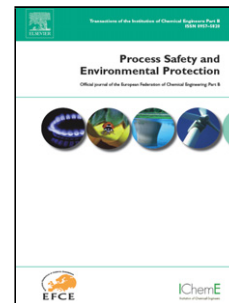


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Identification of malachite green in industrial wastewater using lignocellulose biomass composite bio-sorbent and UPLC-MS/MS: a green environmental approach

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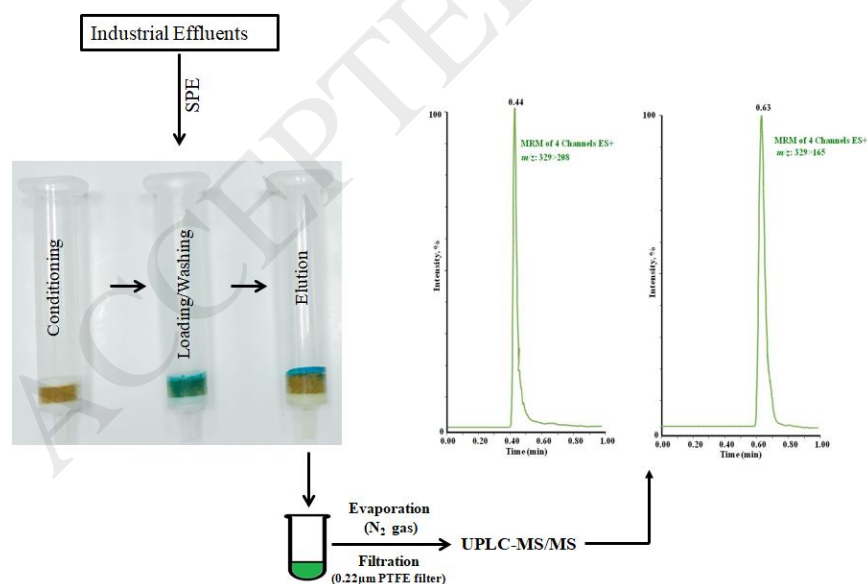
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Graphical abstract



Highlights

- SPE and UPLC-MS/MS was validated for analysis of malachite green in wastewater

- SPE bio-sorbent, a lignocellulose biomass was prepared from *moringa oleifera* pods
- Methanol was found to be the most efficient extracting solvent
- Analysis time was found to be <1 min with excellent peak resolution and symmetry
- Optimized method has been applied for the analysis of industrial wastewater samples

Abstract

A method based on eco-friendly solid-phase extraction (SPE) and ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) was validated for the trace analysis of malachite green (MG) in industrial wastewater samples. For the extraction of MG, a lignocellulose biomass composite material was prepared from *moringa oleifera* pods, which were modified with CuFe_2O_4 , and used as solid phase extraction bio-sorbent. The chromatographic separation of MG was optimised using an Acquity[®] BEH C₁₈ analytical column in isocratic separation conditions (65% water with formic acid (0.1%, v/v) and 35% methanol). The total separation time was <1 min with satisfactory resolution and symmetry. The proposed method offers suitable validation parameters in terms of linearity ($r^2 > 0.999$), precision (run-to-run and day-to-day) <4%, and sensitivity with a limit of detection of 0.1 $\mu\text{g/L}$. The optimized method has been applied in the analysis of industrial wastewater samples (printing press, paper, textile and laundry), and the amounts obtained were in the range of 0.62 to 1.68 mg/L with recovery rates ranging from 99 to 103%. The achieved outcomes confirmed the applicability of the proposed analytical methodology as a novel advantageous approach for the routine analysis of MG in industrial effluents. In the future, this method could be comprehensively applied in the efficient removal of colored pollutants as well as for the quantification of the exposure to MG.

Keywords: Malachite green; *Moringa oleifera* pods; lignocellulose biomass composite; wastewater; solid phase extraction; liquid chromatography-mass spectrometry

1. Introduction

Colored compounds in industrial effluents are very visible and a common type of pollution. At present, more than thousands types of commercial dyes including malachite green (MG) and pigments are used by various industries (e.g. printing, paper, textile and laundry industry), and important amounts of these compounds (7×10^5 tons/year) are manufactured globally [1, 2]. A fraction of them ends up in aquatic environments and their presence has adverse effects on aquatic life, reduces photocatalytic activity of underwater plants and makes water unusable for irrigation, drinking and recreation purposes [3,4]. MG has high solubility in water, therefore organisms can ingest this class II health hazard. Indeed, the Canadian Food Agency identified a significant amount of MG in fish at levels that were a major health threat [5]. In 2006, the US Food and Drug Administration (FDA) found MG in seafood from different countries. In the following years, the FDA stopped importing numerous kinds of seafood owing to the presence of MG [6]. The United States of America and United Kingdom have barred the use of MG in food products [7].

MG is resistant to biological degradation which makes it difficult its removal from wastewater. Treatments such as precipitation [8], degradation [9], decolorization [10], nanofiltration [11] and adsorption [12] have been used for removing MG from contaminated water. Among these, adsorption is well established and considered a good approach because it is simple, cost-effective and provides efficient removal with minimum sludge production, and most importantly, the sorbent can be reused (generally when the sorbent is costly). A variety of materials including zeolite [13], polymer [14], hydrotalcite clay [15], protein nanofibers [16] and nanocomposites [17] have been used as sorbents for the abatement of MG from industrial wastes. However, their cost or difficulties in their regeneration have driven research towards the

development of low cost sorbents for MG.

The approaches employed for the removal of MG are independent from the strategies used for the quantification of MG, however these are related because both require capturing MG efficiently. The analysis of MG comprises sample clean-up (typically including liquid–liquid extraction [18-20] or solid phase extraction (SPE) using commercial cartridges [21-23] in combination with the separation of the purified extracts (with liquid chromatography, including ion chromatography [24,25] or capillary electrophoresis [26]) and its detection (based on spectroscopy or mass spectrometry (typically with quadrupole, ion trap or time of flight analysers) [27-29]. These extraction and separation techniques have some limitations as other substances from the wastewater sample matrix are often be co-extracted, can co-elute and hinder the accurate determination of the target analyte; tedious sample treatment with use of large volumes of organic solvents are required [29]. Most significantly, several false peaks may appear in the chromatogram with similar retention time than MG. Besides, conventional determination methods are time consuming and require large volumes of organic solvents and hence have an important environmental impact.

In this work, to address some of these selectivity limitations in the extraction of dyes from wastewater, and to reduce sample extraction cost and the environmental impact of using disposable synthetic SPE bio-sorbent, a composite derived from lignocellulosic *moringa oleifera* pods and spinel ferrite (copper ferrite, CuFe_2O_4) was prepared for the enhanced extraction of MG. The performance of the new bio-sorbent was assessed in different types of industrial wastewater samples, and quantified the extracted MG with UPLC-MS/MS.

