Research article

Occurrence and risk assessment of fullerene colloidal nanoparticles by ultrasonic-assisted dispersive liquid-liquid extraction and high-performance liquid chromatography in surface waters

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ABSTRACT

This paper presents a developed analytical technique for risk assessment of colloidal fullerene in surface waters by ultrasonic-assisted dispersive liquid-liquid extraction (UADLLE) and high-performance liquid chromatography ultraviolet-visible detector (HPLC-UV). Fullerene colloidal nanoparticles were synthesised and characterized by high-resolution transmission electron microscopy (HRTEM) and ultraviolet-visible spectroscopy (UV-Vis). Ultra-sonication step, disperser solvent, and sodium chloride salt enhance the surface area of fullerene derivative aggregates for better contact and lowers the solubility of fullerene derivative to the aqueous solution, respectively promoting mass transfer of fullerene from aqueous into the organic phase. Several extraction parameters were optimized, and the optimal conditions were established: 5 mL toluene as extraction solvent (2 cycles); 200 mL water sample; 1% sodium chloride salt; 15 min ultrasonication, and 400 μL methanol as disperser solvent. The mean absolute recoveries established in drinking water, wastewater, and river water were 117%, 103%, and 93%, respectively. The proposed analytical technique was linear in the ranges between 0.25 μg L^{-1} – 250 μg L^{-1} with an r-squared of 0.9958. The limit of detection (LOD) determined from the signal-to-noise ratio of 3 was 0.11 μg L^{-1} and the limit of quantification (LOQ) from a signal-to-noise ratio of 10 was 0.38 μg L^{-1}. The precision ranges from 2% to 11% and accuracy percent error ranged from 7%–14% for spiked concentration levels of 0.25 μg L^{-1}, 50 μg L^{-1}, and 250 μg L^{-1}. The measured environmental concentration (MECs) for the fullerene in water samples ranged from not detected to 10.54 μg L^{-1} and ecological assessment showed the concentration level of the fullerene can pose risk. Overall, according to the author's knowledge, this is the earlier work on the occurrence and risk assessment of fullerene colloidal nanoparticles (C61-PCBM) in potable and wastewater on the African continent.

1. Introduction

Fullerenes are carbonaceous nanomaterials discovered in 1985 (Kroto et al., 1985). They possess certain unique properties, which differentiates them from other nanomaterials (Wang et al., 2018). The well-known physicochemical property of fullerene is a time-dependent transition from a hydrophobic form to a polar form (Zouboulaki and Psillakis, 2016). Although fullerenes are typically insoluble in water, upon long time exposure in aqueous matrices they produce stable colloidal nanoparticles due to van der Waals attraction between fullerene nanoparticles (Zouboulaki and Psillakis, 2016). Fullerenes colloidal nanoparticles form hydrophilic and complex hydrated aggregates of C_{60} (Astefanei et al., 2015).

A pathway of fullerenes into the environment from anthropogenic sources is through wastewater treatment plants effluent and leachate from landfills (Kim et al., 2020). However, naturally occurring events that result in the generation of fullerenes are the main sources of the fullerenes in surface waters. Natural processes that result in the formation of fullerenes include forest fires and volcanic eruptions. In some cases, they are formed accidently by human activities such as incomplete combustion leading to soot containing fullerene in diesel exhaust (Gataullin et al., 2019; Zhang et al., 2021). Moreover, Yang et al. reported that the increasing application of fullerene in industry, and in many areas of research such as biochemical, solar cells, optics, and formulation in personal care products have led to the surge in the occurrence of fullerenes in surface waters (Yang et al., 2013). Keller et al. and Wigger et al. have found that various fullerenes enable products to reach wastewater treatment plants and landfills contributing to the increased release from these sectors (Keller et al., 2014) (Wigger et al., 2015).

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The usage of sewage sludge contaminated with fullerenes in agricultural fields is another prominent pathway of the fullerenes into the environment (Wang et al., 2020). Although uniqueness together with the nano-sized scale of fullerene gives them better characteristics, the ecological risk associated with them in the ecosystem when released into the receiving water is becoming a concern to public health in general (Kim et al., 2020). According to (Pakarinen et al., 2011) fullerenes in the river ecosystem, form aggregates and settle into sediments, thereby increasing the exposure of benthic organisms to fullerenes (Chen and Elimelech, 2006). Moore et al. concluded that, the presence of fullerenes in an ecological system should be classified as contaminants of emerging concerns (CECs) (Von der Kammer et al., 2012; Moore et al., 2019).

Keller et al. and Wigger et al. suggested that the potentially adverse environmental impacts of fullerenes might be equally variable due to their diverse sources (Keller et al., 2014; Wigger et al., 2015). Von der Kammer et al. proposed that toxicological studies of CECs must use biological species that play a role in ecosystem processes and services (Von der Kammer et al., 2012). Auwerter et al. investigated the Effects of nanosized titanium dioxide (TiO2) and fullerene (C60) on wastewater microorganisms activity (Auwerter et al., 2017). This study focused on the aerobic microorganisms from the wastewater treatment plant in the stage of the first sedimentation (primary). The study established that the amount and the time that the organisms are exposed to the nanoparticles plays a huge role on the toxic effect (Auwerter et al., 2017).

An important aspect of environmental ecological risk assessment is an analytical technique that can study the fate and occurrence of fullerenes in surface waters at relevant concentration levels (Sanchis et al., 2020). There is also a shortage of reliable analytical protocols for the measurement of fullerenes sizes, shape determination, and concentration in the environmental and biota samples (Gataullin et al., 2019). Gottschalk et al. studied the occurrence of fullerenes in the environmental samples and found the concentration to range from 0.003 ng L^{-1}–5.2 ng L^{-1} in surface water and wastewater (Gottschalk et al., 2009). Also, many researchers have reported the occurrence of fullerenes in the environment (Benn et al., 2011; Carboni et al., 2014; Emke et al., 2015; Pérez et al., 2013; van Wezel et al., 2011). There is diversity in analytical protocols that have been described to extract fullerenes from surface waters (Astefanei et al., 2015; Yi et al., 2017). Often used analytical methods to extract fullerenes from surface waters are liquid-liquid extraction and solid-phase extraction. Emerging novel analytical methods such as ultrasonic-assisted dispersive liquid-liquid microextraction have been employed with success for the extraction of fullerenes from surface waters (Sedov et al., 2020). HPLC is a technique of choice for most studies for the separation of fullerene, but some techniques like capillary electrophoresis and asymmetric flow field fractionation are emerging. Mass spectrometry and UV-vis are mostly used detectors to study fullerenes in surface waters. Wang et al. developed an analytical technique for analysis of fullerenes in surface waters employing dispersive liquid-liquid microextraction followed by detection with HPLC (Wang et al., 2018). Also, Wang et al. quantified fullerene colloidal nanoparticles in wastewater using liquid-liquid extraction and solid phase extraction, UV-vis and mass spectrometer were used as the detectors, analytes were separated by HPLC method (Wang et al., 2010). Many studies have used these techniques for the detection of fullerenes in surface waters (Pérez et al., 2013; Wang et al., 2018; Shareef et al., 2010).

