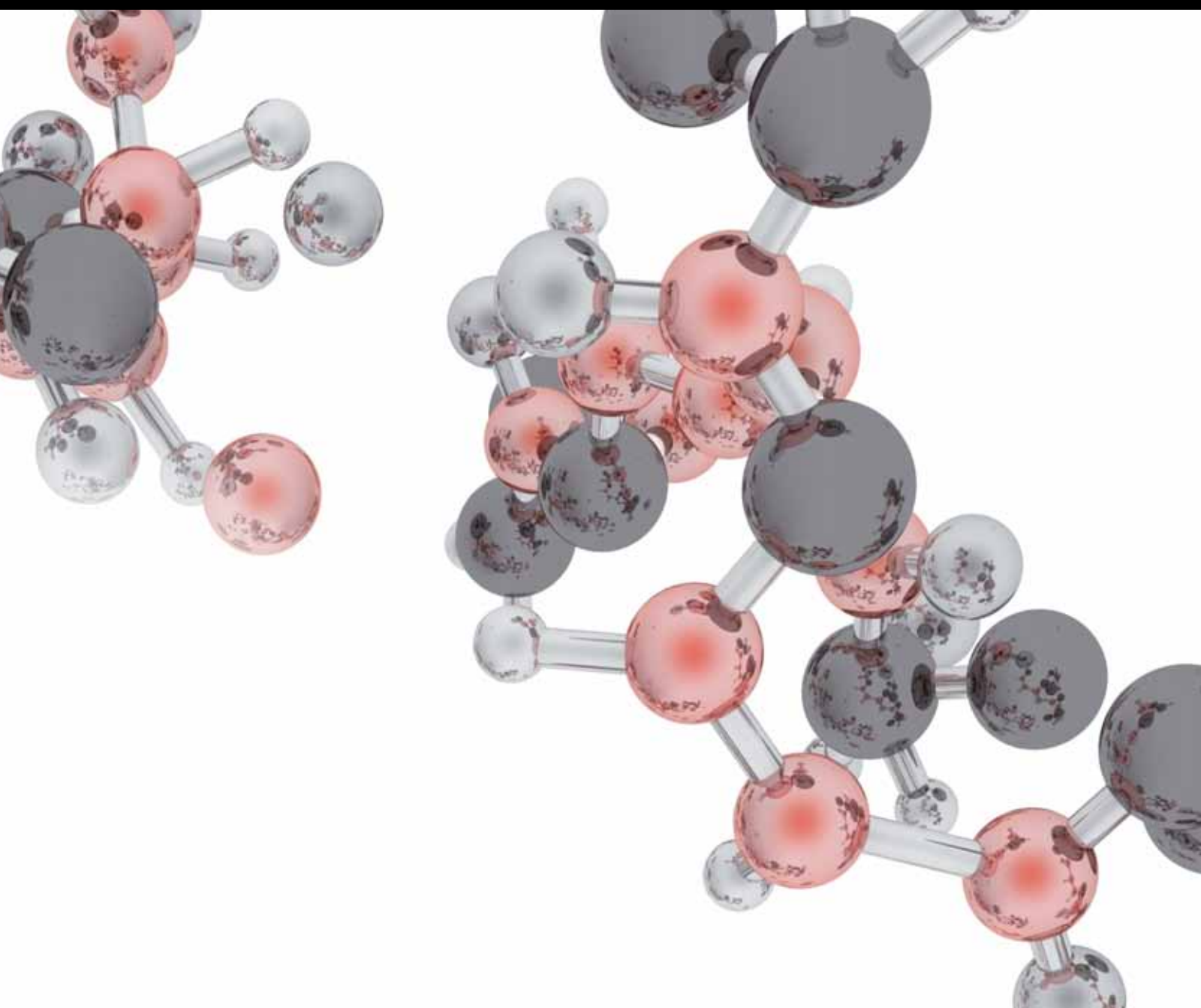


# ANALYSIS AND FATE OF EMERGING POLLUTANTS DURING WATER TREATMENT

GUEST EDITORS: FEI QI, GUANG-GUO YING, KAIMIN SHIH, JOLANTA KUMIRSKA,  
ELIF PEHLIVANOGLU-MANTAS, AND XIAOJUN LUO





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# **Analysis and Fate of Emerging Pollutants during Water Treatment**

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Guest Editors: Fei Qi, Guang-Guo Ying, Kaimin Shih,  
Jolanta Kumirska, Elif Pehlivanoglu-Mantas, and Xiaojun Luo



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## Editorial

# Analysis and Fate of Emerging Pollutants during Water Treatment

**Fei Qi,<sup>1</sup> Guang-Guo Ying,<sup>2</sup> Kaimin Shih,<sup>3</sup> Jolanta Kumirska,<sup>4</sup>  
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Emerging pollutants defined as compounds that are not currently covered by existing water-quality regulations all over the world, have not been studied widely before, and are thought to be potential threats to environmental ecosystems and human health. This special issue compiles 5 exciting papers, which are very meticulously performed researches.

Generally, emerging pollutants encompass a diverse group of compounds, including pharmaceuticals, drugs of abuse, personal-care products (PCPs), steroids and hormones, surfactants, perfluorinated compounds (PFCs), flame retardants, industrial additives and agents, gasoline additives, new disinfection byproducts (DBPs), nanomaterials, and the toxic minerals.

The analysis methods, occurrence, and fate of hormonal and endocrine disruptors compounds (EDCs) were discussed in two papers of this special issue. R. Guedes-Alonso et al. determine the hormonal residues in treated water by ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS) and evaluate the efficiency of the conventional wastewater treatment for the removal of hormonal compounds. Moreover, Y. Liu et al. study another kinds of PPCPs, named as phthalate esters that is typical kind of EDCs. The occurrence in a surface water and the removal efficiency in a traditional drinking water treatment plant are studied. According to results of the two papers,

the occurrence and fate of hormonal and EDCs in water or wastewater treatment are very clear.

J. Xing et al. report a new wastewater treatment technology, bioflocculation, for the removal of sulfamethoxazole that is a typical pharmaceutical in wastewater. The performance and the reaction mechanism of the biodegradation of sulfamethoxazole by the bioflocculation are discussed in depth. Moreover, the optimum reaction condition is obtained.