2. Materials and methods

2.1. Chemicals and materials

All the solvents and chemicals used in this experiment were of analytical reagent grade. Malachite green (MG: purity >82%); crystal violet (CV: purity >90%), used as an internal standard (IS); and formic acid (purity, >98%) were from Sigma-Aldrich (St. Louis, MA, USA). The chemical structures of the MG and CV are shown in Figure 1. Methanol, dichloromethane, acetone, acetonitrile and ethyl acetate were purchased from Merck (Darmstadt, Germany). Water was purified through a Milli-Q water purification system (Millipore Corporation, Bedford, USA). Extrelut-20 empty columns were obtained from (Darmstadt, Germany), whereas the coupling pieces and stopcocks were supplied by Varian (Harbor City, USA). The Visiprep™ and Visidry™ (Supelco, Gland, Switzerland) vacuum manifolds were used for SPE method and solvent evaporation purposes, respectively.

Stock solution of MG (100 mg/L) was prepared in methanol and used for further dilutions. Standard mixtures of MG at concentration levels 0.001–5 mg/L were prepared to establish the calibration in UPLC-MS/MS. The optimisation of the purification with SPE was carried out by using the 100 mg MG/L solution for spiking water samples. All samples and solutions were filtered through a 0.22 µm PTFE syringe filter (Chromafil® Xtra, Macherey-Nagel, Duren, Germany) and injected into the UPLC-MS/MS system. MG quantification was carried out with standard addition (n=3), each including 2 unspiked and 3 spiked samples. Further detail is given in section 2.3.2.

2.2. Instruments

Chromatographic analysis of MG and CV was performed on an Acquity® UPLC system equipped with a quaternary pump (Waters, Milford, USA). The reversed Acquity® BEH C18

column of dimension 50 mm x 2.1 mm id and 1.7 μm particle size (Waters, Milford, USA) was used for separation of the target compound; the columns Acquity[®] BEH C₈ and Acquity[®] BEH Amide, all with the same dimensions and particle size, were also tested. The mass spectrometer used was a triple quadrupole Quattro Premier (Micromass, Milford, USA) equipped with electrospray ionisation source (ESI, Z-spray). Data acquisition was performed by MassLynx V4.1 software (Waters, Milford, USA). Fourier-transform infrared spectroscopy (FT-IR: Thermo Scientific Nicolet 6700) was used to characterize the surface chemistry of lignocellulosic biomass composite. The surface crystallinity of the modified bio-sorbent was determined by powder X-ray diffraction (XRD: Ultima IV, Rigaku). The performance of the method developed was assessed in terms of sensitivity by estimating the limit of detection (LOD) and limit of quantification (LOQ) of MG from the concentrations giving signal-to-noise ratio (S/N) of 3 and 10, respectively. The linearity of the method was measured between 0.001 to 5 mg/L. The precision of the analysis was evaluated from the RSD % of the peak areas with 6 repeated determinations carried out on the same day (run-to-run) and by injecting six replicates of the same standard solution (0.1 mg/L) and wastewater sample during five consecutive days (day-to-day).

2.3. Sample analysis

2.3.1. Preparation of bio-sorbent composite

Fresh *moringa oleifera* pods were collected from local market (Riyadh, Saudi Arabia) and chopped into small pieces. The chopped pods were washed with ultrapure water in order to remove dust particles followed by overnight drying in an oven at 65 °C. The dried *moringa oleifera* pods were mechanically crushed and sieved through 200 μm to get their powder. The powder that passed

through the sieve was treated with hydrogen peroxide (30%, v/v) solution to decompose the organic content present. Then, the powdered bio-sorbent was washed with ultrapure water until reaching neutral pH. The lignocellulosic biomass modified with CuFe_2O_4 was prepared by adopting a previous reported procedure [30]. Briefly, 1 g of *moringa* pods biomass powder was mixed with CuCl_2 and FeCl_3 (mass ratio 3:1) in 500 ml conical flask followed by the addition ultrapure water to make the suspension of 100 ml. The suspension was magnetically stirred at approximately $\sim 85^\circ\text{C}$ for 3 h. Thereafter, NH_4OH solution was added drop wise to the suspension to cause the precipitation of the metals (pH=10) and kept for 1 h with continuous stirring. The precipitate was cooled and washed separately with ultrapure water and ethanol until reaching neutral pH. Finally, composite materials were separated using suction filtration and kept in oven at 70°C for complete dryness. Then, the dried composite materials were allowed to cool and stored in a desiccator until use.

2.3.2. Sample extraction procedure

Wastewater samples were collected in amber glass bottles (500 ml) from different industries located in Saudi Arabia. The samples were then filtered through Whatman filter paper (no. 42), (Maidstone, UK), and stored in refrigerator until analysis. Lignocellulosic biomass- CuFe_2O_4 composite (0.5 g) was weighed and packed into an empty Extrelut column (3 ml). The column was conditioned using water (10 ml) followed by methanol (10 ml) and it was allowed to dry under vacuum. Wastewater samples (20 ml) were then filtered through column packed with lignocellulosic biomass- CuFe_2O_4 composite at a controlled flow rate of 1 ml/min. After complete sample percolation, the column was washed with ultrapure water (20 ml) and dried under vacuum for 10 min. Methanol (20 ml, at 1 ml/min) was used for the elution of the retained target

compounds. The extracted sample was then evaporated to dryness under a gentle stream of nitrogen. The dried sample was reconstituted with 1 ml of CV (0.5 $\mu\text{g/ml}$) in methanol/water (35/65, v/v). Then, the final mixture was filtered through PTFE syringe filter (0.22 μm) and analyzed with UPLC-MS/MS system. To assess the efficacy of the extraction method in terms of MG recovery values and preventing the influence of matrix effects on peak shape, intensity and retention time, two non-spiked and three spiked samples 50% (percentage indicating the increase of MG in the sample after spiking) , (1.0 $\mu\text{g/ml}$), 100% (2.0 $\mu\text{g/ml}$) and 300% (6.0 $\mu\text{g/ml}$) were assessed by standard addition quantification method. The spiking of the samples was carried out at the beginning of each extraction process. All samples were analyzed in triplicate.

3. Results and discussion

SPE is the most widespread sample treatment technique to isolate the compounds of interest from a variety of complex matrices, such as wastewater, biological samples and beverages [31]. The alteration of sorbents with various functional groups or precipitates changes the extraction properties of the sorbents. In the current study, *moringa oleifera* pods biomass/ CuFe_2O_4 composite was prepared and investigated as a possible SPE bio-sorbent for MG. *Moringa oleifera* is frequently available in low altitude countries.