The challenge in ecological risk assessment of fullerenes in surface waters is to have an analytical technique that can determine the occurrence of fullerenes at trace levels. Currently, environmental investigations are based on C_{60} or C_{70} types of fullerenes, however analytical techniques that can analyse fullerene colloidal nanoparticles derivatives (functionalized) in surface waters are limited. This work presents an analytical method development and optimization of extraction parameters for a cheap and simple UADLLE/HPLC-UV-vis analytical method for occurrence and risk assessment of fullerene colloidal nanoparticles in South African surface waters. The developed method is promising and can be used for monitoring fullerene colloidal nanoparticles in water treatment plant's effluents and receiving environmental waters. This is first report on utilization of the developed extraction method to study risk assessment of fullerene in surface waters. According to author's earlier work on occurrence of fullerene nanoparticles in African Surface Waters.

2. Experimental

2.1. Chemicals and reagents

Fullerene standard C61 fullerene, [6, 6]-phenyl C61 butyric acid methyl ester (C61-PCBM), of >99.5% purity, sodium chloride (≥99.5% ACS reagent), toluene, acetone, and methanol were Sigma-Aldrich (Steinheim, Germany) chemicals supplied by Merck, South Africa. All solvents used were of CHROMASOLV® (99%) grade. Millipore water was collected from a Millipore Elix system bought from Microsep South Africa and had a resistivity of 18 MΩ cm.

2.2. Apparatus and glassware

Glassware; separating funnel, volumetric flasks, and beakers were washed and rinsed with acetone and heated (150 °C) for 24 h to prevent emulsion. Amber bottles were used for sampling, after being pre-cleaned with acid and dried. Small volumes were delivered by a micropipette plus kit Sorox lab (China) starting from 100 to 1000 µL, and 5 mL. All Millipore filters were purchased from Anatech, South Africa. HPLC vials (amber) were bought from Chemetrix, South Africa.

2.3. Instruments

The identification and the quantification of the analyte was conducted with an Agilent 1200 series HPLC system equipped with an auto-injector coupled with an 1100 series MSD Trap UV-vis (Agilent Technologies). High-Resolution Transmission Electron Microscopy (HRTEM), UV-Vis spectrometer (optics, Netherlands through Narich suppliers), an ultrasonic bath was Scientech (frequency of 50 kHz, and a nominal power 150 W) bought from DLD scientific, pH meter (Eutech) bought from Labfriend, and hot air oven (50–150 °C) bought from Separations.

2.4. Sampling

River water samples were collected at various sampling points along Msunduzi and Mgeni Rivers, coordinates are presented in T. 1 supplementary paper. Wastewater samples were taken from the wastewater treatment plant (WWTP) in Pietermaritzburg City, and the drinking water was sampled using grab sampling method the drinking treatment plants in Durban. All the water samples were collected in amber glass bottles, and were well labelled. The samples were transported under 4 °C to the laboratory for further analysis. The sample extraction commenced immediately after reaching the laboratory. Details of sampling points are summarised in the flow diagrams presented in F. 1 and 2 of the supplementary paper.

2.5. Preparation of fullerene solutions

2.5.1. Preparation of fullerene stock solution and C61-PCBM standards in toluene

A 100 mg L^{-1} stock solution of fullerene was made by dissolving 10 mg of C61-PCMB in 100 mL of toluene in a volumetric flask. Thereafter, working solutions of 10 mg L^{-1} were prepared by transferring 100 µL of 100 mg L^{-1} into a 1 mL volumetric flask, and 1 mg L^{-1} working solution was prepared by transferring 100 µL of 10 mg L^{-1} into a 1 mL volumetric flask. To calibrate the instrument, working solutions were used. The successive dilution was employed for the preparation of the working standard solution used in HPLC-UV-vis analyses and calibration. All standards for external calibration curves were prepared in toluene.

Working standards on concentrations of 5 mg L^{-1}, 10 mg L^{-1}, 20 mg L^{-1}, 30 mg L^{-1}, 40 mg L^{-1}, and 50 mg L^{-1} were prepared from the 100 mg L^{-1} stock solution of fullerene. Fullerene solutions of 0.5 mg L^{-1} and 1 mg L^{-1} were prepared from 5 mg L^{-1} and 10 mg L^{-1}, respectively.

2.5.2. Preparation of fullerene colloidal nanoparticles standards in aqueous solution

Fullerene colloidal nanoparticles standards for the internal calibration curve were prepared following the method of (Chen et al., 2008) and (Wang et al., 2018) with slight modifications after optimisation. To prepare colloidal fullerene nanoparticles standards in an aqueous solution, 1 mL of varying concentration were added into Millipore water such that the final volume was 200 mL and sonicated for 15 min to make colloids. The final concentration of standards in 200 mL was between 0.25 μg L^{-1}–250 μg L^{-1}.

2.6. Spiking and sample extraction

2.6.1. Spiking

The spiked 200 mL aqueous solutions of fullerene colloidal nanoparticle standards (25 μg L^{-1}, 50 μg L^{-1}, 250 μg L^{-1}), as well as; the river, drinking, and the wastewater were also spiked for determination of recoveries, precision, and accuracy. All the spiked aqueous solutions were extracted and analysed the same as the samples.

