Special Issue on CleanWAS 2015 **Open Access**

Liu Xuqing*, Li Yayun, Liu Chao, Zhang Li, Yuan Hao, Fu Chao, Muhammad Aqeel Ashraf

Identification of residual non-biodegradable organic compounds in wastewater effluent after two-stage biochemical treatment

DOI 10.1515/biol-2016-0053
Received March 2, 2016; accepted August 21, 2016

Abstract: The main non-biodegradable compounds (soluble microbial product – SMP) of wastewater from the Maotai aromatic factories, located in the Chishui river region, were analyzed by UV spectroscopy, and by solid-phase extraction followed by gas chromatography coupled to mass spectrometry, after a two-stage biochemical treatment. The UV-Vis spectra revealed that the wastewater contained two double-bonds in conjugated systems (conjugated diene or α, β- unsaturated ketone, etc.) and simple non-conjugated chromophores containing n electrons from carbonyl groups or the like. The residual organic non-biodegradable substances were identified using SPE-GC/MS analysis as complex polymers containing hydroxyl, carbonyl, and carboxyl functional groups with multiple connections to either benzene rings or heterocyclic rings. As these compounds are difficult to remove by conventional biochemical treatments, our findings provide a scientific basis for the design of efficient new strategies to remove SMP from wastewater.

Keywords: wastewater, two-stage biochemical treatment, SMP

1 Introduction

Wastewater from the production of wine-making contains highly concentrated easily degradable organic compounds [1]. In environmentally sensitive areas, such as the region of the Chishiu river where aromatic liquor enterprises are clustered, the Chinese government limits the discharge to a maximum carbon oxygen demand (COD) of 50 mg/L (GB27631-2011) [2,3]. Currently, the wastewaters of wine-making factories are submitted to a two stage biochemical treatment, which can reduce the COD of liquor wastewater below concentrations of 100 mg/L. However, as the technology mostly targets microorganisms, it is not effective for the removal of organic compounds, even when the hydraulic retention time is extended. Indeed, Li et al. reported that liquor wastewaters, even treated by a two-stage biochemical treatment, contained between 65% and 71% of COD from SMP [3]. High values of COD mainly originate from soluble microbial products (SMP) stemming from the metabolism of the microorganisms [4,5]. These SMP are ineffectively removed by the two stage biochemical treatment and new techniques should be developed in order to meet increasingly rigorous environmental protection regulations. Therefore, determining the composition of SMP is essential in order to design a proper strategy for an advanced treatment process.

The objective of this study was to establish the composition of the SMP and the remaining non-biodegradable substances of wastewater coming from aromatic wine-making factories in the Chishui river region using UV and SPE-GC/MS to analyze concentrated wastewater samples following a two-stage biochemical treatment.

***Corresponding author: Liu Xuqing,** School of Chemical and Environmental Engineering, Wuhan Institute of Technology, Wuhan 430074, Hubei, E-mail: lxqkyq1997@163.com
Li Yayun, Liu Chao, Zhang Li, Yuan Hao, Fu Chao, School of Chemical and Environmental Engineering, Wuhan Institute of Technology, Wuhan 430074, Hubei
Muhammad Aqeel Ashraf, Faculty of Science and Natural Resources, University Malaysia Sabah 88400 Kota Kinabalu, Sabah, Malaysia

© 2016 Liu Xuqing et al., published by De Gruyter Open.
This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 License.

2 Material and methods

2.1 Experimental wastewater

Mixed wastewaters were collected from an industrial aromatic wine park located in Maotai Town from the city Renhui (Guizhou province, China) and submitted to a two-stage biochemical treatment resulting in the here-named experimental wastewater. The quality of the initial mixed wastewaters is detailed in a previous study [3].

2.2 Experimental instruments and reagents

The analyses were conducted using ultraviolet and visible spectrophotometers (UV-2802PC, Unico (Shanghai) Instruments Co., Ltd., China; UV-1601, Shimadzu Co., Japan) and, a GC-MS instrument (6890-5975, Agilent Technologies Inc., USA).

All reagents used were of analytical purity and were purified by solid-phase extraction. The organic solvents used for GC-MS analyses were of HPLC grade.

2.3 Methods of measurements and analysis

The effluent was collected from the aerobic reactor and centrifuged for 5 min at 5000 rpm. The supernatant was then filtered through a 0.45 μm Millipore filter which was then analyzed by UV spectroscopy or submitted to a solid-phase extraction (SPE). The concentrated solution after SPE was then analyzed by GC-MS. The samples which were unsuitable for routine GC-MS analytical conditions were stored at 4°C for standby measurement.