The heavy metal and toxic mineral are also important pollutants in the environment, especially in the mining area. Two papers of this issue are focused on this. K. Naemullah et al. report a green preconcentration method for the determination of cobalt and lead in water. Y. Liu et al. do a novel research on the electrochemical reaction of pyrite as a simulation of the natural environmental.

By compiling this special issue, we hope to enrich our readers and researchers on the analysis and fate of emerging pollutants during water treatment.

Fei Qi  
Guang-Guo Ying  
Kaimin Shih  
Jolanta Kumirska  
Elif Pehlivanoglu-Mantas  
Xiaojun Luo

## Research Article

# Occurrence and Removal Characteristics of Phthalate Esters from Typical Water Sources in Northeast China

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The presence of phthalate esters (PAEs) in the environment has gained a considerable attention due to their potential impacts on public health. This study reports the first data on the occurrence of 15 PAEs in the water near the Mopanshan Reservoir—the new and important water source of Harbin city in Northeast China. As drinking water is a major source for human exposure to PAEs, the fate of target PAEs in the two waterworks (Mopanshan Waterworks and Seven Waterworks) was also analyzed. The results demonstrated that the total concentrations of 15 PAEs in the water near the Mopanshan Reservoir were relatively moderate, ranging from 355.8 to 9226.5 ng/L, with the mean value of 2943.1 ng/L. DBP and DEHP dominated the PAE concentrations, which ranged from 52.5 to 4498.2 ng/L and 128.9 to 6570.9 ng/L, respectively. The occurrence and concentrations of these compounds were heavily spatially dependent. Meanwhile, the results on the waterworks samples suggested no significant differences in PAE levels with the input of the raw waters. Without effective and stable removal of PAEs after the conventional drinking water treatment in the waterworks (25.8% to 76.5%), the risks posed by PAEs through drinking water ingestion were still existing, which should be paid special attention to the source control in the Mopanshan Reservoir and some advanced treatment processes for drinking water supplies.

## 1. Introduction

Phthalate acid esters, a class of chemical compounds mainly used as plasticizers for polyvinyl chloride (PVC) or to a lesser extent other resins in different industrial activities, are ubiquitous in the environment and have evoked interest in the past decade due to endocrine disrupting effects and their potential impacts on public health [1–3].

Worldwide production of PAEs is approximately 6 million tons per year [4]. As PAEs are not chemically bound to the polymeric matrix in soft plastics, they can enter the environment by losses during manufacturing processes and by leaching or evaporating from final products [5]. Therefore, the occurrence and fate of specific PAEs in natural water environments have been observed, and also there are a lot of considerable controversies with respect to the safety of PAEs in water [5–10].

Six PAE compounds, including dimethyl (DMP), diethyl (DEP), dibutyl (DBP), butylbenzyl (BBP), di(2-ethylhexyl)

(DEHP), and di-n-octyl phthalate (DNOP), are classified as priority pollutants by the U.S. Environmental Protection Agency (EPA). Though the toxicity of PAEs to humans has not been well documented, for some years, the Ministry of Environmental Protection in China has regulated phthalates as environmental pollutants. In addition, the standard in China concerning analytical controls on drinking waters does not specifically identify any PAEs as organic pollutant indexes to be determined by the new drinking water standard in 2007 (Standard for drinking water quality; GB5749-2006), which was forced to be monitored for the drinking water supplies in 2012. Consequently, official data about the presence of these pollutants in the aquatic environment of some cities are not available.

Harbin, the capital of Heilongjiang Province, is a typically old industrial base and economically developed city with a population of over 3 million in Northeast China. As the newly enabled water source for Harbin city, Mopanshan waterworks supply the whole city with drinking water through long



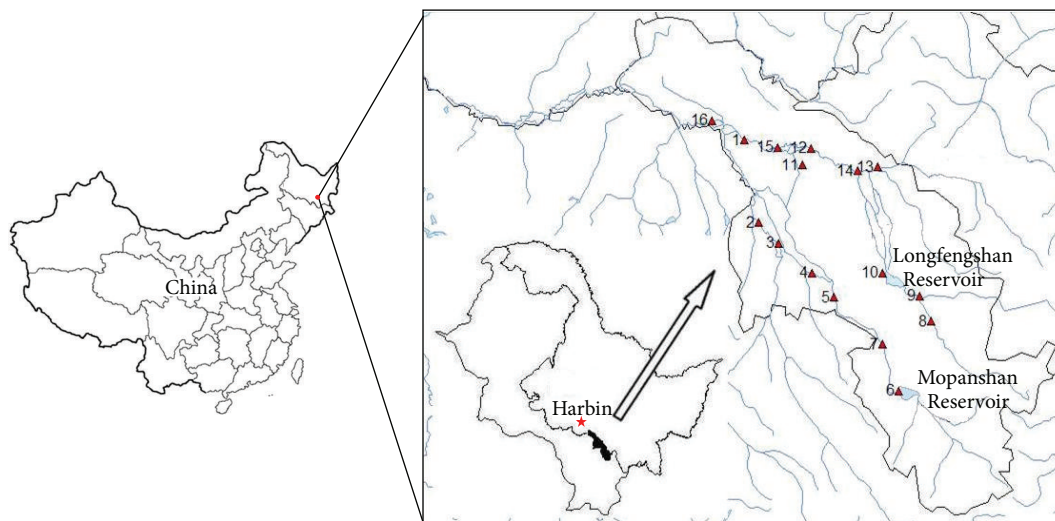


FIGURE 1: Spatial distribution of the 16 sampling sites near the Mopanshan Reservoir in Northeast China.

distance transfer from the Mopanshan Reservoir. To the authors' knowledge, the occurrence and fate of PAEs in the water near this area and its relative waterworks have not previously been examined.

The objectives of this study were (i) to determine the occurrence of PAEs and clarify the fate and distribution of the pollutants in the water source, (ii) to examine the two waterworks, where traditional drinking water treatment step was evaluated on the PAEs removal efficiencies, and (iii) to evaluate the potential for adverse effects of PAEs on human health. Therefore, the investigation of PAEs in the water can provide a valuable record of contamination in Harbin city.