3.1. Characterization of bio-sorbent

3.1.1. Surface chemistry

The major constituents of the *moringa oleifera* powder are proteins, carbohydrates, cellulose, fatty acids, lignin and absorbed water [32-34]. The protein content of this bio-sorbent contains nitrogen that can be involved in the removal of MG. The FT-IR spectra of a 1% dilution of the composite

in KBr (Figure 2a) shows a strong and broad band at 3329 cm^{-1} that is typical from the vibration stretch of the O-H bond of hydroxyl groups present in the aforementioned biomolecules and it could also include N-H stretching from amines and amides [32-34]. The bands at about 2928 cm^{-1} correspond to C-H stretching vibration in alkanes [35]. The bands in a region of $1700\text{-}1520\text{ cm}^{-1}$ may be due to the stretching of C=O in ketones, amides and carboxylic groups [33]. The band at 1057 cm^{-1} was attributed to the stretching of C-O, which is in agreement with the presence of lignin [36]. Pagnanelli's research group had confirmed that these functionalities have a capability to bind with metals [37]. The adsorption of dyes onto *moringa oleifera* pods powder-CuFe₂O₄ composite may be attributed to Van der Waals interactions between the aromatic π system of the dye and lone pairs of electrons from the nitrogen groups in MG with the aromatic system of lignin and the metals in the in the composite, and also with OH groups from the composite [30].

3.1.2. X-ray diffraction (XRD) analysis

The XRD patterns of the *moringa oleifera* pods powder confirmed the existence of amorphous material in the sample due to the presence of lignin and protein (Figure not shown). The X-Ray diffractogram of the modified *moringa oleifera* pods has confirmed the effective precipitation of CuFe₂O₄ (Figure 2b).

3.2. Optimization of SPE method

Since there were no earlier studies reporting the extraction of MG from wastewater samples using modified biomass as SPE bio-sorbent, and UPLC-MS/MS, preliminary studies were carried out on MG standard solutions. To optimize the extracting solvent, ethyl acetate, dichloromethane, acetone, ethanol and methanol were tested. Among the tested solvents, methanol showed

comparatively better recovery (103%), the rest presented recoveries < 60%, and methanol was selected for further studies. Statistically similar recoveries were obtained when 10 times higher mass of bio-sorbent was used for the extractions ($p < 0.05$), which indicates that the bio-sorbent did not become saturated by the analyte or other components in the wastewater samples. The high recovery achieved with the *moringa oleifera* pods powder - CuFe_2O_4 may be due to the new interaction between Cu and Fe with the aromatic structure of the analyte; the coordination between the metals and the lone pair of electrons from the tertiary amine, which would be affected by pH. Indeed, when additional extractions were performed by adding 0.1% formic acid to each extraction solvent, then MG was not recovered, probably due to the ionisation of the tertiary amine which may have decreased the interaction with the bio-sorbent and caused its elution during the washing step with water. Previous work by the authors suggested possible electrostatic interaction between hydroxyl groups of the bio-sorbent and the quaternary amines in the dye; and between carbonyl groups of the bio-sorbent and the tertiary amine in the MG [30].

3.3. Optimization of chromatographic separation

The development of a high-throughput method for the quantification of MG using UPLC, in combination with a greener extraction technique, is an advance in the analysis of MG with respect to previous methodology [30]. Different reversed phase columns (Acquity® UPLC BEH C_8 , C_{18}) were tested. BEH columns produce superior stability at low-pH; their stationary phase tri-functionally bonded to the particle and also suffer from minimum column bleed. Furthermore, this type of columns are end-capped which minimises peak tailing. Among these hydrophobic stationary phases (C_8 and C_{18}) tested, the C_{18} column was selected for further studies because it

offered greater resolution between MG and CV. Overall, the peak width and symmetry were appropriate (see Figure 4), and the narrow peaks favored high sensitivity in the analysis.

Another Acquity® UPLC column, with hydrophilic interaction liquid chromatography (HILIC) with amide as stationary phase, was also investigated. The HILIC column is normally designed to separate highly polar compounds and the separation mechanism in this type of stationary phase can be affected by the concentration of ions [38]; therefore the analysis of wastewater samples with variable concentration of salts could potentially affect the retention and robustness of the determination of MG. Although the analysis of MG with the HILIC column was fast (its retention time was 2 min when using 35% methanol in water with 0.1 % formic acid at a flow rate of 0.25 ml/min), the possible variability in retention due to variable presence of ions led to select the BEH C₁₈ column for the analysis of MG. The effect of the mobile phase on the separation of MG was assessed at different proportions of organic solvent/ aqueous phase on the signal of MG and CV solution (1 µg/ml). Mixtures of methanol/water; acetonitrile/water; and tetrabutyl ammonium hydroxide/methanol/acetonitrile were tested and the combination methanol/water, comparatively, gave the best results although peak tailing was present in the chromatogram. The addition of formic acid (0.1–0.01%) in mobile phase was tested and it considerably reduced the tailing of both MG and CV chromatographic peaks, with the best symmetry achieved when 0.1% formic acid was added to the aqueous phase. Hence, for optimal separation, the amines need to be protonated, as observed in previous works for dyes that, like MG, have tertiary amines [28]. The best separation in terms of resolution, peak symmetry, reproducibility and high signal-to-noise ratio were obtained for the study dye when using a mobile phase of 65% of water with formic acid at 0.1%, v/v, and 35% of methanol, in isocratic elution mode. The effect of column oven temperature on the

separation of MG and CV was also tested between 25 and 50 °C but improvements in the separation were not noticed when increasing the temperature above 25 °C.

The flow of mobile phase also plays crucial role in chromatographic separation. Flow rate ranging from 0.1 to 1 ml/min were tested, and despite that UPLC can provide maximum speed at the higher flow rates with minimum sacrifice of plate height, a relatively low flow rate of 0.25 ml/min was selected for the analysis of MG and CV. This is because less volume of mobile phase reaching the electrospray (ESI) provided more efficient desolvation and ionic evaporation in the ionisation source, while maintaining narrow and symmetric chromatographic peaks. Using all these optimal parameters, the separation of both dyes was achieved in less than 1 min (Figure 4). The sample injection volume for each run was 5 μ l.

3.4. Optimization of MS detection parameters

The detection with mass spectrometry was optimized by direct infusion of a mixture of methanolic solutions of MG and CV at concentration levels 10 mg/L. The detection of target compounds was made in both positive and negative ionisation modes in order to detect the best signal. Primarily, full scan was carried out to select the most abundant precursor ion and optimum cone voltage. The highest sensitivity of the investigated compounds was achieved in positive ionisation mode given the cationic nature of the analytes. The addition of acid did not modify the molecular ion (MG and CV) but it improved their intensity, as previously observed with methylene blue [28]. Multiple reaction monitoring (MRM) mode was optimised for the detection of MG and CV precursor ions and at least two product ions as required by the European Commission [39]. The most abundant transitions for MG and CV, and corresponding collision voltages, are given in Table 1. The mass spectra and fragmentation pattern for the investigated MG and CV dyes have been proposed

in Figure 3. For the analysis of MG and CV. The optimal MS source conditions, which are affected by the composition of the mobile phase were: cone voltage, 74 V; capillary voltage, 3.5 kV; desolvation temperature, 300 °C source temperature, 130 °C; desolvation gas flow rate; 600 L/h; and cone gas flow rate, 60 L/h.