2.6.2. Sample extraction by UADLLE

About 200 mL of aqueous sample (drinking water, wastewater, and river water) was quantitatively transferred into a 250 mL conical flask, followed by the addition of 1 g of sodium chloride and 400 μL methanol. Sodium chloride and methanol were added to the water samples with the fullerenes to control the ionic strength and as disperser solvents respectively. After the samples were sonicated for 15 min, then 5 mL of the extraction solvent (toluene) was added and the samples were mixed and sonicated again for 15 min to transfer the colloidal fullerene nanoparticle into the toluene phase. The phases were allowed to separate in the separating funnels for 30 min. The aqueous phase of each sample was collected in the conical flasks, while the organic phase was transferred into a vial. The extraction step with 5 mL toluene was done in two cycles and the extracts collected were combined. The combined extracts were concentrated using the solvent exchange method, to avoid analyte loss and eliminate traces of water in the extract. The resulting extracts were then left in the dark overnight to allow pre-concentration to 200 μL–500 μL and were made to 1 mL of constant volume using toluene. The samples were then analysed with HPLC-UV-vis.

2.7. Instrumental analysis

The analysis was conducted on an HPLC (Agilent 1200 series) coupled to a UV-Vis detector and elution was done on a C18 with 100% toluene as the mobile phase. The elution was isocratic with a 0.400 mL min^{-1} flow rate. The aliquot injection volume was 2 μL, and the detector wavelength of 338 nm. The retention time of C61-PCBM was established to be around 0.955 min.

2.8. Risk assessment determination

In this present study, the highest observed no effect concentration (HONEC) and the environmental concentration was used to establish the risk characterisation ratio (RCR) for ecological risk assessment of fullerene in surface waters. Other researchers conducted the risk assessment by using the probabilistic Species Sensitivity Distributions (pSSDs) (Coll et al., 2016; Gottschalk et al., 2009, 2013; Andreani et al., 2021, Hong et al., 2021). In these studies, pSSDs are normally used to predict the predicted environmental concentration (PEC) and predicted no effect concentration (PNEC). The measured environmental concentration (MEC) was obtained from occurrence investigation in this study

and HONEC values were adapted from the previous toxicity studies in wastewater by (Auwerter et al., 2017), river and drinking water from (Coll et al., 2016). These values were used in Eq. (1) for ecological risk assessment of C61-PCBM:

$$RCR = \frac{MEC}{HONEC} \qquad (1)$$

The low risk occurs when RCR is less than 0.1 in the studied matrix, medium risk occurs when RCR is equal to 0.1–1 in the system and there is a high risk when RCR is greater than 1. Risk management should be put into place to manage the environmental impact when the RCR is greater than 1. The MEC values used were found in the experimental in the realistic river water, wastewater, and drinking water samples.

3. Results and discussion

3.1. Characterization of fullerene colloidal nanoparticles

3.1.1. UV-vis characterization of fullerene in water

Fullerenes colloidal nanoparticles were prepared in an aqueous solution using the method described by (Wang et al., 2018). To confirm the formation of colloidal nanoparticles, fullerene aqueous solution was analysed with UV-vis spectrophotometer. In addition, C61-PCBM dissolved in hexane was determined to compare the characteristic peak shift normal observed in organic solvents. The representative UV-vis adsorption spectra of fullerene dispersed colloidal nanoparticles (FDCNPs) in aqueous solution and fullerene in hexane are shown in Figure 1. All absorbance bands in the spectra of FDCNPs are broader and are shifted to the longer wavelength. This could be attributed to the creation of colloidal nanoparticles of fullerenes in solution and their chemical derivatives described by (Andrievsky et al., 2002). In addition, there is a new absorption band in the spectra of FDNPs at 430 nm, which is associated with the creation of weak donor-acceptor complexes in the fullerene. The donors of electrons may include alcohols and water molecules used in the synthesis of fullerene colloidal nanoparticles (Andrievsky et al., 2002).

3.1.2. HRTEM characterization of fullerene in water

HRTEM was used to confirm the formation of fullerene colloidal nanoparticles after extraction of fullerene colloidal nanoparticles from water into the toluene phase by ultrasonic-assisted liquid-liquid extraction. Fullerene colloidal nanoparticles were spherical with an average diameter of 3 nm as shown in Figure 2. These results confirm that the observed wavelength shift was the result of the formation of fullerene colloidal nanoparticles in water.
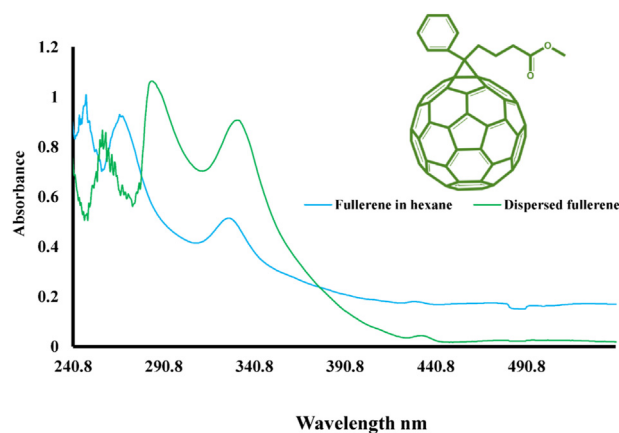

Figure 1. UV-vis absorption spectra of C61-PCBM in hexane and C61-PCBM colloidal nanoparticles extracted into toluene.
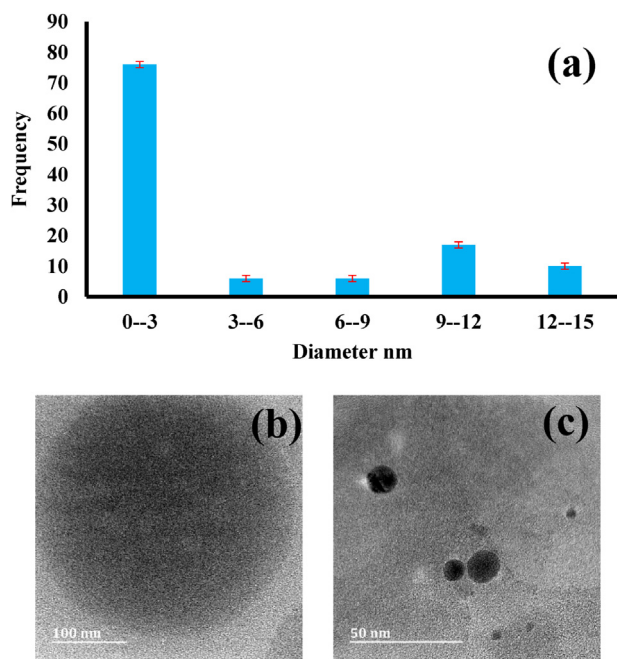
Figure 2. C61-PCBM colloidal nanoparticles (a) histogram and (b, c) HRTEM images.