## 2. Materials and Methods

**2.1. Chemical.** Fifteen PAEs standard mixture, including dimethyl phthalate, diethyl phthalate, diisobutyl phthalate, di-n-butyl phthalate, di(4-methyl-2-pentyl) phthalate, di(2-ethoxyethyl) phthalate, di-n-amyl phthalate, di-n-hexyl phthalate, butyl benzyl phthalate, di(hexyl-2-ethylhexyl) phthalate, di(2-n-butoxyethyl) phthalate, dicyclohexyl phthalate, di(2-ethylhexyl) phthalate, di-n-nonyl phthalate, di-n-octyl phthalate at 1000  $\mu\text{g}/\text{mL}$  each, and surrogate standards, consisting of diisophenyl phthalate, di-n-phenyl phthalate, and di-n-benzyl phthalate, in a mixture solution of 500  $\mu\text{g}/\text{mL}$  each, were supplied by AccuStandard Inc. As the internal standard, benzyl benzoate, was also purchased from AccuStandard Inc. All solvents (acetone, hexane, and dichloromethane) used were HPLC-grade and were purchased from J. T. Baker Co. (USA). Anhydrous sodium sulfate (Tianjin Chengguang Chemical Reagent Co., China) was cleaned at 600°C for 6 h and then kept in a desiccator before use.

**2.2. Sample Collection and Preparation.** Harbin, the capital of Heilongjiang province in China, imports nearly all of its drinking water from two sources: the Mopanshan Reservoir

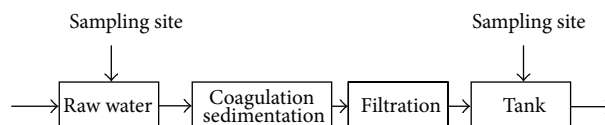


FIGURE 2: Schematic diagram of the waterworks.

(long-distance transport project) and the Songhua River (old water source).

Water samples from the Mopanshan Reservoir and Mopanshan waterwork were collected in 2008. The locations of the sampling sites near the Mopanshan Reservoir are presented in Figure 1; as a comparison, sampling from another Harbin water supply—Seven waterworks with water source from the Songhua River was performed in 2011. In each waterworks, with considering the hydraulic retention time, three sets of raw water samples (influent) were taken, and then the final finished waters (effluent) after the whole treatment process were carried out for the collections. The flow diagram of the waterworks is shown in Figure 2.

Samples were collected using 2.0 L glass jars from 0.5 m below the water surface. During the whole sampling process, the global position system was used to locate the sampling stations (details in Table 1). All samples were transferred to the laboratory directly after sampling and stored at 4°C prior to extraction within 2 d.

Water samples were filtered under vacuum through glass fiber filters (0.7  $\mu\text{m}$  pore sizes). Prior to extraction, each sample was spiked with surrogate standards. The water samples were extracted based on a classical liquid phase extraction method (USEPA, method 8061) with slight modifications. Briefly, 1 L of water samples was placed in a separating funnel and extracted by means of mechanical shaking with 150 mL dichloromethane, and then, with filtration on sodium sulfate (about 20 g), the organic extracts were concentrated using a rotary evaporator. The exchange of solvent was done by replacing dichloromethane with hexane. Finally, they were

TABLE 1: Detailed descriptions of the sampling locations.

Number	Sampling site	Name	Latitude	Longitude
1#	Lalin, Jinsan Bridge	Mangniu River	E127°02'53"	N45°5'45"
2#	Xingsheng Town, Gaojiatun	Lalin River	E127°06'11"	N44°51'57"
3#	Dujia Town, Shuguang	Lalin River	E127°10'44"	N44°48'41"
4#	Shanhe Town, Taipingchuan	Lalin River	E127°18'36"	N44°43'50"
5#	Xiaoyang Town, Qichuankou	Lalin River	E127°23'54"	N44°39'46"
6#	Shahezi Town	Mopanshan Reservoir	E127°38'59"	N44°24'17"
7#	Shahezi Town, Gali	Lalin River	E127°35'17"	N44°31'56"
8#	Chonghe Town, Changcuizi	Mangniu River	E127°46'36"	N44°35'46"
9#	Chonghe Town, Xingguo	Mangniu River	E127°35'17"	N44°31'57"
10#	Longfeng Town	Mangniu River	E127°35'19"	N44°43'48"
11#	Changpu Town, Zhonghua	Mangniu River	E127°16'30"	N45°1'42"
12#	Erhe Town, Shuanghe	Mangniu River	E127°18'32"	N45°4'28"
13#	Zhiguang Town, Songjiajie	Mangniu River	E127°34'3"	N45°1'20"
14#	Zhiguang Town, Wuxing	Mangniu River	E127°29'20"	N45°0'43"
15#	Changpu Town, Xingzhuang	Mangniu River	E127°10'42"	N45°4'36"
16#	Yingchang Town, Xingguang	Lalin River	E126°55'8"	N45°9'0"

reduced to 0.5 mL under gentle nitrogen flow. The internal standard was added to the sample prior to instrumental analysis.

**2.3. Chemical Analysis.** The extracted compounds were determined by gas chromatography coupled to mass spectrometer analysis as described in other publications [11, 12]. Briefly, extracted samples were injected into an Agilent 6890 Series GC equipped with a DB-35MS capillary column (Agilent; 30 m  $\times$  0.25 mm i.d.; 0.25  $\mu$ m film thickness) and an Agilent 5973 MS detector, operating in the selective ion monitoring mode. The column temperature was initially set at 70°C for 1 min, then ramped at 10°C/min to 300°C and held constant for 10 min. The transfer line and the ion source temperature were maintained at 280 and 250°C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/min. The extracts (2.0  $\mu$ L) were injected in splitless mode with an inlet temperature of 300°C.

**2.4. Quality Assurance and Quality Control.** All glassware was properly cleaned with acetone and dichloromethane before use. Laboratory reagent and instrumental blanks were analyzed with each batch of samples to check for possible contamination and interferences. Only small levels of PAEs were found in procedural blanks in some batches, and the background subtraction was appropriately performed in the quantification of concentration in the water samples. Calibration curves were obtained from at least 3 replicate analyses of each standard solution. The surrogate standards were added to all the samples to monitor matrix effects. Recoveries of PAEs ranged from 62 to 112% in the spiked water and the surrogate recoveries were  $75.1 \pm 12.7\%$  for diisophenyl phthalate,  $72.3 \pm 14.3\%$  for di-n-phenyl phthalate, and  $102.6 \pm 10.4\%$  for di-n-benzyl phthalate in the water samples. The determination limits ranged from 6 to 30 ng/L. A midpoint calibration check standard was injected

as a check for instrumental drift in sensitivity after every 10 samples, and a pure solvent (methanol) was injected as a check for carryover of PAEs from sample to sample. All the concentrations were not corrected for the recoveries of the surrogate standards.