2.3.1 UV absorbance and ultraviolet and visible (UV-Vis) light scanning

The absorbance at 254 nm was measured to detect the concentration of organic matter or SMP, specifically those that contain aromatic rings or unsaturated bonds (double and triple) in their molecular structures. Ultraviolet light at the 254 nm wavelength crosses a quartz cell containing the sample water. The intensity of the attenuated light was measured with a sensor and divided by a pure water measurement prior to logarithmic calculation, reported as absorbance per path length (abs/cm), indicating the concentration of the characteristic organic matter in the water sample [6].

We define in our study the absorbance value (Abs) of the water samples as the UV254 absorbance. The maximum absorbance value was 3.100, while we obtained a maximum absorbance of 4.000 when performing a UV-scan from 190 to 600 nm [7]. All absorbance values were calculated using distilled water as blank controls.

2.3.2 Solid Phase Extraction (SPE)

Solid phase extraction (SPE), also known as solid-liquid microextraction technology, is a widely used pretreatment of water for quality-control analysis. This technology is mainly used for the purification and concentration of analytes at high recovery [8].

The SPE column containing Gea gel (1 g, The Great Eur-Asia Sci & Tech Development Co., Ltd, China) was activated by first washing the column with 100 mL methanol and then equilibrated with ultrapure water (100 mL). The water sample was delicately injected into the activated column and eluted with n-hexane (10 mL), methylene chloride (10 mL), and methanol (10 mL), respectively. The collected effluents were concentrated, dried over anhydrous sodium sulfate (Tianjin Wei-Yi Chemical Co., Ltd, China) and filtered. The respective organic solvent was evaporated on the rotary evaporator and the concentrated samples were stored in 0.5 mL sample bottles purged with nitrogen gas.

2.3.3 GC-MS

The temperature of the column was maintained at 40°C for 2 min, elevated at a rate of 10°C/min to 300°C and maintained for 2 min. The vaporization temperature was set to 250°C using helium gas as a carrier at a flow of 1.0 mL/min. The extracted sample (1.0 μL) was injected to the column in a non-splitting mode. The samples were detected by mass spectrometer using an electron impact (EI) source operating at an electron energy of 70 eV, with source, transmission line, and quadrupole rod temperatures set to 230°C, 280°C, and 150°C, respectively. The ions were scanned within the range of 50~550 amu and the attribution of the peaks was based on the standard NIST08 Mass Spectral Library.

3 Results

3.1 UV-visible spectroscopy scans

The UV-Vis scan of the raw wastewater and the one degraded in an aerobic reactor were recorded and are depicted in Fig. 1 and 2, respectively.

The spectrum of untreated wastewater exhibits a single absorption band between 400 and 420 nm. When the wastewater was submitted to a two-stage biochemical treatment, two absorption bands appeared and are attributed to the absorption band of SMP. The absorption band between 200 and 220 nm is typical of two double-bond conjugated systems such as conjugated diene or α,β-enone. The absorption band in the range of 260-280 nm is characteristic of simple non conjugated N-electron chromophores containing chemical groups such as carbonyl groups. To determine the detailed composition and relative content of the compounds containing carbonyl groups, the treated wastewater sample was submitted to a solid phase extraction (SPE) and the collected fractions were analyzed by GC-MS after elution with n-hexane, methylene dichloride, and methanol, respectively.

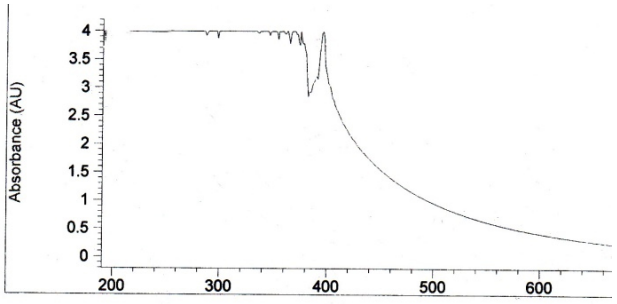

Fig. 1. UV-visible absorption spectrum of the raw liquor wastewater.