3.5. Method validation

The proposed method was validated in terms of linearity, LOD, run-to-run and day-to-day precision, and recovery. The linearity of the method was tested over a wide range of MG concentrations from 1 µg/L and covering 3 orders of magnitude. High linearity (r^2 0.999) value was achieved, and the instrumental LOD and LOQ values for the developed technique were found to be 0.1 and 0.4 µg/L, respectively. Relating to the precisions, run-to-run precision of the method was established by injecting six replicates of MG standard (0.1 mg/L) and a wastewater sample (printing press) with a known concentration (0.79 mg/L) on the same day. The determination of run-to-run precision was found to be 1.96% in the analysis of the standard and 2.42% in the analysis of wastewater sample. The day-to-day precision was calculated by injecting six replicates of the same solutions and sample along five consecutive days. The obtained values were 2.25% in the analysis of the standard and 3.58% in the analysis of the wastewater samples.

For evaluating the effect of sample matrix on the quantification of MG, a total of 4 industrial wastewater samples were analysed and the obtained recoveries (comparison between the amount of MG added as part of the quantification with standard addition, and the amount of MG found with external calibration) were found to be in the range between 99 and 103%, as listed in Table 2. The negligible matrix effect on the signal of target compounds can be attributed to the

special configuration (Z-spray) of the electrospray ionisation source, which minimised the entry of neutral compounds in the MS and the effective clean-up with the developed composites.

3.6. Application to the wastewater sample analysis

The applicability of the proposed SPE and UPLC-MS/MS approach using low cost modified *moringa oleifera* pods with CuFe_2O_4 as SPE bio-sorbent was quantified in a range of industrial wastewater samples. The experimental results for the detection of MG indicate that negligible matrix effects were noticed by the investigated sample matrices. The quantified MG concentrations are given in Table 2. The highest MG level (1.68 mg/L) was found in textile industry sample and the lowest MG level (0.62 mg/L) was determined in wastewater from a paper industry. To check for possible cross contamination during the analysis, blanks water samples were analyzed in parallel in every batch and they showed negligible contamination. As an example of the quality of the analysis developed, Figure 4 displayed the UPLC-MS/MS chromatograms of MG and CV obtained in textile industry sample purified with *moringa oleifera* pods - CuFe_2O_4 composite.

4. Conclusions

A rapid, sensitive, low-cost, green and reliable methodology based on extraction and purification of MG with SPE (*moringa oleifera*- CuFe_2O_4 composite), and analysis with UPLC-MS/MS, has been established for the quantitative analysis of MG in various industrial wastewater samples. The proposed method shows negligible matrix effect despite the complexity of the initial wastewater samples. The low cost and ecofriendly SPE bio-sorbent developed makes it an excellent alternative to synthetic disposable SPE bio-sorbent and provides sufficient sensitivity, recovery and

selectivity for the quantification of MG in complex sample such as wastewater. Validation shows that the methods is precise (RSD <4% RSD) for the analysis of MG in wastewater samples; and negligible matrix effect was found for the analysis of the study wastewater samples with the developed method. The results obtained confirm the applicability of the proposed analytical methodology for the routine analysis of MG in industrial effluents. In the future, this proposed bio-sorbent (*moringa oleifera*- CuFe₂O₄ composite) could be comprehensively applied both for the efficient removal of colored pollutants in large scale and the quantitation of the exposure of both humans and aquatic organisms to MG. Further work should address the study of the reproducibility in the preparation of the bio-sorbent and strategies for its re-use.

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References:

- [1] M. Naushad, M.A. Khan, Z.A. Allothman, M.R. Khan, M. Kumar, Adsorption of methylene blue on chemically modified pine nut shells in single and binary systems: isotherms, kinetics, and thermodynamic studies, *Desal. Wat. Treat.* 57 (2016) 15848–15861.
- [2] A. Roy, S. Chakraborty, S.P. Kundu, B. Adhikari, S.B. Majumder, Adsorption of anionic-azo dye from aqueous solution by lignocelluloses-biomass jute fiber: equilibrium, kinetics, and thermodynamics study, *Ind. Eng. Chem. Res.* 51 (2012) 12095–12106.
- [3] R.T. Gomez, D.A.R. Ramírez, V.H. Montoya, A.B. Petriciolet, C.J.D. Valle, M.A.M. Mora'n, Synergic adsorption in the simultaneous removal of acid blue 25 and heavy metals from water using a Ca(PO₃)₂-modified carbon, *J. Hazard. Mater.* 199–200 (2012) 290–300.
- [4] S.M.Wabaidur, M.A. Khan, M.R.Siddiqui, Z. A. Allothman, S. Vasudevan, M.S. Al-Gamdi, I. H. Al-Sohami, Dodecyl sulfate chain anchored bio-char to sequester triaryl methane dyes: equilibrium, kinetics, and adsorption mechanism. *Desal. Wat. Treat.* 67 (2017) 357–370.
- [5] W.C. Andersen, S.B. Turnipseed, J.E. Roybal, Quantitative and Confirmatory Analyses of Malachite Green and Leucomalachite Green Residues in Fish and Shrimp, *J. Agric. Food Chem.* 54 (2006) 4517–4523.
- [6] Chinese fish crisis shows seafood safety challenges, https://www.usatoday.com/money/industries/food/2007-06-28-fish-cover-usat_N.htm (accessed 6th April 2019)
- [7] Veterinary Residues Committee. Annual Report on Surveillance for Veterinary Residues in Food in the UK for 2001, 2002, and 2003.
- [8] Y.-C. Lee, E.J. Kim, J.-W. Yang, H.-J. Shin, Removal of malachite green by adsorption and precipitation using aminopropyl functionalized magnesium phyllosilicate, *J. Hazard. Mater.* 192 (2011) 62–70.

- [9] F. Sayılkan, M. Asiltürk, P. Tatar, N. Kiraz, E. Arpac, H. Sayılkan, Photocatalytic performance of Sn-doped TiO₂ nanostructured mono and double layer thin films for malachite green dye degradation under UV and vis-lights, *J. Hazard. Mater.* 144 (2007) 140–146.
- [10] C. Bai, W. Xiao, D. Feng, M. Xian, D. Guo, Z. Ge, Y. Zhou, Efficient decolorization of malachite green in the Fenton reaction catalyzed by [Fe (III)-salen] Cl complex, *Chem. Eng. J.* 215 (2013) 227–234.
- [11] T.J. Nwabanne, F.C. Uzoh, Effective Decolorization of Eriochrome Black T, Furschin Basic and Malachite Green Dyes from Synthetic Wastewater by Electrocoag-nanofiltration, *Chem. Pro. Eng. Res.* 21 (2014) 22–24.
- [12] G. Kiani, M. Dostali, A. Rostami, A.R. Khataee, Adsorption studies on the removal of Malachite Green from aqueous solutions onto halloysite nanotubes, *App. Clay Sci.* 54 (2011) 34–39.
- [13] X. Wu, Y. Wang, J. Liu, J. Ma, R. Han, Study of malachite green adsorption onto natural zeolite in a fixed-bed column. *Desalin. Water Treat.* 20 (2010) 228–233.
- [14] S. Patel, G. Hota, Adsorptive removal of malachite green dye by functionalized electrospun PAN nanofibers membrane, *Fiber Polym.* 15 (2014) 2272–2282.
- [15] Y.C. Lee, E.J. Kim, J.W. Yang, H.J. Shin Removal of malachite green by adsorption and precipitation using aminopropyl functionalized magnesium phyllosilicate, *J. Hazard. Mater.* 192 (2011) 62–70.
- [16] W.H. Leung, W.H. Lo, P.H. Chan, Amyloid fibrils as rapid and efficient nanobiosorbents for removal of dye pollutants, *RSC Adv.* 5 (2015) 90022–90030.