### 3. Results and Discussion

**3.1. PAEs in Water Source.** The PAEs in the waters from the sampling sites near the Mopanshan Reservoir were investigated and the results are presented in Table 2. The  $\sum_{15}$ PAEs concentrations ranged from 355.8 to 9226.5 ng/L, with the geometric mean value of 2943.1 ng/L. Among the 15 PAEs detected in the waters, DIBP, DBP, and DEHP were measured in all the samples, with the average concentrations being 196.6, 801.4, and 1774.1 ng/L, respectively. The three PAE congeners are important and popular additives in many industrial products including flexible PVC materials and household products, suggesting the main source of PAE contaminants in the water [13]. The correlations of the concentration of DEHP, DBP, and DIBP with the concentrations of total PAEs in the water samples are shown in Figure 3, and a relatively significant correlation between  $\sum_{15}$ PAEs and DEHP concentration was found, suggesting the important part played by DEHP in total concentrations of PAEs in the water bodies near the Mopanshan Reservoir. In other words, the contribution of DEHP to total PAEs was higher than that of other PAE congeners in the water samples. In addition, DMP, DEP and DNOP, with the mean value of 14.0, 28.4, and 54.1 ng/L, respectively, only detected at some sampling sites, and have also attracted much attention as the priority pollutants by the China National Environmental Monitoring Center. On the contrary, the concentrations of 6 PAEs (DMEP, BMPP, BBP, DBEP, DCHP, and DNP) were below detection limits in all the water samples near the Mopanshan Reservoir, which is easily explained in terms of much lower quantities of present use in China.

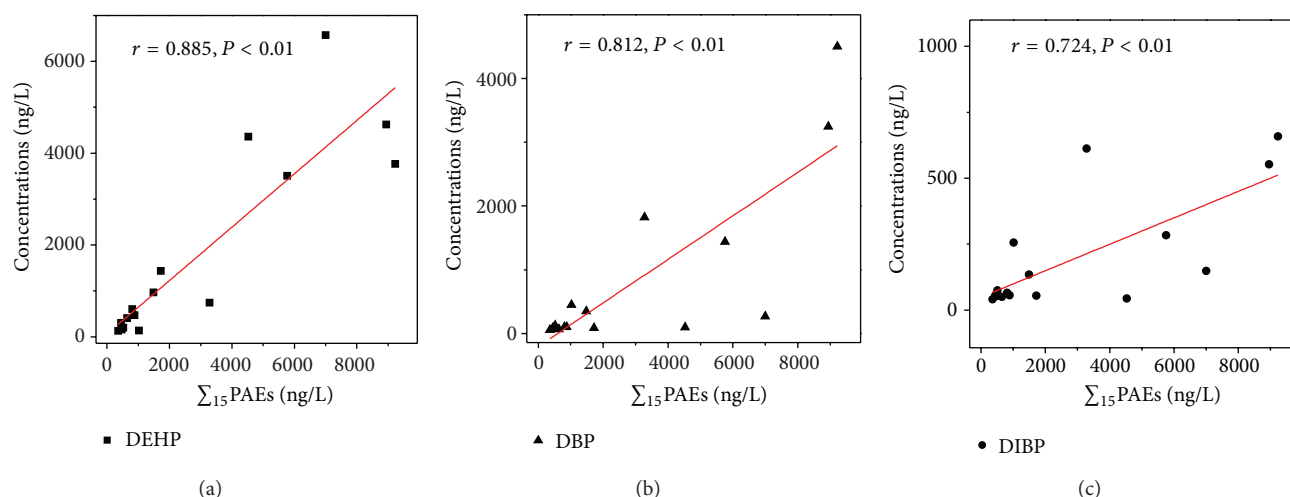


FIGURE 3: Correlations of the concentration of the major PAEs (DEHP, DBP, and DIBP) with the concentrations of total PAEs in the water samples.

TABLE 2: Concentrations of 15 PAEs in water samples near the Mopanshan Reservoir.

PAEs	Abbreviation	Water (ng/L)		
		Range	Mean	Frequency
Dimethyl phthalate	DMP	nd–42.4	14.0	8/16
Diethyl phthalate	DEP	nd–55.0	28.4	15/16
Diisobutyl phthalate	DIBP	40.0–658.8	196.6	16/16
Dibutyl phthalate	DBP	52.5–4498.2	801.4	16/16
bis(2-methoxyethyl)phthalate	DMEP	nd	nd	0/16
bis(4-methyl-2-pentyl)phthalate	BMPP	nd	nd	0/16
bis(2-ethoxyethyl)phthalate	DEEP	nd–54.6	12.8	5/16
Dipentyl phthalate	DPP	nd–92.5	45.5	10/16
Dihexyl phthalate	DHXP	nd–65.1	16.2	5/16
Benzyl butyl phthalate	BBP	nd	nd	0/16
bis(2-n-butoxyethyl)phthalate	DBEP	nd	nd	0/16
Dicyclohexyl phthalate	DCHP	nd	nd	0/16
bis(2-ethylhexyl)phthalate	DEHP	128.9–6570.9	1774.1	16/16
Di-n-octyl phthalate	DNOP	nd–448.2	54.1	5/16
Dinonyl phthalate	DNP	nd	nd	0/16
Σ <sub>15</sub> PAEs		355.8–9226.5	2943.1	—

The distribution of 15 PAEs ( $\Sigma_{15}$ PAEs) studied and 6 US EPA priority PAEs ( $\Sigma_6$ PAEs; including DMP, DEP, DIBP, DBP, DEHP, and DNOP) in the waters from different sampling sites are shown in Figure 4. There was an obvious variation in the total  $\Sigma_{15}$ PAEs concentrations in water samples near the Mopanshan Reservoir; the concentrations of  $\Sigma_6$ PAEs from the same sampling sites varied from 269.0 to 9086.0 ng/L, with an average of 2868.6 ng/L, the distribution spectra of which observed for all the sampling sites were similar to  $\Sigma_{15}$ PAEs. The highest levels of PAEs contamination were seen on the sampling site 3, followed by some relatively heavily polluted sites (site 16, 13, 9, 10, and 11), indicating that these sites served as important PAE sources, and the spatial distribution of PAEs was site specific. The levels in the waters examined varied over a wide range, especially in the Mangniu

River near the Mopanshan Reservoir (in some case over more than one order of magnitude). In general, it should be noted that there might be a relation of PAEs levels with the input of local waste, such as sewage water, food packaging, and scrap material near the sampling point, which were found during the sampling period.