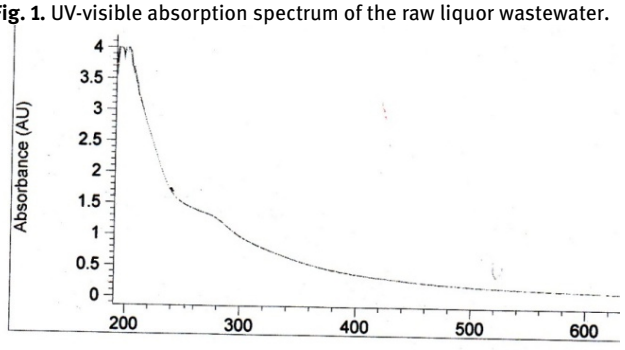

Fig. 2. UV-visible absorption spectrum of the wastewater treated in an aerobic reactor.

3.2 SPE-GC/MS results

All the peaks of the total ion current (TIC) chromatogram were identified based on the mass spectra of individual peak components at different retention times and on a standard mass spectral library.

3.2.1 SPE-GC/MS results (elution with n-hexane)

The TIC chromatogram of the treated wastewater after an elution with n-hexane is shown in Fig. 3. Through a GC-MS automated retrieval system, the most probable detected chemical formula, molecular structures, and relative content of major detected substances are summarized in Table. 1, and Fig. 4.

The experimental data indicate that after the biochemical treatment, the major organic compounds remaining in wastewater include esters, benzene derivatives, and small amounts of hydrocarbons, ketones,

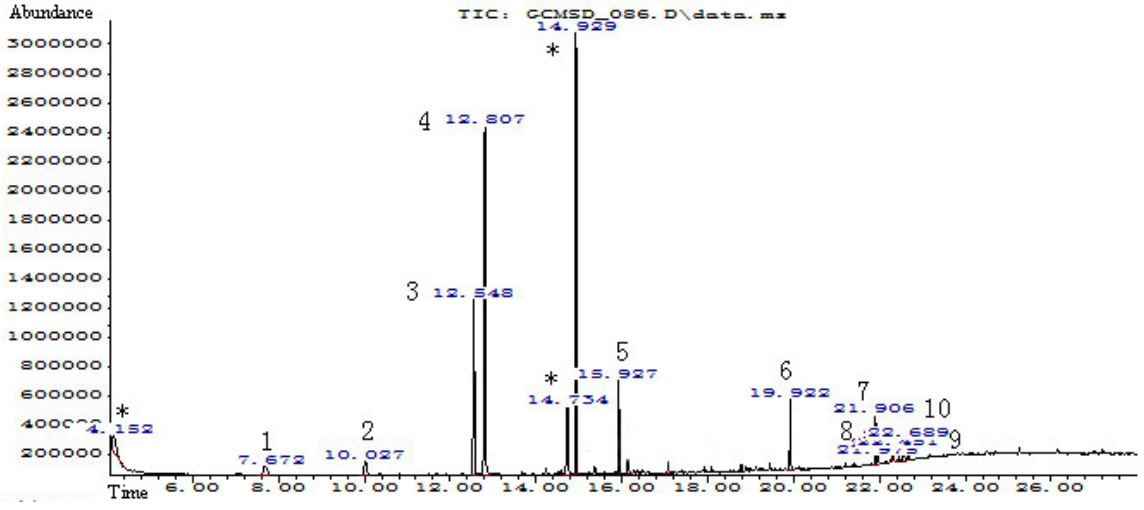

Fig. 3. The total ion current chromatogram of the treated wastewater submitted to SPE -GC/MS after an elution with n-hexane.

Table 1. Detected SMP and related characteristics after an elution with n-hexane.

No.	Retention Time (min)	Name of detected organic matter	Molecular formula	Relative content (%)
1	7.672	decane	$C_{10}H_{22}$	2.03
2	10.027	octyl aldehyde	$C_8H_{16}O$	2.06
3	12.548	4-Methylphenol	C_7H_8O	13.68
4	12.807	1,2-Propanediol cyclic carbonate	$C_4H_6O_3$	27.80
5	15.927	1-Hydroxy-2-butanone	$C_4H_8O_2$	5.15
6	19.922	1,2,11,12- two epoxy-4,9-two oxygen twelve	$C_{10}H_{18}O_4$	3.73
7	21.906	Phthalic acid dibutyl ester	$C_{16}H_{22}O_4$	2.70
8	22.295	1,2-Benzenedicarboxylic acid	$C_{12}H_{14}O_4$	0.39
9	22.451	(9Z,11E)-9,11-methyl octadecadienoate diphenyl disulfide	$C_{19}H_{34}O_2$	0.72
10	22.578	diphenyl disulfide	$C_{12}H_{10}S_2$	1.59

Note a: proportion of the peak area of each component from the total peak area

and aldehydes. The esters were the most abundant followed by phenols, exhibiting relative contents of 31.61% and 15.27% of the total detected organic matters, respectively. The rest of the organic compounds were present in minimal concentrations.

