageJ was to provide a reliable, less time-consuming, the vacuum filtration of MPs is avoided since the quantification is carried out while MPs are in liquid samples, and simple protocol for the quantification of MPs in liquid samples; but also, the results show that this protocol is reliable. It is important to highlight that the proposed protocol is an automated alternative for quantifying MPs that does not require specialized and expensive equipment like microscopes, spectrophotometers, stereoscopes. Moreover, this method could be used to quantify all kinds of MPs due to NR having affinity to another types of MPs. The main advantage is that it does not require visual quantification of the MPs, improving both the accuracy and repeatability of the results and the ability to process numerous samples. This protocol would aid the development of a method alongside the equipment to study plastic pollution.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support provided by Tecnológico Nacional de México, for funding this work through Project Number 20011.24-P and to CONACYT for the National Scholarship 2020-1, Number 2020-000013-01NACF-00146.

REFERENCES

Abràmoff, M.D., Magalhães, P.J. & Ram, S.J. (2004). Image processing with imageJ. *Biophotonics International, 11* (7), 36–41. https://imagej.net/ij/docs/pdfs/Image_Processing_with_ImageJ.pdf

Anger, P.M., von der Esch, E., Baumann, T., Elsner, M., Niessner, R. & Ivleva, N.P. (2018). Raman microspectroscopy as a tool for microplastic particle analysis. *TrAC - Trends in Analytical Chemistry, 109*, 214–226. https://doi.org/10.1016/j.trac.2018.10.010

Bankhead, P. (2014). *Analyzing fluorescence microscopy images with ImageJ*. ImageJ. https://confocal.uconn.edu/wp-content/uploads/sites/1081/2016/02/2014-05_Analyzing_fluorescence_ microscopy _images.pdf

Chen, S., Li, Y., Mawhorter, C. & Legoski, S. (2021). Quantification of microplastics by count, size and morphology in beverage containers using Nile Red and ImageJ. *Journal of Water and Health, 19* (1), 79–88. https://doi.org/10.2166/wh.2020.171

Cruz, A.A, Álvarez, J.C., Tapia, J., Pérez, B., Martínez, C., Vázquez, A., *et al*. (2023). Measures to prevent across-contamination in the analysis of microplastics: A short literature review. *Revista Internacional de Contaminación Ambiental, 39*, 241–256. https://doi.org/10.20937/RICA.54740

De Frond, H., Cowger, W., Renick, V., Brander, S., Primpke, S., Sukumaran, S., *et al*. (2023). What determines accuracy of chemical identification when using microspectroscopy for the analysis of microplastics? *Chemosphere, 313*, 137300. https://doi.org/10.1016/j.chemosphere.2022.137300

EFSA CONTAM, P. P. on C. in the F. C. (CONTAM). (2016). Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA Journal, 14* (6). e04501. https://doi.org/10.2903/j.efsa.2016.4501

Environment and Climate Change Canada (2019). *Environment and Climate Change Canada. Canada's Plastics Science Agenda*., 1–31. https://wedocs.unep.org/bitstream/handle/20.500.11822/41263/Plastic_Science_E.pdf

Erni-Cassola, G., Gibson, M.I., Thompson, R.C. & Christie-Oleza, J.A. (2017). Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 μm) in Environmental Samples. *Environmental Science and Technology, 51* (23), 13641–13648. https://doi.org/10.1021/acs.est.7b04512

Forero, M.G., Pennack, J.A., Learte, A.R. & Hidalgo, A. (2009). DeadEasy Caspase: Automatic Counting of Apoptotic Cells in Drosophila. *PLoS ONE, 4* (5), e5441. https://doi.org/10.1371/journal.pone.0005441

Huppertsberg, S. & Knepper, T.P. (2018). Instrumental analysis of microplastics—benefits and challenges. *Analytical and Bioanalytical Chemistry, 410* (25), 6343–6352. https://doi.org/10.1007/s00216-018-1210-8

Jenkins, T., Persaud, B.D., Cowger, W., Szigeti, K., Roche, D.G., Clary, E., *et al*. (2022). Current State of Microplastic Pollution Research Data: Trends in Availability and Sources of Open Data. *Front. Environ. Sci. 10:912107.* https://doi.org/10.3389/fenvs.2022.912107

Kaile, N., Lindivat, M., Elio, J., Thuestad, G., Crowley, Q.G. & Hoell, I.A. (2020). Preliminary Results from Detection of Microplastics in Liquid Samples Using Flow Cytometry. *Front. Mar. Sci. 7,* 552688. https://doi.org/10.3389/fmars.2020.552688

Leonard, J., Koydemir, H.C., Koutnik, V.S., Tseng, D., Ozcan, A. & Mohanty, S. K. (2022). Smartphone-enabled rapid quantification of microplastics. *Journal of Hazardous Materials Letters, 3*, 100052. https://doi.org/10.1016/j.hazl.2022.100052

Li, J., Liu, H. & Paul Chen, J. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Research, 137*, 362–374. https://doi.org/10.1016/j.watres.2017.12.056

Liang, J-f., Wang, J., Qin, S., & Qu, J-p. (2025). Super toughness and reinforcement of recycled high-density polyethylene/poly(ethylene terephthalate) composite achieved by oriented spherical crystal structure. *Composites Part B: Engineering*, 294(6550):112158. https://doi.org/10.1016/j.compositesb.2025.112158

Maes, T., Jessop, R., Wellner, N., Haupt, K. & Mayes, A.G. (2017). A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Sci. Rep. 7,* 44501. https://doi.org/10.1038/srep44501