**3.2. PAE Congener Profiles in the Water.** Different PAE patterns may indicate different sources of PAEs. Measurement of the individual PAE composition is helpful to track the contaminant source and demonstrate the transport and fate of these compounds in water [11]. The relative contributions of the 9 detectable PAE congeners to the  $\Sigma_{15}$ PAEs concentrations in the water are presented in Figure 5. It is clear that DEHP was the most abundant in the water samples with

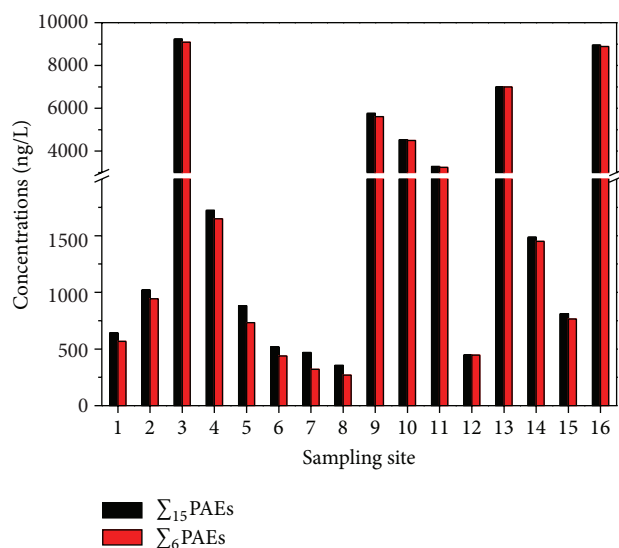


FIGURE 4: Spatial distributions of the 16 sampling sites near the Mopanshan Reservoir.

the exception of site 2, 3 and 11, contributions ranging from 33.1% to 96.3%, followed by DBP, ranging from 2.1% to 36.3%. The results are consistent with the above data for overall analysis that DBP and DEHP are the dominant components of the PAEs distribution pattern in each sampling site, which reflected the different pattern of plastic contaminant input during the sampling period. Similarly, DIBP and DBP are used in epoxy resins or special adhesive formulations, with the different proportions of these two PAE congeners, which were also the important indicator of the information polluted by PAEs for the sampling locations. Although the limited sample number draws only limited conclusions, there is still reason to note that a leaching from the plastic materials into the runoff water is possible, and that water runoff from the contaminated water is a burden pathway from different sources of PAEs [14].

**3.3. Comparison with Other Water Bodies.** Comparison with the total PAEs concentrations is nevertheless very limited due to the different analysis compounds. However, the individual PAE, namely, DBP and DEHP, is by far the most abundant in other researches, and it is possible to make a comparison with our findings. In this case, the results of DBP and DEHP concentrations published in literatures for kinds of water bodies are presented together in Table 3.

The DEHP and DBP concentrations of the present study showed, to some extent, lower concentration levels than those reported in the other water bodies in China. In comparison, the results were comparable or similar to those from the examinations described in other foreign countries. For example, as shown in Table 3, the DEHP concentrations were similar to the surface waters from the Netherlands and Italy described in the literature. Meanwhile, these concentrations in this study were quite lower than the Yangtze River and Second Songhua River in China.

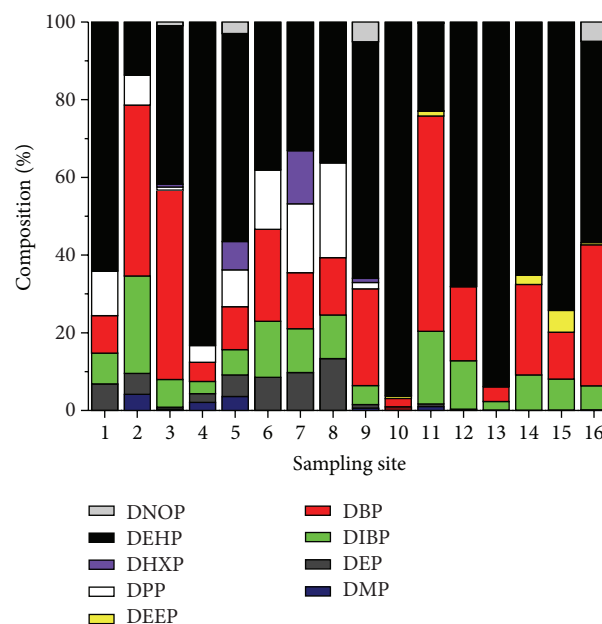


FIGURE 5: PAE composition of the water samples in 16 sampling sites.

In conclusion, as compared to the results of other studies, the waters near the Mopanshan Reservoir were moderately polluted by PAEs. Therefore, there is a definite need to set up a properly planned and systematic approach to water control near the Mopanshan Reservoir.

**3.4. PAE Levels in the Waterworks.** The Mopanshan Waterworks (MPSW), equipped essentially with the water source of the Mopanshan Reservoir, has been investigated in order to assess the fate of the PAEs during the drinking water treatment process. For comparison, a sampling campaign for the determination of PAEs levels in the Seven Waterworks (SW, waterworks with the old water source of the Songhua River) was carried out. Both waterworks operate coagulation, sedimentation and followed by filtration treatment process, which are typical traditional drinking water treatment.