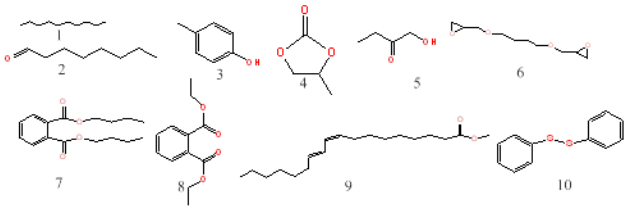

Fig. 4. Molecular structures of the main detected SMP after elution with n-hexane

3.2.2 SPE-GC/MS results (elution with dichloromethane)

The TIC chromatogram of the treated wastewater after elution with dichloromethane is shown in Fig. 5. Through a GC-MS automated retrieval system, the most probable detected chemical formula, molecular structures, and relative content of major detected substances are summarized in Table 2 and Fig. 6.

The results show that after the two-stage biochemical treatment, the major organic compounds in the wastewater included benzene derivatives, accounting for 51.37% of the total detected organic matter, and minimal concentrations of esters and alkanes.

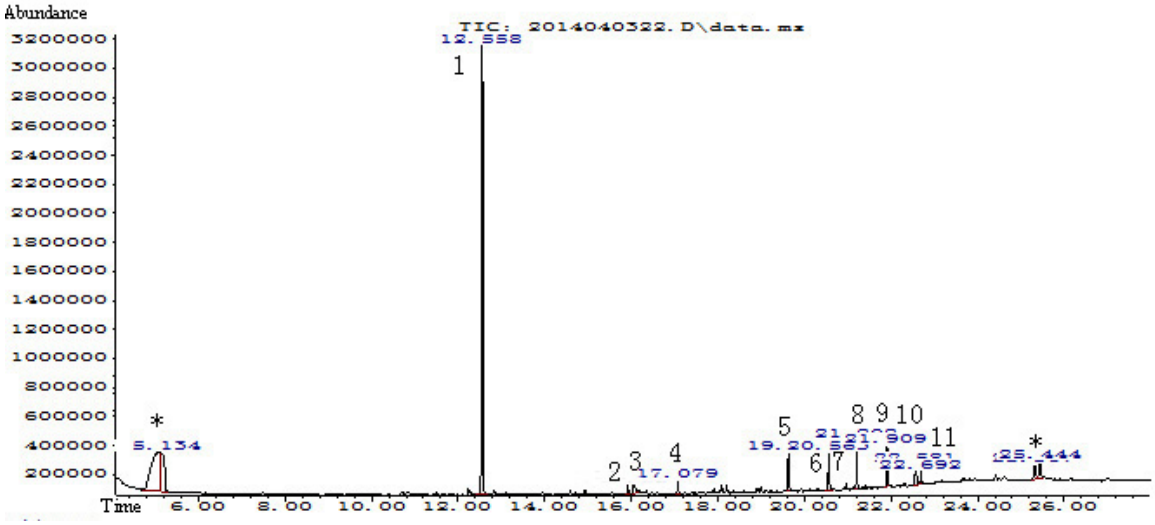

Fig. 5. The total ion current chromatogram of the treated wastewater submitted to SPE -GC/MS after elution with dichloromethane.

Table 2. Detected SMP and related parameters characteristics after an elution with dichloromethane.

No.	Retention Time (min)	Name of detected organic matter	Molecular formula	Relative content (%)
1	12.568	4-methyl phenol	C_7H_8O	41.92
2	15.924	pentadecane	$C_{15}H_{32}$	0.71
3	16.065	5-butyl-5-e lactone	$C_9H_{16}O_2$	1.43
4	17.077	hexadecane	$C_{16}H_{34}$	0.88
5	19.628	2, 3-dihydro-4-methyl indole	$C_9H_{11}N$	2.94
6	20.559	2-methyl-7-amino coumarone	C_9H_9ON	3.48
7	21.207	1-(6-methyl-1-4-butyl phenyl-amino-2-thio-1, 2-dihydro pyrimidine base)-1-ethyl ketone	$C_{17}H_{21}NS$	3.03
8	21.905	Phthalic acid dibutyl ester	$C_{16}H_{22}O_4$	2.67
9	21.910	1, 2-phthalate 2-methyl propyl alcohol butanol ester	$C_{16}H_{22}O_4$	2.82
10	22.558	1,1-dimethoxy-cyclododecan	$C_{14}H_{30}O_2$	2.20