Mahadevan, T.S., Milosevic, M., Kojic, M., Hussain, F., Kojic, N., Serda, R., *et al.* (2013). Diffusion transport of nanoparticles at nanochannel boundaries. *J. Nanopart. Res. 15,* 1477. https://doi.org/10.1007/s11051-013-1477-9

Martínez-Francés, E., van Bavel, B., Hurley, R., Nizzetto, L., Pakhomova, S., Buenaventura, *et al*. (2023). Innovative reference materials for method validation in microplastic analysis including interlaboratory comparison exercises. *Analytical and Bioanalytical Chemistry, 415* (15), 2907–2919. https://doi.org/10.1007/s00216-023-04636-4

Mason, S.A. Welch, V.G. & Neratko, J. (2018). Synthetic Polymer Contamination in Bottled Water. *Front. Chem. 6,* 407. doi: 10.3389/fchem.2018.00407

Minitab (2019). Soporte de Minitab® 18. https://support.minitab.com/es-mx/minitab/18/

Mintenig, S.M., Löder, M.G.J., Primpke, S. & Gerdts, G. (2019). Low numbers of microplastics detected in drinking water from ground water sources. *Science of the Total Environment, 648*, 631–635. https://doi.org/10.1016/j.scitotenv.2018.08.178

Mukhanov, V.S., Litvinyuk, D.A., Sakhon, E.G., Bagaev, A.V., Veerasingam, S. & Venkatachalapathy, R. (2019). A new method for analyzing microplastic particle size distribution in marine environmental samples. *Ecologica Montenegrina, 23*, 77–86. https://doi.org/10.37828/em.2019.23.10

Munoz-Pineiro, M.A. (2018). *Microplastics - Focus on Food and Health*. Publications Office of the European Union, Luxembourg, ISBN N/A, JRC110629. https://publications.jrc.ec.europa.eu/repository/handle/JRC110629

Nichele, L., Persichetti, V., Lucidi, M. & Cincotti, G. (2020). Quantitative evaluation of ImageJ thresholding algorithms for microbial cell counting. *OSA Continuum, 3* (6), 1417.

Pastor, C.B. & Agulló, D.V. (2019). Presence of microplastics in water and the potential impact on public health. *Revista Española de Salud Pública, 93*. https://scielo.isciii.es/pdf/resp/v93/1135-5727-resp-93-e201908064.pdf

Periyasamy, A.P. (2023). Environmentally Friendly Approach to the Reduction of Microplastics during Domestic Washing : Prospects for Machine Vision in Microplastics Reduction. *Toxics, 11* (575), 15–31. https://doi.org/10.3390/toxics11070575

Picó, Y. & Barceló, D. (2019). Analysis and prevention of microplastics pollution in water: Current perspectives and future directions. *ACS Omega, 4* (4), 6709–6719. https://doi.org/10.1021/acsomega.9b00222

Prata, J.C., Alves, J.R., da Costa, J.P., Duarte, A.C. & Rocha-Santos, T. (2020). Major factors influencing the quantification of Nile Red stained microplastics and improved automatic quantification (MP-VAT 2.0). *Science of the Total Environment, 719*, 137498. https://doi.org/10.1016/j.scitotenv.2020.137498

Prata, J.C., Reis, V., Matos, J.T.V., da Costa, J.P., Duarte, A.C. & Rocha-Santos, T. (2019). A new approach for routine quantification of microplastics using Nile Red and automated software (MP-VAT). *Science of the Total Environment, 690*, 1277–1283. https://doi.org/10.1016/j.scitotenv.2019.07.060

Rezania, S., Park, J., Md Din, M. F., Mat Taib, S., Talaiekhozani, A., *et al*. (2018). Microplastics pollution in different aquatic environments and biota: A review of recent studies. *Marine Pollution Bulletin*, 133, 191–208. https://doi.org/10.1016/j.marpolbul.2018.05.022

Rocha-Santos, T., & Duarte, A. C. (2015). A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. *TrAC - Trends in Analytical Chemistry, 65*, 47–53. https://doi.org/10.1016/j.trac.2014.10.011

Schymanski, D., Goldbeck, C., Humpf, H.U. & Fürst, P. (2018). Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water. *Water Research, 129*, 154–162. https://doi.org/10.1016/j.watres.2017.11.011

Shim, W.J., Song, Y.K., Hong, S.H. & Jang, M. (2016). Identification and quantification of microplastics using Nile Red staining. *Marine Pollution Bulletin, 113* (1–2), 469–476. https://doi.org/10.1016/j.marpolbul.2016.10.049

Silva, A.B., Bastos, A.S., Justino, C.I.L., da Costa, J.P., Duarte, A.C. & Rocha-Santos, T.A.P. (2018). Microplastics in the environment: Challenges in analytical chemistry - A review. *Analytica Chimica Acta*, 1017, 1–19. https://doi.org/10.1016/j.aca.2018.02.043

Stanton, T., Johnson, M., Nathanail, P., Gomes, R.L., Needham, T. & Burson, A. (2019). Exploring the Efficacy of Nile Red in Microplastic Quantification: A Costaining Approach. *Environmental Science and Technology Letters, 6* (10), 606–611. https://doi.org/10.1021/acs.estlett.9b00499

Wang, W. & Wang, J. (2018). Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. *TrAC - Trends in Analytical Chemistry, 108*, 195-202. https://doi.org/10.1016/j.trac.2018.08.026

Wiggin, K.J. & Holland, E.B. (2019). Validation and application of cost and time effective methods for the detection of 3–500 Mm sized microplastics in the urban marine and estuarine environments surrounding Long Beach, California. *Marine Pollution Bulletin, 143*, 152–162. https://doi.org/10.1016/j.marpolbul.2019.03.060