- [17] D. Wang, L. Liu, Xinyu Jiang, Jingang Yu, Xiaoqing Chen, Adsorption and removal of malachite green from aqueous solution using magnetic β -cyclodextrin-graphene oxide nanocomposites as adsorbents, *Colloids Surf. A* 466 (2015) 166–173.
- [18] J.-H. Bae, Y.-S. Yun, S.-H. Yoon, K.-J. Choi, J.-S. Lee, C.-W. Lim, Y.-K. Kim, H.-Y. Park, Extraction Methods for Recovering Malachite Green and Leucomalachite Green, *Korean J. Fish. Aquatic Sci.* 41 (2008) 13-19.
- [19] C. Das, M. Rungta, G. Arya, S. DasGupta, S. De, Removal of Dyes and Their Mixtures from Aqueous Solution Using Liquid Emulsion Membrane. *J. Hazard. Mater.* 159 (2008) 365–371.
- [20] Y.J. Xu, X.H. Tian, X.Z. Zhang, X.H. Gong, H.H. Liu, H.J. Zhang, H. Huang, L.M. Zhang, Simultaneous determination of malachite green, crystal violet, methylene blue and the metabolite residues in aquatic products by ultra-performance liquid chromatography with electrospray ionization tandem mass spectrometry, *J. Chromatogr. Sci.* 50 (2012) 591–597.
- [21] V.J.P. Vilar, C.M.S. Botelho, R.A.R. Boaventura, Methylene blue adsorption by algal biomass based materials: biosorbents characterization and process behavior, *J. Hazard. Mater.* 147 (2007) 120–132.
- [22] Z. Zhang, Z. Zhang, Y. Fernandez, J. A. Menendez, H. Niu, J. Peng, L. Zhang, S. Guo, Adsorption isotherms and kinetics of methylene blue on a low-cost adsorbent recovered from a spent catalyst of vinyl acetate synthesis, *Appl. Surf. Sci.* 256 (2010) 2569–2576.
- [23] F. A. Ozdemir, B. Demirata, R. Apak, Adsorptive removal of methylene blue from simulated dyeing wastewater with melamine-formaldehyde-urea resin, *J. Appl. Polym. Sci.* 11 (2009) 3442–3448.

- [24] N. López-Gutiérrez, R. Romero-González, P. Plaza-Bolaños, J.L. Martínez-Vidal, A. Garrido-Frenich, Simultaneous and Fast Determination of Malachite Green, Leucomalachite Green, Crystal Violet, and Brilliant Green in Seafood by Ultrahigh Performance Liquid Chromatography–Tandem Mass Spectrometry, *Food Anal. Meth.* 6 (2013) 406–414.
- [25] J. Deng, X. Yang, L. Li, J. Cen, S. Hao, Y. Wei, Sh. Xin, Determination of the malachite green in sediment by high performance liquid chromatography with fluorescence detection, *Adv Mater. Res.* 781-784 (2013) 942–946.
- [26] S.E. Mylon, G. Benoit, Subnanomolar Detection of Acid-Labile Sulfides by the Classical Methylene Blue Method Coupled to HPLC, *Environ. Sci. Technol.* 35 (2001) 4544–4548.
- [27] N.F. Gaudette, J.W. Lodge, Determination of methylene blue and leucomethylene blue in male and female Fischer 344 rat urine and B6C3F1 mouse urine, *J. Anal. Toxicol.* 29 (2005) 28–33.
- [28] M.R. Khan, M.A. Khan, Z.A. Allothman, I.H. Alsohaimi, M. Naushada, N.H. Al-Shaalan, Quantitative determination of methylene blue in environmental samples by solid-phase extraction and ultra-performance liquid chromatography tandem mass spectrometry: a green approach, *RSC Adv.* 4 (2014) 34037–34044.
- [29] X.H., Zhou, J.R. Zhang, Z.L. Pan, D.L. Li, Review of methods for the detection and determination of Malachite Green and Leuco-Malachite green in aquaculture, *Crit. Rev. Anal. Chem.* 49 (2019)1-20.
- [30] M.A. Khan, M. Otero, M. Kazi, A.A. Alqadami, M.R. Siddiqui, Z.A. Allothman, S. Sumbul. Unary and binary adsorption studies of lead and malachite green onto a nanomagnetic copper ferrite/drumstick pod biomass composite. *J. Hazard. Mater.* 365 (2019) 759 – 770.

- [31] A. Abbas, I. Schneider, A. Bollmann, J. Funke, J. Oehlmann, C. Prasse, U. Schulte-Oehlmann, W. Seitz, T. Ternes, M. Weber, H. Wesely, M. Wagner, What you extract is what you see: Optimising the preparation of water and wastewater samples for in vitro bioassays, *Water Res.* 152 (2019) 47-60.
- [32] A.C. Gonçalves Jr., H. Nacke, V.T. Fávere, G.D. Gomes, Comparison between an anionic exchanger of chitosan quaternary ammonium salt and a commercial exchanger in the extraction of available phosphorus in soils, *Quim. Nova* 33 (2010) 1047–1052.
- [33] R. Han, L. Zhang, C. Song, M. Zhang, H. Zhu, L. Zhang, Characterization of modified wheat straw, kinetic and equilibrium study about copper ion and methylene blue adsorption in batch mode, *Carbohydr. Polym.* 79 (2010) 1140–1149.
- [34] C.S.T. Araújo, V.N. Alves, H.C. Rezende, I.L. Almeida, R.L. de Assunção, C.R. Tarley, M.G. Segatelli, N.M. Coelho, Characterization and use of *Moringa oleifera* seeds as biosorbent for removing metal ions from aqueous effluents, *Water Sci. Technol.* 62 (2010) 2198–2203.
- [35] L.C.A. Barbosa, Infrared spectroscopy for characterization of organic compounds, Ed. UFV: Viçosa, 2007, 189 p.
- [36] X. Guo, S. Zhang, X. Shan, Adsorption of metal ions on lignin, *J. Hazard. Mater.* 15 (2008) 134–142.
- [37] F. Pagnanelli, S. Mainelli, F. Veglio, L. Toro, Heavy metal removal by olive pomace: biosorbent characterization and equilibrium modeling, *Chem. Eng. Sci.* 58 (2003) 4709–4717.
- [38] Y. Guo and S. Gaiki, Retention behavior of small polar compounds on polar stationary phases in hydrophilic interaction chromatography, *J. Chromatogr. A* 1074 (2005) 71–80.