3.2. Analytical method development

3.2.1. Instrumental method

The instrument parameters such as injection volume, flow rate, and detector wavelength were optimised in developing the HPLC-UV-vis method.

3.2.1.1. Selection of wavelength. UV-vis detector is widely used for the analysis of fullerenes in the environment due to the strong absorption of fullerenes in the UV range. However, the lambda maximum (λ_{max}) of compounds changes with nanoparticle sizes. To evaluate suitable wavelength spectra of 3 nm, colloidal nanoparticles of fullerene solution in

toluene was investigated, results are presented in Figure 3(C). The spectra were found to have four peaks with λ_{max} at 265 nm, 288 nm, 332 nm, and 431 nm. Also, a toluene solvent was determined with the UV-vis because it is used as a mobile phase and showed a peak from 252 nm to 336 with λ_{max} of 278 nm. After the lambda maximum was established, the following wavelengths were chosen and used as channels in the UV-vis HPLC detector; 332 nm, 335 nm, 338 nm, 340 nm, 345 nm, 348 nm, and 349 nm. Although the highest λ_{max} of fullerene is 288 nm, it was not used as the channel due to that it is overlapping with the toluene peak. The wavelengths 340–349 nm did not detect concentrations less than 1 mg L^{-1}. While shorter wavelengths (332 nm, 335 nm, and 338 nm) concentrations detected up to 0.1 mg L^{-1}. The wavelength 338 nm was selected to be used for further analysis because the peaks were narrow and symmetric and it was not overlapping with the toluene absorption range, which helps to avoid baseline noise. This wavelength (338 nm) was closer to 332 nm the wavelength selected by (Carboni et al., 2014).

3.2.1.2. Flow rate. The Gemini NX (C18) column is efficient for the analysis of fullerene and its derivatives hence, it was used in this work (Dönmez and Grennberg, 2020) (Gerstung et al., 2020). Toluene was employed as the mobile phase because aromatic solvents have better solvating power for fullerenes. Since the elution of fullerenes depends on the partitioning interaction of the analyte with the stationary and the mobile phase, it is important to select a mobile phase and stationery with high interaction with the analyte, the higher the interaction with the stationary phase, it will retain for a long time in the stationary phase. Although the solubility of fullerenes is low in the water and aliphatic organic solvents, researchers have to use aromatic solvents successfully in extraction. Aromatic solvents such as toluene, 1.2-dichlorobenzene, and 1.2.3-tribromopropane show better solvating power hence their use as extraction solvents. Toluene is normally used for the elution of fullerenes when a C18 column is used.

Sub et al. investigated the flow rate, they found that it is necessary to determine the optimum flow rate, since it can affect the elution and separation of analytes (Sub et al., 2019). In this work, the effect of mobile phase flow rate was assessed between 0.2–0.4 mL min^{-1} injecting 2 μL of 50 μg L^{-1} fullerene colloidal nanoparticles solution. Chromatograms of 0.2 mL min^{-1} and 0.4 mL min^{-1} are shown in Figure 3(a, b). As expected, the retention times decreased with increasing flow rate, between 0.2 mL
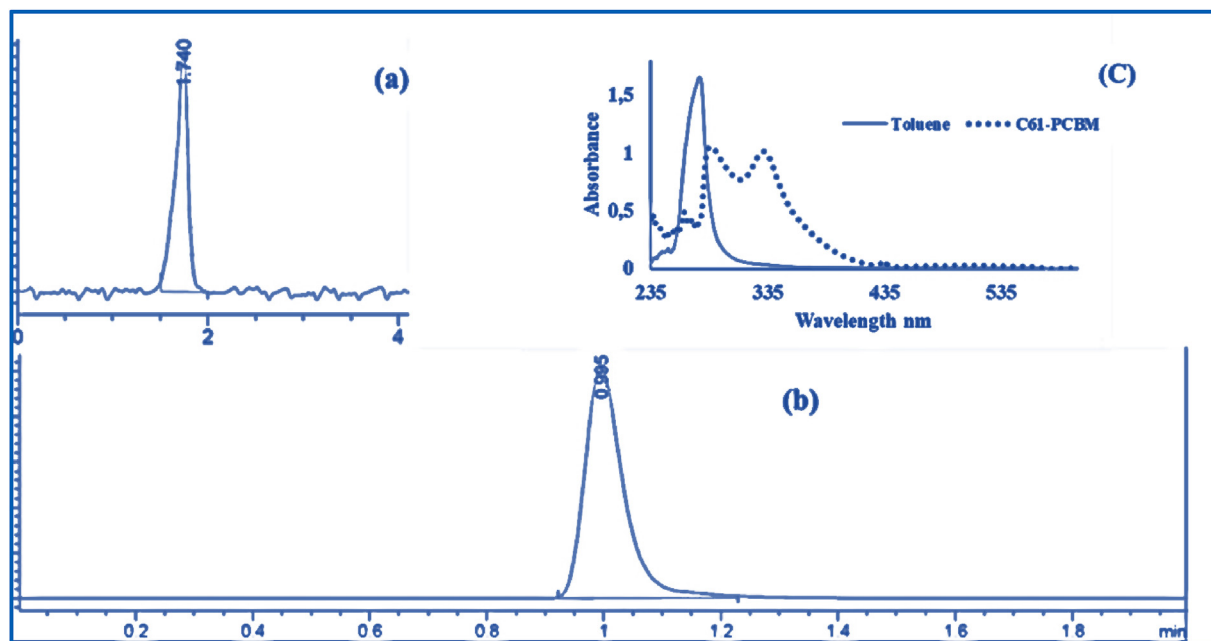

Figure 3. Chromatogram of 50 μg L^{-1} with flow rate of 0.2 mL min^{-1} (a) and 0.4 mL min^{-1} (b), (C) UV-vis spectra of C61-PCBM colloidal nanoparticles and toluene.

min^{-1} and 0.4 mL min^{-1} the analyte was eluted at 1.740 min and 0.955 min, respectively. The flow rate of 0.4 mL min^{-1} was chosen because it showed a narrow peak (1.5 min–2 min) compared to a broader peak (0.95 min–1.2 min) at 0.2 mL min^{-1}.

The fullerene peaks depend on the distribution of colloidal nanoparticles sizes. When 50 μg L^{-1} was injected with varying volumes from 1 μL–10 μL peak area varied, the peak resolution increased from lower to higher injection volume. At higher concentrations fullerene colloidal nanoparticles aggregated and there were many peaks as they eluted in different retention times as shown in F. 3 of the supplementary paper. The injection volume of 2 μL was selected because it showed a better resolution and a single narrow peak.