Note a: proportion of the peak area of each component from the total peak area

3.2.3 SPE-GC/MS results (elution with methanol)

The TIC chromatogram of the treated wastewater after an elution with dichloromethane is shown in Fig. 7. Through a GC-MS automated retrieval system, the most probable detected chemical formula, molecular structures, and relative content of major detected substances are summarized in Table. 3 and Fig. 8.

The results show that wastewater submitted to the two-stage biochemical treatment contained as main organic pollutants esters, benzene derivatives, acids, and small amount of ketone and alkanes. The esters represented 25.24% of the total detected organic matters.

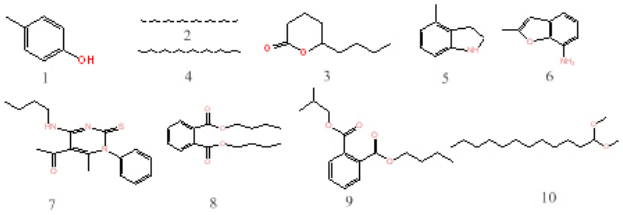

Fig. 6. Molecular structures identified after elution with n-hexane.

Benzene derivatives were ranked second with 15.96%, and the acids accounted for 13.86%. The other compounds were present only in small amounts.

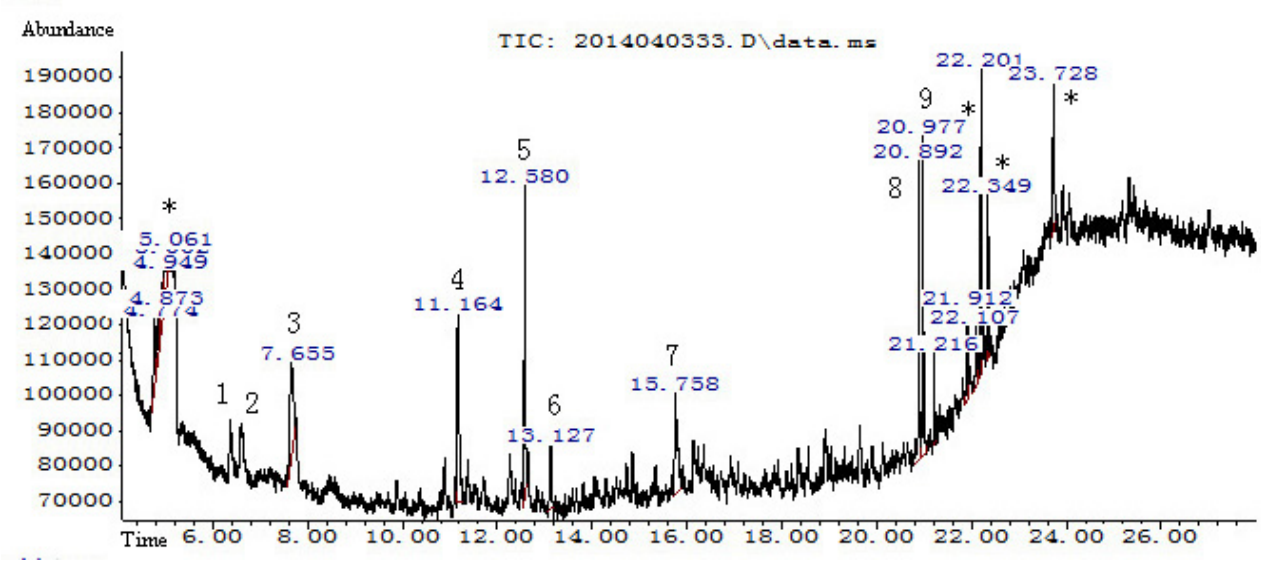

Fig. 7. The total ion current chromatogram of the treated wastewater submitted to SPE -GC/MS after an elution with methanol eluted.

Table 3. Detected SMP and related parameters characteristics after an elution with methanol.