[39] EU Commission Decision 2002/657/EC of 12 August 2002, implementing Council Directive (EC) 96/23 concerning the performance of analytical methods and the interpretation of the results (text with EEA relevance), Off. J. Eur. Comm.L221 (2002) 8.

Figure captions:

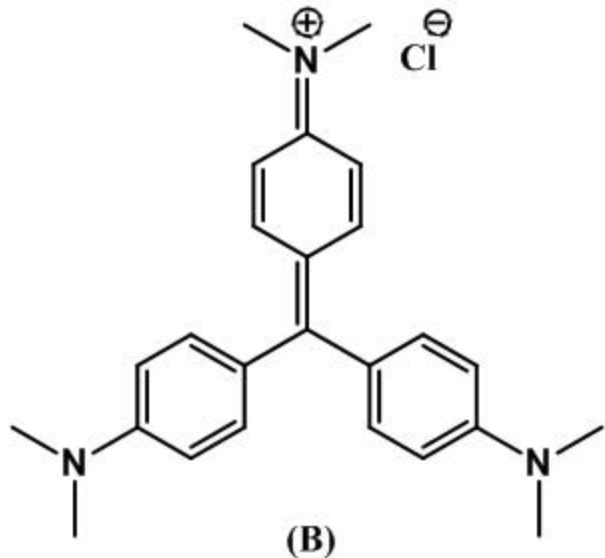
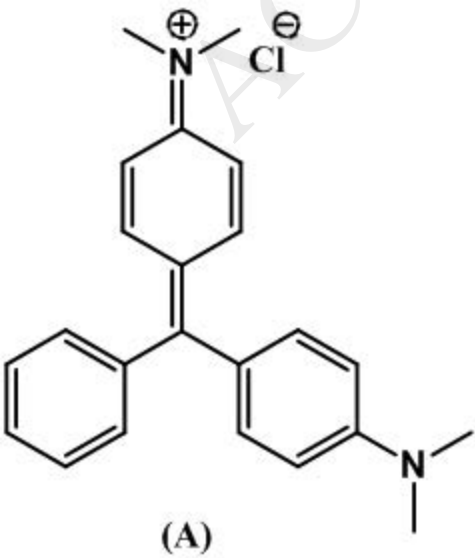
Figure 1. Chemical structures of investigated dyes: malachite green (A) and crystal violet (B).

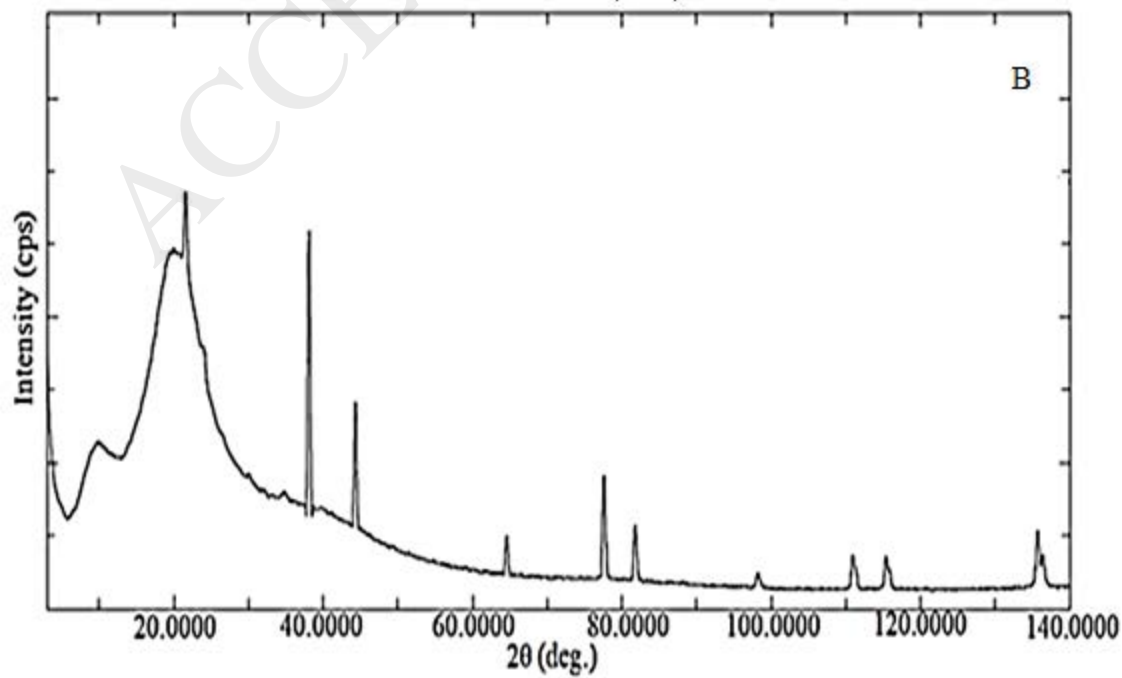
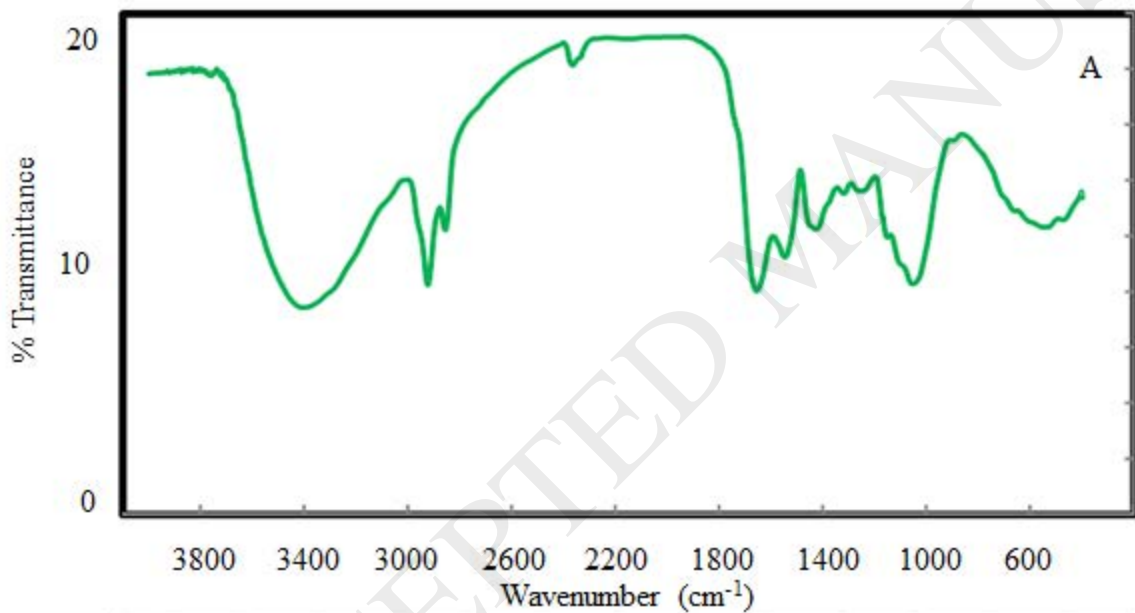
Figure 2. Fourier transform infra-red (FT-IR) spectra (A), and X-ray (XRD) diffractogram (B) of composite bio-sorbent.