3.2.1.3. Instrument stability. Fullerenes are prone to transformation when they come into contact with the mobile phase, stationary phase, and UV detector (Sanchis et al., 2015). The stability of the chromatographic method and fullerene analyte was evaluated by injecting 2 μL of the fullerene standard solution (200 μg L^{-1}) 10 times in 5 min intervals over 50 min results are shown in F. 4 of the supplementary paper. The percent standard deviations obtained for elution times were less than 0.1% and the relative standard deviation of peak areas was 1.4%, this proves that the method is stable and robust. The obtained results were comparable to chromatographic stability studies (Pérez et al., 2013).

3.2. Extraction method

The efficient extraction technique is important to shorten the throughput of the entire analytical procedure. Ultrasonic-assisted and liquid-liquid techniques are often used as pre-treatment methods in the extraction of C_{60} from surface waters. However, these techniques are time-consuming, hence, they require extraction parameters optimization to improve throughput. In this work extraction parameters including, toluene as an extraction solvent, methanol as a disperser solvent, and sodium chloride for the ionic strength were optimised to improve the extraction efficiency of the ultrasonic technique.

3.2.2.1. Optimisation of toluene volume and extraction cycles. The analyte recovery at equilibrium increases with sample amount and this is more noticeable for compounds with high K_{ow} (Zouboulaki and Psillakis, 2016). Other researchers have used 50 mL, 100 mL, and 200 mL (Emke et al., 2015; Wang et al., 2010, 2018; Chen et al., 2008). Due to the hydrophobicity of fullerenes, usually, toluene and benzene are used for their extraction from an aqueous matrix. Considering that toluene is eco-friendly compared to benzene and has demonstrated good chromatographic conditions as a mobile phase during the instrumental method. Hence, in this work toluene was used as the extraction solvent. In this work 200 mL sample volume was used, the extraction solvent volume is important for the entire performance of the UADLLE method. To establish the optimal extraction solvent volume of toluene; 2.5 mL–10 mL were used to extract the analyte from the aqueous solution. The extraction efficiency was not influenced by the extraction solvent volume resulting in no effect to recoveries (Wang et al., 2018). However, at higher volumes (10 mL), precipitation of the salt (NaCl) was observed, which interfered with dispensing and separation of the toluene phase from the aqueous phase during extraction. The 5 mL was chosen as the optimum extraction volume.

3.2.2.2. Selection of the amount of disperser solvent. An optimal disperser solvent should be soluble in both organic (toluene) and aqueous phase and have little or no interference with the analysis. The normally used disperser solvents are acetone, acetonitrile, and methanol. The performance of different solvents during dispersive extraction has been investigated in previous studies (Wang et al., 2010). In this study, methanol was chosen as the disperser solvent and its required volume was investigated as necessary for higher efficiency. Moreover, the investigation was conducted as the amount of methanol may affect the formation of the three-phase emulsion (toluene-methanol-aqueous phases) and the distribution of toluene drops together with their sizes in aqueous media. The influence of methanol volumes 100 μL, 200 μL, 300 μL, 400 μL, 500 μL, and 600 μL on the extraction of C61-PCBM is shown in Figure 4(a). The graph shows that the recoveries increase with the disperser solvent volume up to 400 μL, then decrease. Methanol contributes to the creation of a cloudy solution that is attributed to small sizes of toluene droplets for better contact with the aqueous phase. The observed decrease in the recovery in methanol above 400 μL was due to the higher volume of methanol increasing the solubility of toluene in water, which in turn decreases the amount of extraction solvent and the recoveries are affected. Hence, 400 μL of methanol was the optimal volume needed to disperse the extraction solvent with the fullerene from the aqueous phase.

3.2.2.3. Effect of ionic strength. Ionic strength and pH greatly influence colloidal nanoparticles sizes and surface charge in the aqueous phase. In order to overcome emulsion challenges as well as partitioning in the middle of two immiscible phases, pH and ionic strength are normally optimised (Zouboulaki and Psillakis, 2016). Instead of optimising the pH, the glassware was heated up to 150 °C to avoid emulsion (Wang et al., 2010). To aid the separation of phases, sodium chloride was used to control the ionic strength of the extraction media. The sodium chloride concentrations varied were; 0.5%, 1%, 5% and 10% and the results are shown in Figure 4(b). The results show that increasing sodium chloride concentration by up to 1% improves the recoveries of C61-PCBM. Figure 4(b) shows that increasing the concentration of sodium chloride beyond 1% decreases recoveries, this observation is comparable to what was found by (Xiao et al., 2011). As previously discovered that the introduction of salt compresses the electrostatic double layer at the fullerene interface, resulting in the rapid formation of aggregates. Hence,
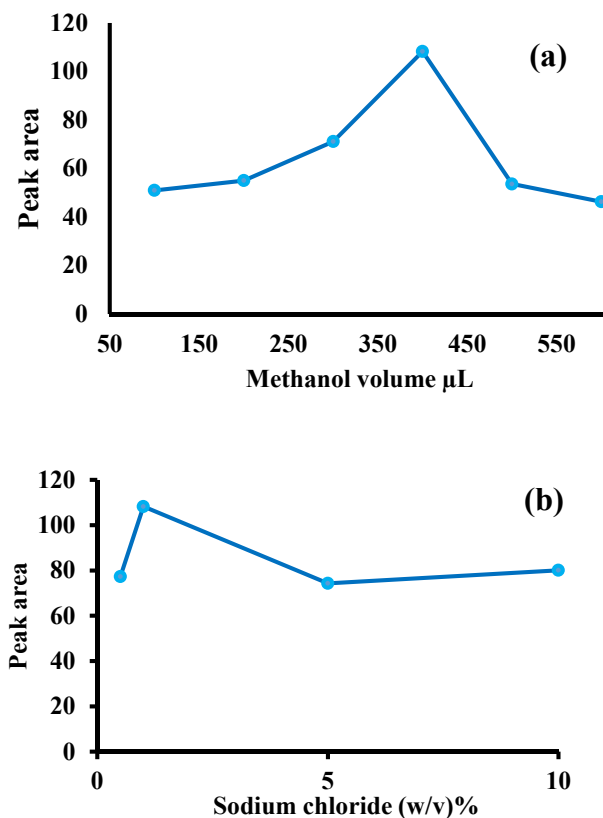

Figure 4. (A) Effect of disperser solvent (methanol) volume during extraction and (B) Variation of sodium chloride (w/v) % to establish the effect of the ionic strength in extraction.