No.	Retention time (min)	Name of detected organic matter	Molecular formula	Relative content (%)
1	6.364	heptylic acid	$C_7H_{14}O_2$	5.34
2	6.602	ethyl formate	$C_3H_6O_2$	5.62
3	7.665	decane	$C_{10}H_{22}$	9.76
4	11.202	2- hydroxy -2,4,6- cyclohepta -1- three allyl ketone hydroxy-2 minus three-ring g ene-1-ketone	$C7H6O_2$	10.13
5	12.579	4-methyl phenol	C_7H_8O	10.84
6	13.115	1-phenyl-2-amino-1-acetone	$C_9H_{11}NO$	5.12
7	15.747	2-phenyl acetic acid	$C_8H_8O_2$	8.52
8	20.888	methyl hexadecanoate	$C_{17}H_{34}O_2$	9.74
9	20.974	(Z)-9-hexadecenoic acid methyl ester	$C_{17}H_{32}O_2$	9.88

Note a: proportion of the peak area of each component from the total peak area

4 Discussion

According to the results of UV spectroscopy and SPE-GC/MS analysis, the absorption band at 260-280 nm can be attributed to the carbonyl groups of the SMP. After a two-stage biochemical treatment, the remaining non-biodegradable substances were mainly composed of a complex mixture of organic compounds presenting hydroxy, carbonyl, and carboxyl groups, and connected to benzene rings or heterocycles, which do not easily. Therefore, we suggest performing a forced degradation by catalytic oxidation on advanced treatment units at the final stage of the wastewater treatment. If the organic compounds are still not removed after a forced degradation, they could be filtered on activated carbon.

Acknowledgements: We would like to express our gratitude to the many people who made invaluable contributions, both directly and indirectly to our research. We would like to express our heartfelt gratitude to Professor DaYin Liu for providing us with instructive suggestions and valuable comments on the writing of this paper.

Conflict of interests: The authors declare no conflict of interests.

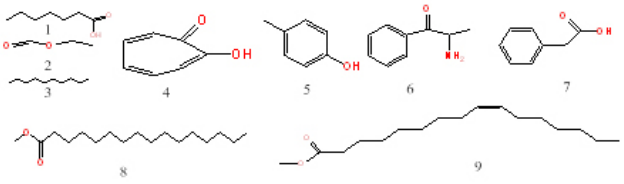

Fig. 8. Molecular structures of the main detected materials SMP after elution with methanol.

References

[1] Faryal M., Iqra J., Uzaira R., Noshabah T., Maliha S., Sher Z. S., Ismail Y., Ashraf, M. A., New method for the adsorption of organic pollutants using natural zeolite incinerator ash (ZIA) and its application as an environmentally friendly and cost-effective adsorbent, Desalin. Water Treat., 2016, 57, 6230-6231.

[2] GB27631-2011, Standard of Water Pollutants Discharge in Fermentation Alcohol and Liquor Industry. Ministry of Environmental Protection, People S' Republic of China., Available online http://english.mep.gov.cn/standards_reports/standards/water_environment/Discharge_standard/201201/W020111116454988514732.pdf

[3] Li Y., Liu X., The law of the residual COD in distilled spirit wastewater after two stages of biochemical treatment, J. App. Mechanics Mater, 2014, 1, 596-601.

[4] Kousar P., Uzaira R., Sher Z. S., Ashraf, M. A (2016). A novel method for synthesis of functionalized hybrids and their application for wastewater treatment, Desalin. Water Treat., 2016, 57, 161-170.

[5] Huixing L., Aihui C., Zhaoxia L., Ashraf M. A., Cheng D., Influences of 1,2-dichlorobenzene on bacterial community structure in wetland soil, Sains Malaysiana, 2016, 45, 129-134.

[6] Iqra J., Faryal M., Uzaira R., Noshabah T., Balkhair K. S., Ashraf M. A., Synthesis of zeolite from marble powder waste: a greener approach and its application for the removal of inorganic metals from wastewater, Desalin. Water Treat., 57 (22) 10422-10431.

[7] Anjum R., Farah F., Uzaira R., Saima N., Ashraf M. A., Analysis of sorption efficiency of activated carbon for removal of anthracene and pyrene for wastewater treatment, Desalin. Water Treat., 57, 145-150.

[8] Tahira Q., Najma M., Saima Q. M., Ashraf M. A., Deconta-mination of ofloxacin: optimization of removal process onto sawdust using response surface methodology, Desalin. Water Treat., 57, 221-129.

[9] Huo L., A novel determination method for GC/MS and high performance capillary electrophoresis of certain harmful substance in food, Hebei Uni. J., 2010, 1, 12-20.