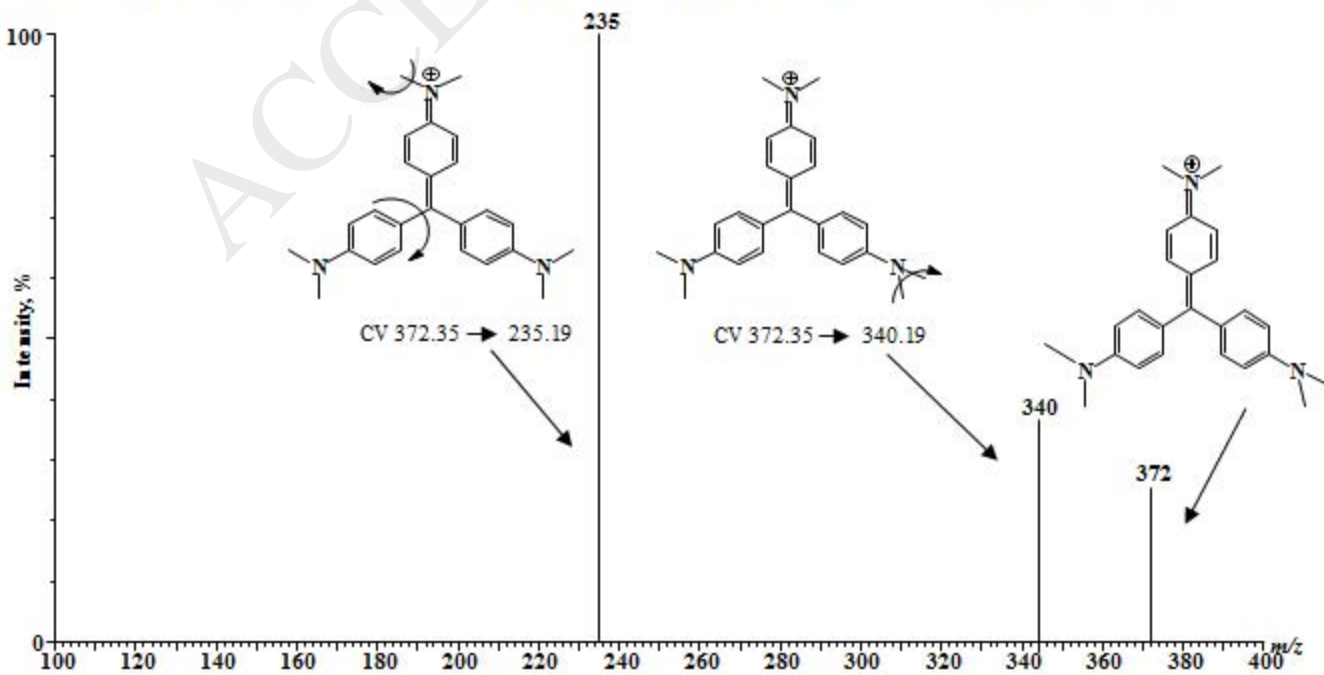
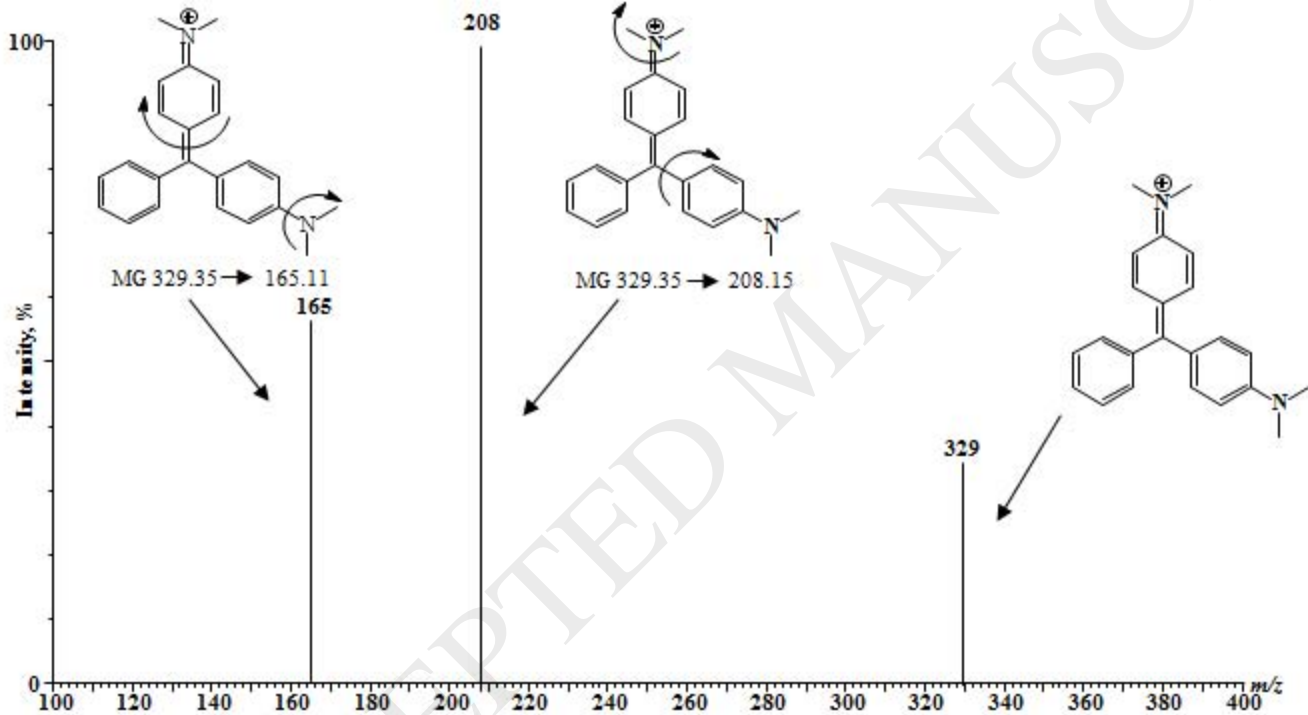
Figure 3. MS/MS spectra of malachite green precursor ions, m/z 329.35 (top), and crystal violet, m/z 372.35 (bottom) with the proposed fragmentation pattern.

Figure 4. UPLC–MS/MS chromatograms of a textile sample where malachite green and crystal violet (the latter added as internal standard) were at 1.68 and 3.00 mg/L, respectively. The Total Ion Current chromatogram for malachite green and crystal violet is shown on the top; the corresponding extracted-ion chromatograms from the dominant fragmentation of malachite green (bottom left) and crystal violet (bottom right) used for the quantification are also shown.