1% of sodium chloride was selected as the optimum concentration to control the ionic strength.

3.3. Method validation

The proposed analytical method was validated according to IUPAC harmonised guidelines by (Thomson et al., 2002), focusing on the limit of quantification, limits of detection, recovery, accuracy, precision, linearity, and matrix effects.

3.3.1. Assessment of precision

A 200 mL of aqueous samples were spiked with C61-PCBM standard solution to obtain the final concentration of 25 µg L^{-1}, 50 µg L^{-1}, and 250 µg L^{-1} to perform precision studies. To perform the matric effect and accuracy in wastewater, drinking water, and river water, the final spiked concentration in 200 mL was 50 µg L^{-1}. All these spiked solutions together with their un-spiked blank were extracted with the developed method and all the extractions and spiking were done in triplicates results are shown in T. 4 of the supplementary paper.

3.3.2. Assessment of recoveries

These results are shown in Figure 5(a), the recoveries range from 66%–83% with a relative standard deviation of 11%, when absolute recoveries were determined from the standard prepared in toluene. This showed that these matrixes were not significantly different from each other but had an impact on the recoveries as an aqueous media, due to the matrix complexity. The standard was prepared in Millipore water and extracted as spiked samples. The standard was further used for the calculation of absolute recoveries, these results are shown in Figure 5(b). The recoveries improved and ranged from 93%–117% (calculated using Eq. (2)) similar to other studies when an aqueous calibration curve is used and they were within the recommended range in IUPAC by Thomson et al. and Kolkman et al. of 60%–120% (Thomson et al., 2002; Kolkman et al., 2013).
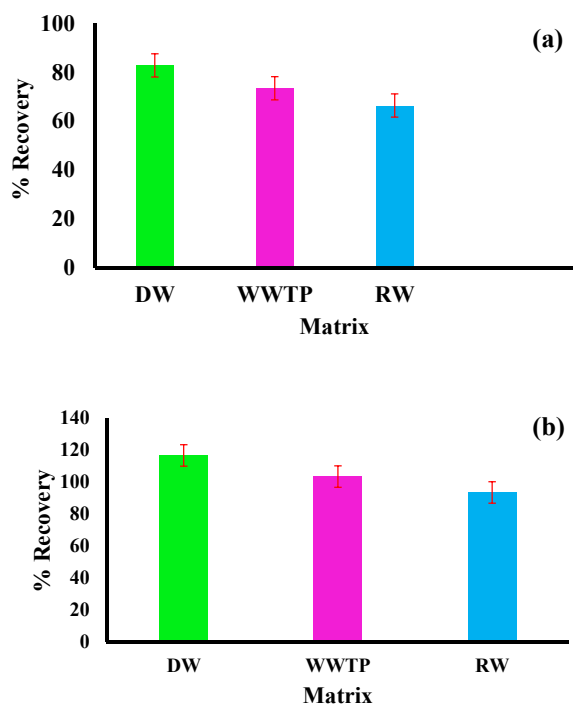

Figure 5. The effect of the matrix on the extraction of C61-PCBM (a) calculated using external standard prepared in toluene (b) calculated using internal standard in prepared in Millipore H$_2$O.

$$Absolute\ recovery\ (\%) = \frac{Peak\ area\ (Spiked) - Peak\ area\ (blank)}{Peak\ area\ (Standard)} \times 100\%$$

(2)

where:

Peak area (spiked) is the peak area intensity obtained by spiking the matrices with the C61-PCBM standards, The peak area blank is the peak area of intensity obtained by analysis un-spiked matrix and the peak area (standard) is the peak area intensity obtained by analysis the standards prepared in toluene (organic calibration curve) or the standard prepared in Millipore water, extracted (aqueous calibration curve).

3.3.3. Linearity, the limit of detection and quantification

An aqueous calibration curve was established by spiking 200 mL of an aqueous solution with fullerene standards (0.25 µg L^{-1}, 2.5 µg L^{-1}, 25 µg L^{-1}, 50 µg L^{-1}, 150 µg L^{-1} and 250 µg L^{-1}) to make a varying concentration of fullerene colloidal nanoparticles solutions. Fullerenes colloidal nanoparticles were extracted using the developed extraction method and determined using the HPLC-UV-vis. The obtained peak areas were plotted against the concentration, this graph of the aqueous calibration curve is shown in F. 6 of the supplementary paper. The linearity of the method was established to be between 0.25 µg L^{-1} and 250 µg L^{-1} and regression analysis were 0.9958.

The method limit of detection (MLOD) and method limit of quantification (MLOQ) were based on the slope of the calibration curve and standard deviation of the lowest concentration that fulfills the criteria: signal-to-noise ratio, at least, 3 and 10, respectively. The MLOD and MLOQ were calculated using Eqs. (3) and (4), respectively. They were obtained to be 0.11 µg L^{-1} and 0.38 µg L^{-1}, respectively.

$$MLOD = 3 \times \frac{\sigma}{S}$$

(3)

$$MLOQ = 10 \times \frac{\sigma}{S}$$

(4)

where MLOD is the method limit of detection and MLOQ is the method limit of quantification, σ is the standard deviation of the lowest standard, and S is the slope of the organic calibration curve.

These results shown in T. 3 of the supplementary paper are comparable with other literature methods where fullerenes were determined in the environment (van Wezel et al., 2011; Xiao et al., 2011).

3.3.4. Accuracy and precision

Method repeatability, inter- and intra-day precision were calculated using percentage error Eq. (5) as the mean percentage of the %RSD of the standard solutions (six replicates) at three concentrations (25 µg L^{-1}, 50 µg L^{-1}, and 250 µg L^{-1}) levels prepared independently from organic calibration curve over three days. A known concentration (50 µg L^{-1}) was prepared in three different matrices; drinking water (DW), river water (RW), and wastewater (WW), they were extracted and analysed with the developed method using an aqueous calibration curve to determine the accuracy of the entire method. These results for precision and accuracy are shown in T. 4 of the supplementary paper.

$$\%Error = \frac{C_e - C_a}{C_a} \times 100\%$$

(5)

where C_e is the concentration of the spiked standard obtained after experimental extraction and calculated using the internal calibration curve and C_a is the actual concentration of the prepared standard.