The measured concentrations in the raw and finished water of the two investigated waterworks are shown in Figure 6. Six out of fifteen PAEs were detected in the finished water from the two waterworks. The detected PAEs were DMP, DEP, DIBP, DBP, DEHP, and DNOP, and the other investigated phthalates are of minor importance, with concentrations all below the limit of detection. As shown in Figure 6, the measured concentrations of the analyzed PAEs in the finished water of the two waterworks varied strongly. The most important compound in the finished water was DEHP, with the mean concentration of 3473.7 and 4059.2 ng/L for the MPSW and SW, respectively, suggesting the highest relative composition of total PAE concentrations in the drinking water.

For the raw water from the different water sources, DMP and DIBP concentrations in the MPSW were much lower than the ones in the SW, and the concentrations of DEP, DBP, DEHP, and DEHP were relatively comparable in the two

TABLE 3: Comparison of the concentrations of DEHP and DBP in the water bodies (ng/L).

Location	DEHP			DBP			Reference
	Range	Median	Mean	Range	Median	Mean	
Surface water, Germany	330–97800	2270	—	120–8800	500	—	[14]
Surface water, the Netherlands	nd–5000	320	—	66–3100	250	—	[15]
Seine River estuary, France	160–314	—	—	67–319	—	—	[16]
Tama River, Japan	13–3600	—	—	8–540	—	—	[17]
Velino River, Italy	nd–6400	—	—	nd–44300	—	—	[2]
Surface water, Taiwan	nd–18500	—	9300	1000–13500	—	4900	[18]
Surface water, Jiangsu, China	556–15670.7	—	—	16–5857.5	—	—	[19]
Yangtze River, mainstream, China	3900–54730	—	—	nd–35650	—	—	[20]
Middle and lower Yellow River, China	347–31800	—	—	nd–26000	—	—	[21]
Second Songhua River, China	nd–1752650	370020	—	nd–5616800	—	717240	[22]
Urban lakes, Guangzhou, China	87–630	170	240	940–3600	1990	2030	[11]
Xiangjiang River, China	620–15230	—	—	—	—	—	[23]
This study	128.9–6570.9	671.0	1774.1	52.5–4498.2	110.3	801.4	

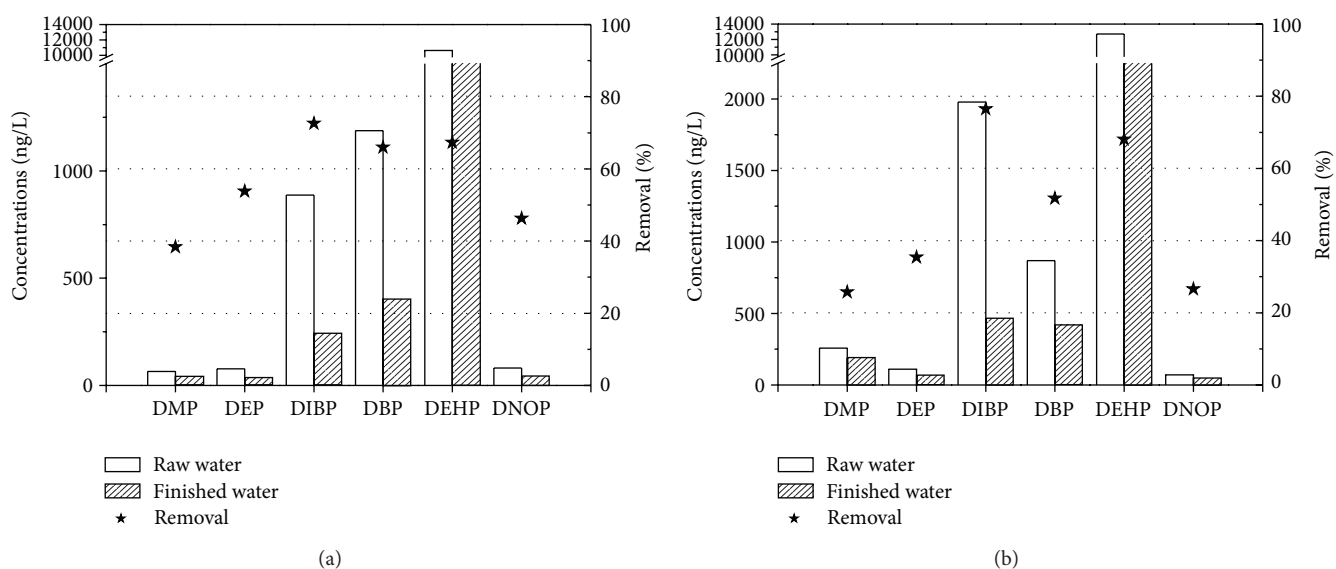


FIGURE 6: PAEs detected in the water samples from the MPSW (a) and SW (b).

waterworks. The removal of PAEs by these two waterworks ranged from 25.8% to 76.5%, which varied significantly without stable removal efficiencies. The lower removal efficiencies for DMP and DNOP were observed in the SW, with the removal less than 30%. For both the waterworks, no sound removal efficiencies were obtained for the PAEs, indicating that the traditional drinking water treatment cannot show good performance to eliminate these micro pollutants, which has nothing to do with the type of the water source.

Traditional drinking water treatment focuses on dealing with the particles and colloids in terms of physical processes. Many studies of the environmental fates of PAEs have demonstrated that oxidation or microbial action is the principal mechanism for their removal in the aquatic systems [24–26]. Therefore the treatment process should be the combinations with the key techniques for removing PAEs from the water. On the other hand, since the removal efficiencies of PAEs by

these advance drinking water treatments in waterworks has not been systematically studied to date, further research in this direction would seem to be required.

**3.5. Exposure Assessment of PAEs in Water.** To evaluate the potential and adverse effects of PAEs, quality guidelines for surface water and drinking water standard were used. The results indicated that the mean concentrations of DBP and DEHP at levels were well below the reference doses (RfD) regarded as unsafe by the EPA for the surface water. The levels were not also above the RfD recommended by China (Environmental Quality Standard for Surface Water of China, GB3838-2002). On the other hand, the amounts of DEHP present in public water supplies should be lower than the drinking water standard (0.006 mg/L for EPA and 0.008 mg/L for China). According to the results, the concentrations of



DEHP in drinking water samples from the MPSW were lower than the limited value.