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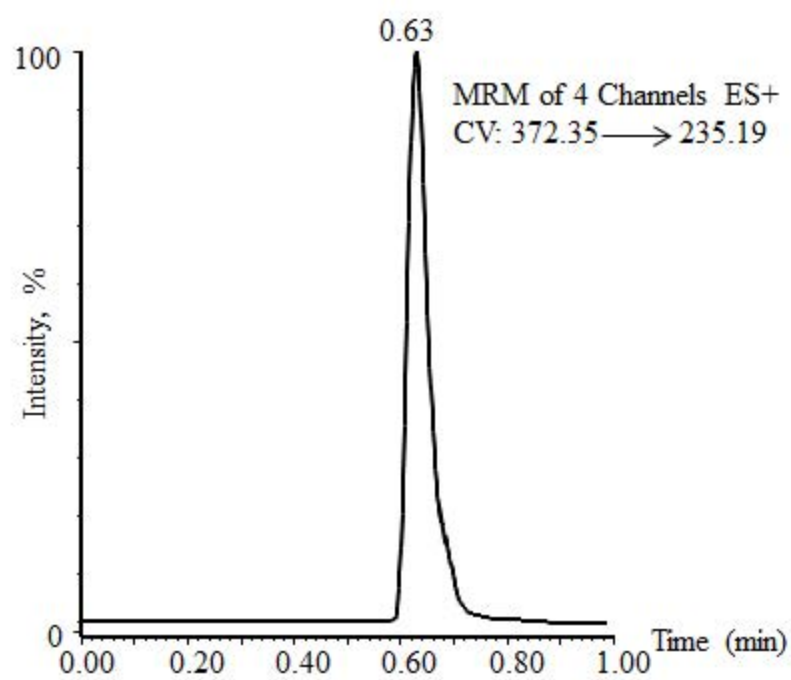
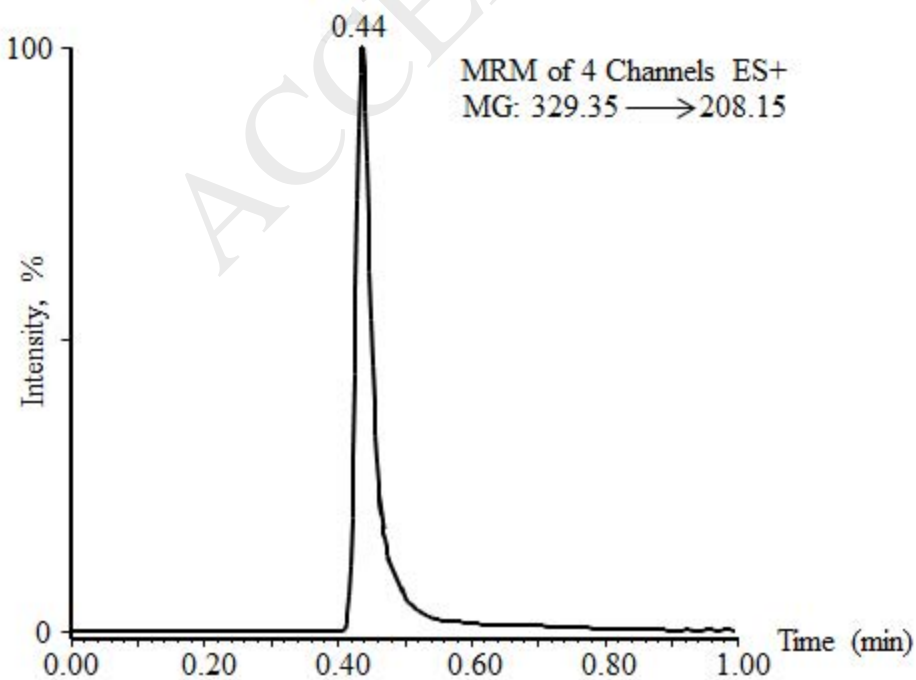
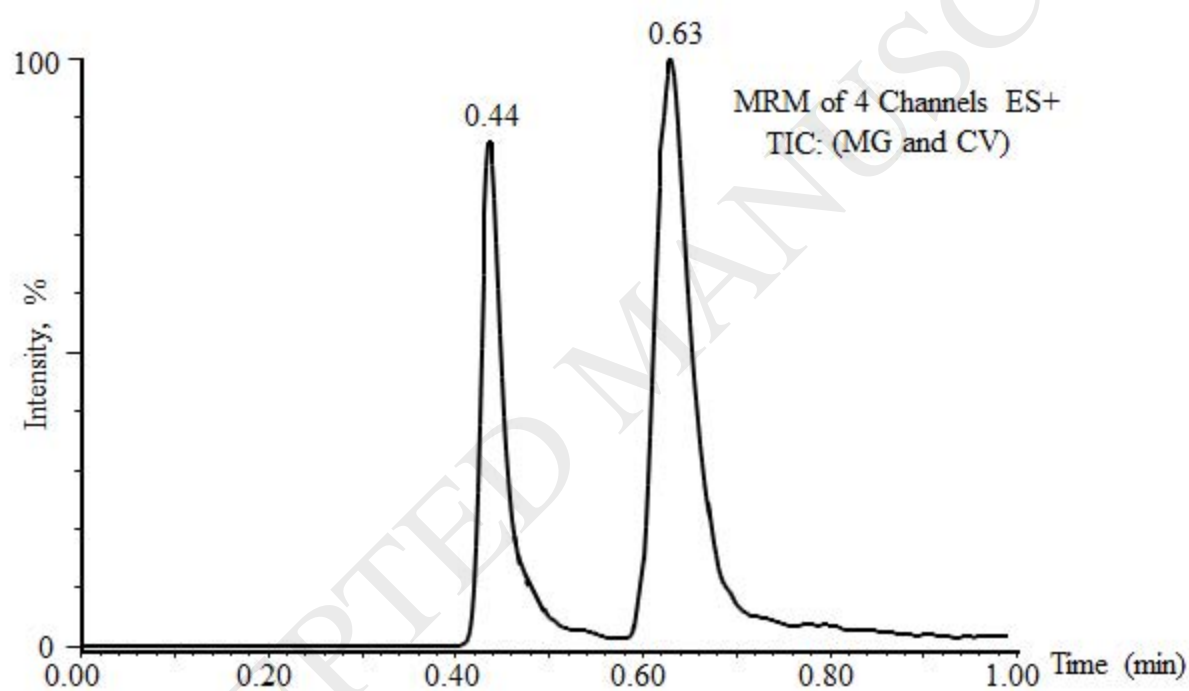


Table 1. Conditions used in the analysis of MG and CV with mass spectrometry.

Analyte	Precursor (<i>m/z</i>)	Quantification		Confirmation	
		Collision energy (V)	Product ion (<i>m/z</i>)	Collision energy (V)	Product ion (<i>m/z</i>)
MG	329	32	208	62	152
CV	372	62	235	52	340

Dwell time (0.025s) in all cases.

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Table 2. MG and recovery values in industrial wastewater

Sample	MG mean \pm s* (mg/L)	Recovery (%)
Laundry	1.32 \pm 0.04	100
Printing press	0.79 \pm 0.02	99
Paper industry	0.62 \pm 0.02	99
Textile industry	1.68 \pm 0.06	103

*standard deviation of the quantification with standard addition (n=3)

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