The precision of the developed method ranged from 2.3%–11.3% and the precision was below the 20% set value for a precise method (Thomson et al., 2002). The method had an acceptable level of accuracy in determining the concentration of the known standards in all the matrices; in WW 51.05 µg L^{-1}, RW 46.28 µg L^{-1}, and DW 57.39 µg L^{-1}.

The percentage error was within range in all the matrices; WW 2.1%, River water 7.4%, and drinking water 14.8% concerning the spiked level of 50 µg L^{-1}. These validation results allow the method to be applied in environmental samples, the conditions are suitable for environmental analysis and are comparable with other studies.

3.4. Application in surface waters

Aqueous samples were collected at different stages of treatment process in the drinking water and wastewater treatment plants to determine the presence and occurrence level of the fullerene in water being processed at the treatment plants. Water samples were also collected along the Umgeni River to determine the environmental contamination level of fullerene. These sampling sites provide insight into fullerene status in South African waters. The proposed method was applied in the detection and quantification of C61-PCBM in collected samples. The results are shown in Table 1. The concentration of C61-PCBM in real samples was found to range from not detected to 10.54 µg L^{-1}.

3.4.1. Occurrence of fullerene in drinking water treatment plants

Water samples were collected from drinking water treatment plants at EThekwini and Msunduzi Municipalities. The raw water samples collected by the inlet to the treatment plant contained 2.66 µg L^{-1} and 2.29 µg L^{-1} in plant A and plant B, respectively. Fullerene C61-PCBM was also detected in samples collected before and after the sand bed in both plants A and B. However, only the sample obtained after the sand bed of plant B was above the quantification limit with a concentration of 2.15 µg L^{-1}. In the drinking water treatment processing plant, sand is used to remove nanomaterials and other suspended particles after the coagulation process, This could be the reason why plant B had a quantifiable concentration of fullerene after sand bed since a different treatment process is used. The water sample collected from the water reservoir (last point) in plant A was found to contain 10.54 µg L^{-1} fullerene, while it was not detected in that from plant B. This was attributed to the fact that in plant A, the reservoirs are in series (as shown in F. 1(a) of the supplementary paper, which allows enough time for the settling of nanomaterials, while in plant B they are in parallel (as shown in F. 1(b) of the supplementary paper).

3.4.2. Occurrence of fullerene in wastewater

The wastewater samples analysed were collected in the influent, effluent as well as before and after the reactor. In the influent and effluent, the fullerene concentration was found to be 5.85 µg L^{-1} and 6.06 µg L^{-1}, respectively. Fullerene was not detected before and after the

reactor which indicates that it was partitioned with biosolids in the primary settling tank as only the seepage goes into the reactor. The presence of C61-PCBM in the final effluent could be due to the introduction of thickeners and digester in the process, which might have contained the fullerene that initially settled with biosolid as shown in F. 2 of the supplementary paper. The observed increase of fullerenes in the effluent shows that the activated sludge used for waste treatment introduces carbonaceous nanomaterials.

3.4.3. Occurrence of fullerene in river water

The river water samples were collected at different points along Mgeni and Msunduzi rivers (Msunduzi/Mgeni Tributary, before Inanda Dam, after Inanda Dam, Mgeni Estuary). The highest concentration 0f 3.56 µg L^{-1} was found before Inanda Dam, followed by concentrations obtained were 2.70 µg L^{-1} in the tributary, 2.62 µg L^{-1} after Inanda Dam while the lowest concentration was 2.12 µg L^{-1} was found in the Estuary. The lower concentration in the estuary may be due to high salinity as this point is closer to the sea. Generally, NaCl has been shown to affect the partitioning of fullerene in aqueous media during extraction.

The concentration of fullerene found in the Mgeni tributary (2.70 µg L^{-1}) is lower than the concentration obtained in the effluent, this indicates that the concentration of fullerene discharged from the wastewater treatment plant into Umgeni River was decreased, which could be attributed to the dilution by river water. The highest concentration of fullerene was observed in the Inanda Dam inlet (3.56 µg L^{-1}). This could be due to that this point is prone to contamination since the downstream geographical location of the Inanda Dam. The concentration of fullerene at the point of entry into Inanda Dam was 2.62 µg L^{-1}, which decreased in water exiting the Inanda Dam. The level of fullerene in Inanda Dam was found to be almost the same as the inlet of raw water feeding the drinking water treatment plant. Inanda Dam is the source of raw water supply to the drinking water treatment plants. The dam is known to allow for some degree of water self-purification hence, it acts as a natural treatment process. The natural process in Inanda Dam contributed to the partial reduction of fullerene in Umgeni River water.

3.5. Comparison of the current study with literature

A comparison of the levels of fullerene observed in this study with some of those reported in previous studies elsewhere in the world is presented in Table 2. This table lists and compares the occurrence of fullerenes in aqueous matrices using different extraction methods and HPLC coupled mostly to a UV-vis detector. The mobile phase, column types (stationary phase), detection limits, quantification limit, linear regression, and environmental concentration of fullerenes are also indicated. The proposed method uses low volumes of extraction solvents and shorter retention time compared to literature methods in Table 2. But the disadvantage of this method uses 100% toluene as mobile phase, which is problematic if to be used in routine monitoring of fullerenes in the environment. Aside from the use of HPLC-UV-vis, which is the most used instrument for detection and quantification of fullerenes using toluene as a mobile phase and a C18 column, the LC-MS is also a well-utilised instrument. However, it expensive but an important technique in the environmental analysis of fullerene as method developed based on UV-vis suffer from interferences and low sensitivity. These limitations have resulted to many method developed using UV-vis not be applied in the environmental analysis of fullerene as shown in Table 2. However, because of the separation technique we employed in our proposed method we were able to successful analysed fullerene in the environment.

3.6. Ecological risk assessment of fullerene in surface waters

Due to the accumulative production and usage of nanomaterials, there is a high probability of them entering the environment causing various toxic effects. There is a need to monitor them and assess the

Table 1. Environmental samples analysis using the developed analytical method.