PAEs are also considered to be endocrine disrupting chemicals (EDCs), whose effects may not appear until long-term exposure. According to the results, PAEs were detected in the drinking water constantly ingested in daily life, indicating that drinking water is an important source of human exposure to PAEs contaminants. Assuming a daily water consumption rate of 2 L and an average body weight of 60 kg for adults, the average daily intake of DEHP, DBP, and DIBP by way of drinking water from MPSW was calculated to be 115.8, 13.52, and 8.1 ng/kg/d, respectively. In comparison, the values of SW with the water source of the Songhua River were relatively higher, with the calculated results of 135.3, 14.0, and 15.5 ng/kg/d for DEHP, DBP, and DIBP, respectively. In this study, the estimated daily intake levels of DEHP from the drinking water were quite lower than the RfD of 20000 ng/kg/d released by the EPA. However, some of PAEs are partly metabolised in the organism, and future experiments should be focused on determining the potential effects of the metabolites [27].

Currently treated water from the Songhua River is for nonpotable uses, and MPSW is the exclusive waterworks run for the water supply of Harbin city. However, population growth and drought cycle are limited by the availability of raw water from the Mopanshan Reservoir. To meet the increasing demand, local and regional water authorities have begun a campaign of second water supply project from the Songhua River again, which needs the protection of the Songhua River and advanced water treatment for the source.

#### 4. Conclusions

This study provided the first detailed data on the contamination status of 15 PAEs in the water near the Mopanshan Reservoir. The concentration range of 15 PAEs in the samples was from 355.8 to 9226.5 ng/L, with the mean value of 2943.1 ng/L. DEHP and DBP were the main pollutants among 15 PAEs, accounting for the main watershed pollution. The occurrence and distribution of PAEs from different sampling sites in the water source varied largely, suggesting that the spatial distribution of PAEs was site specific. In addition, the monitoring of PAEs in the waterworks also showed that PAEs can be detected in the drinking water, and certain toxicological risks to drinking water consumers were found. These results reported here contribute to an understanding of how PAE contaminants are distributed in the new water source and the waterworks in Harbin city as well as forming a basis for further modeling, risk assessment, and selection of drinking water treatment technology.

In conclusion, the results implied that no urgent remediation measures were required with respect to PAEs in the waters. However, the ecological and health effects of these substances through drinking water at the relatively lower concentrations still need further notice in light of their possible biological magnifications. Therefore, the long-term source control in the water and adding advanced treatment process for drinking water supplies should be given special attention in this area.

#### Acknowledgments

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#### References

- [1] M. Nikonorow, H. Mazur, and H. Piekacz, "Effect of orally administered plasticizers and polyvinyl chloride stabilizers in the rat," *Toxicology and Applied Pharmacology*, vol. 26, no. 2, pp. 253–259, 1973.
- [2] M. Vitali, M. Guidotti, G. Macilenti, and C. Cremisini, "Phthalate esters in freshwaters as markers of contamination sources—a site study in Italy," *Environment International*, vol. 23, no. 3, pp. 337–347, 1997.
- [3] G. Latini, C. de Felice, G. Presta et al., "In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy," *Environmental Health Perspectives*, vol. 111, no. 14, pp. 1783–1785, 2003.
- [4] C. E. Mackintosh, J. A. Maldonado, M. G. Ikononou, and F. A. P. C. Gobas, "Sorption of phthalate esters and PCBs in a marine ecosystem," *Environmental Science and Technology*, vol. 40, no. 11, pp. 3481–3488, 2006.
- [5] M. Clara, G. Windhofer, W. Hartl et al., "Occurrence of phthalates in surface runoff, untreated and treated wastewater and fate during wastewater treatment," *Chemosphere*, vol. 78, no. 9, pp. 1078–1084, 2010.
- [6] M. M. Abdel Daiem, J. Rivera-Utrilla, R. Ocampo-Pérez, J. D. Méndez-Díaz, and M. Sánchez-Polo, "Environmental impact of phthalic acid esters and their removal from water and sediments by different technologies—a review," *Journal of Environmental Management*, vol. 109, pp. 164–178, 2012.
- [7] L. Chen, Y. Zhao, L. Li, B. Chen, and Y. Zhang, "Exposure assessment of phthalates in non-occupational populations in China," *Science of the Total Environment*, vol. 427–428, pp. 60–69, 2012.
- [8] M. J. Teil, M. Blanchard, and M. Chevreuil, "Atmospheric fate of phthalate esters in an urban area (Paris-France)," *Science of the Total Environment*, vol. 354, no. 2–3, pp. 212–223, 2006.
- [9] P. Roslev, K. Vorkamp, J. Aarup, K. Frederiksen, and P. H. Nielsen, "Degradation of phthalate esters in an activated sludge wastewater treatment plant," *Water Research*, vol. 41, no. 5, pp. 969–976, 2007.
- [10] J. Gasperi, S. Garnaud, V. Rocher, and R. Moilleron, "Priority pollutants in wastewater and combined sewer overflow," *Science of the Total Environment*, vol. 407, no. 1, pp. 263–272, 2008.
- [11] F. Zeng, K. Cui, Z. Xie et al., "Occurrence of phthalate esters in water and sediment of urban lakes in a subtropical city, Guangzhou, South China," *Environment International*, vol. 34, no. 3, pp. 372–380, 2008.
- [12] F. Zeng, K. Cui, Z. Xie et al., "Phthalate esters (PAEs): emerging organic contaminants in agricultural soils in peri-urban areas around Guangzhou, China," *Environmental Pollution*, vol. 156, no. 2, pp. 425–434, 2008.
- [13] G. Wildbrett, "Diffusion of phthalic acid esters from PVC milk tubing," *Environmental Health Perspectives*, vol. 3, pp. 29–35, 1973.