Sampling area	Drinking water			
	Raw water µg L^{-1}	Before sand bed µg L^{-1}	After sand bed µg L^{-1}	Reservoir µg L^{-1}
Plant A	2.66 ± 0.1335	NQ	NQ	10.54 ± 0.07
Plant B	2.29 ± 0.021	NQ	2.15 ± 0.03	NQ
Wastewater				
Waste treatment plant	Influent µg L^{-1}	Before reactor µg L^{-1}	After reactor µg L^{-1}	Effluent µg L^{-1}
	5.85 ± 0.08	ND	ND	6.06 ± 0.04
River water				
Umgeni River	Msunduzi/ Umgeni Tributary µg L^{-1}	Before Inanda Dam µg L^{-1}	After Inanda Dam µg L^{-1}	Umgeni Estuary µg L^{-1}
	2.70 ± 0.16	3.56 ± 0.07	2.62 ± 0.04	2.12 ± 0.02

ND- Not detected, NQ-detected.

Table 2. Comparison of the analytical method used in literature with the currently developed method.

Instrument	Extraction method	Mobile phase	column	Analyte	Method detection limit	Method quantification limit	R^2	Environmental concentration of fullerene	Reference
HPLC-UV	Dispersive liquid-liquid micro-extraction	Toluene	C18 (Alltima, 250 × 4.6 mm, 5 μm)	C60	0.19–0.34 μg L^{-1}	0.63–1.15 μg L^{-1}	0.99	N.D–4.29 μg L^{-1}	(Wang et al., 2018)
LC-orbitrap MS/UV	Solid phase extraction / Liquid-liquid extraction	Toluene and acetonitrile (90/10)	Cosmol Japan buckyprep (10 mm × 4.0 mm 5 μm)/(250 mm × 2.0 mm 5 μm)	C60/70 / Functionalized fullerenes	0.00017–0.00028 μg L^{-1}	0.00057 μg L^{-1}	-	Detected- 113%	(Kolkman et al., 2013)
HPLC-UV	Solid phase extraction / Liquid-liquid extraction	Toluene	COSMOL buckyprep (250 mm × 4.6 mm)	C60 & C70	4.2 μg L^{-1}	-	0.99 (LLE) 0.99 (SPE)	-	(Xiao et al., 2011)
HPLC/UV/MS	Solid phase extraction	Toluene and methanol (60/40)	C18 (150 mm × 3.9 mm)	nC60	0.001 & 0.005 μg L^{-1}	-	-	Detected- 120 ng L^{-1}	(van Wezel et al., 2011)
HPLC-UV	Solid phase extraction / Liquid-liquid extraction	Toluene and acetonitrile (55/45)	COSMOL buckyprep (250 mm × 4.6 mm)	C60	0.42–0.64 μg L^{-1}	-	0.99	-	(Wang et al., 2010)
HPLC-UV	**ultrasonic-assisted dispersive liquid-liquid extraction**	**Toluene**	**Gemini column (150 mm × 2.1 mm)**	**C61-PCBM**	**0.11 μg L^{-1}**	**0.38 μg L^{-1}**	**0.99**	**N.D–3.56 μg L^{-1}**	**Current method**

ecological risks in different environmental matrices. Previous studies used different organisms occupying the matrices to study ecological risk assessment (Coll et al., 2016; Auwerter et al., 2017; Zhang et al., 2019). In some studies, wastewater and surface water toxicity assessments were done using the microbial species occupying the matrices. The data obtained from these previous studies is further used for assessment on similar matrices, this is done to avoid future resting of the animals for experimental purposes. In this study, the HONEC value of 10 μg L^{-1} for

C61-PCBM in wastewater was obtained in the study by (Auwerter et al., 2017), this was the lowest concentration level of fullerene that did not cause toxic effects on the microorganism. For the river and drinking water, the HONEC value was 3.84 μg L^{-1} obtained by (Coll et al., 2016).

These values were used with the obtained environmental concentrations found in this work to study the ecological risks, the results are shown in Table 3. In the river samples, all sampling areas were found to be of medium risk. In the drinking water treatment plants, some points were of medium risk whereas others did not possess any risks. Although drinking water treatment plant A it has shown high risk as the risk characterization ratio was greater than one, this area needs further attention for the fullerene levels that are present. In the wastewater, some areas show no ecological risks and in the other areas, the risks were showing medium risks. These ecological risks were done using the presently accepted values on the no-effect concentration limit levels for accurate assessment.

4. Conclusion

A cheap, simple, and sensitive method based on the UADLLE extraction method followed by detection with HPLC-UV-vis, has been developed for risk assessment of C61-PCBM in drinking water, wastewater, and river water. With this developed method, extractions were ultrasonic-assisted and performed with low extraction volumes and disperser solvents, which allowed the determination of fullerene in these matrices, without the need for sophisticated extraction and analysis instruments. High extraction recoveries between 93%–117% were obtained for C61-PCBM in all matrices spiked at different levels. The MLOD and MLOQ were within range of those reported by other authors in surface water studies, using HPLC-UV-vis. This UADLLE method was employed in the analysis of C61-PCBM in South African waters and was found to range from not detected to 10.54 μg L^{-1}. These findings are the first in African water bodies. The developed method assisted in the ecological risk assessment of C61-PCBM in the selected areas. For river water, it had an RCR that ranged from 0.55–0.70 showing a medium risk in all the areas. The drinking water has RCR between 0.6–2.7 indicating a medium to high risk. The wastewater RCR ranged between 0.59–0.60 RCR this indicate a medium risk.

Table 3. Environmental ecological risk assessment for C61-PCBM obtained from MEC and HONEC.

Sampling area		River water Concentration μg L^{-1}	Quotient	Risk assessment
Msunduzi/uMgeni tributary		2.70 ± 0.16	0.70	Medium risk
Before Inanda Dam		3.56 ± 0.07	0.92	Medium risk
After Inanda Dam		2.62 ± 0.04	0.68	Medium risk
uMgeni Estuary		2.12 ± 0.02	0.55	Medium risk
Drinking water				
Sampling area		**Concentration μg L^{-1}**	**Quotient**	**Risk assessment**
Raw water	**Plant A**	2.66 ± 0.1335	0.69	Medium risk
	Plant B	2.29 ± 0.021	0.59	Medium risk
Before sand band	**Plant A**	-	-	No risk
	Plant B	-	-	No risk
After sand bed	**Plant A**	-	-	No risk
	Plant B	2.15 ± 0.03	0.6	Medium risk
Reservoir	**Plant A**	10.54 ± 0.07	2.7	High risk
	Plant B	-	-	No risk
Wastewater				
Sampling area		**Concentration μg L^{-1}**	**Quotient**	**Risk assessment**
Influent		5.85 ± 0.08	0.59	Medium risk
Before reactor		-	-	No risk
After reactor		-	-	No risk
Effluent		6.06 ± 0.04	0.60	Medium risk

Declarations

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