- [14] H. Fromme, T. Küchler, T. Otto, K. Pilz, J. Müller, and A. Wenzel, "Occurrence of phthalates and bisphenol A and F in the environment," *Water Research*, vol. 36, no. 6, pp. 1429–1438, 2002.
- [15] A. D. Vethaak, J. Lahr, S. M. Schrap et al., "An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of The Netherlands," *Chemosphere*, vol. 59, no. 4, pp. 511–524, 2005.
- [16] C. Dargnat, M. Blanchard, M. Chevreuil, and M. J. Teil, "Occurrence of phthalate esters in the Seine River estuary (France)," *Hydrological Processes*, vol. 23, no. 8, pp. 1192–1201, 2009.
- [17] T. Suzuki, K. Yaguchi, S. Suzuki, and T. Suga, "Monitoring of phthalic acid monoesters in river water by solid-phase extraction and GC-MS determination," *Environmental Science and Technology*, vol. 35, no. 18, pp. 3757–3763, 2001.
- [18] S. Y. Yuan, C. Liu, C. S. Liao, and B. V. Chang, "Occurrence and microbial degradation of phthalate esters in Taiwan river sediments," *Chemosphere*, vol. 49, no. 10, pp. 1295–1299, 2002.
- [19] B. Li, C. Qu, and J. Bi, "Identification of trace organic pollutants in drinking water and the associated human health risks in Jiangsu Province, China," *Bulletin of Environmental Contamination and Toxicology*, pp. 1–5, 2012.
- [20] F. Wang, X. Xia, and Y. Sha, "Distribution of phthalic acid esters in Wuhan section of the Yangtze River, China," *Journal of Hazardous Materials*, vol. 154, no. 1–3, pp. 317–324, 2008.
- [21] Y. J. Sha, X. H. Xia, and X. Q. Xiao, "Distribution characters of phthalic acid ester in the waters middle and lower reaches of the Yellow River," *China Environmental Science*, vol. 26, no. 1, pp. 120–124, 2006.
- [22] J. Lu, L.-B. Hao, C.-Z. Wang, W. Li, R.-J. Bai, and D. Yan, "Distribution characteristics of phthalic acid esters in middle and lower reaches of no. 2 Songhua River," *Environmental Science & Technology*, vol. 12, article 014, 2007.
- [23] X. J. Zhu and Y. Y. Qiu, "Measuring the phthalates of xiangjiang river using liquid-liquid extraction gas chromatography," *Advanced Materials Research*, vol. 301, pp. 752–755, 2011.
- [24] B. L. Yuan, X. Z. Li, and N. Graham, "Aqueous oxidation of dimethyl phthalate in a Fe(VI)-TiO<sub>2</sub>-UV reaction system," *Water Research*, vol. 42, no. 6–7, pp. 1413–1420, 2008.
- [25] Z. Yunrui, Z. Wanpeng, L. Fudong, W. Jianbing, and Y. Shaoxia, "Catalytic activity of Ru/Al<sub>2</sub>O<sub>3</sub> for ozonation of dimethyl phthalate in aqueous solution," *Chemosphere*, vol. 66, no. 1, pp. 145–150, 2007.
- [26] H. N. Gavala, U. Yenil, and B. K. Ahring, "Thermal and enzymatic pretreatment of sludge containing phthalate esters prior to mesophilic anaerobic digestion," *Biotechnology and Bioengineering*, vol. 85, no. 5, pp. 561–567, 2004.
- [27] Y. Guo, Q. Wu, and K. Kannan, "Phthalate metabolites in urine from China, and implications for human exposures," *Environment International*, vol. 37, no. 5, pp. 893–898, 2011.

## Research Article

# Simultaneous Determination of Hormonal Residues in Treated Waters Using Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectrometry

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In the last years, hormone consumption has increased exponentially. Because of that, hormone compounds are considered emerging pollutants since several studies have determined their presence in water influents and effluents of wastewater treatment plants (WWTPs). In this study, a quantitative method for the simultaneous determination of oestrogens (estrone,  $17\beta$ -estradiol, estriol,  $17\alpha$ -ethinylestradiol, and diethylstilbestrol), androgens (testosterone), and progestogens (norgestrel and megestrol acetate) has been developed to determine these compounds in wastewater samples. Due to the very low concentrations of target compounds in the environment, a solid phase extraction procedure has been optimized and developed to extract and preconcentrate the analytes. Determination and quantification were performed by ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The method developed presents satisfactory limits of detection (between 0.15 and  $9.35 \text{ ng}\cdot\text{L}^{-1}$ ), good recoveries (between 73 and 90% for the most of compounds), and low relative standard deviations (under 8.4%). Samples from influents and effluents of two wastewater treatment plants of Gran Canaria (Spain) were analyzed using the proposed method, finding several hormones with concentrations ranged from 5 to  $300 \text{ ng}\cdot\text{L}^{-1}$ .

## 1. Introduction

In general, it is supposed that more than 100,000 different chemical compounds can be introduced in the Environment, many of them in very small quantity. However, a lot of these compounds are not included as pollutants in the legislation. Although these compounds, named emerging pollutants, are not regulated as pollutants, they probably will be in the future because of their potential negative effect in the ecosystem. For 20 years, many articles have reported the presence of these “new compounds” in wastewater [1, 2].

The emerging pollutant origin is mainly anthropogenic, considering that the majority of these compounds are biologically active substances that are synthesized to use them in agriculture, industry, and medicine. The main source of these emerging pollutants is the residual urban waters and the wastewater treatment plants effluents because many of these

WWTPs are not designed or optimized to treat this kind of compounds [3].

Hormones are one of the most potent endocrine disrupting compounds as well as are considered also as emerging pollutants. Hormones can be differentiated in oestrogens, androgens, and progestogens. Some of them have limits in their use, but not a specific legislation [4].

The main characteristic of these pollutants is that it is not necessary to remain in the environment to cause negative effects, in view of the fact that their constant introduction in it offsets their removal or degradation [5].

The steroid hormones help controlling the metabolism, inflammations, immunological functions, water and salt balance, sexual development, and the capacity of withstanding illnesses [6]. The term steroid can be used for natural hormones produced by the body as well as for artificially produced medicines that increase the natural steroid effect.



In the last 50 years, the natural and synthetic hormone worldwide consumption has grown, as much as in human medicine as in cattle farming, and they become the most prescribed medicines [7].

A significant quantity of consumed oestrogens leaves the organism through excretions. For example,  $17\beta$ -estradiol (E2) is oxidized rapidly, becoming an estrone (E1) that can turn into estriol afterwards (E3). Besides, the  $17\alpha$ -ethinyl estradiol (EE) is excreted as conjugated [8].

With regard to emission sources, in the first place are the wastewater treatment plants (WWTPs) [9], and secondarily, cattle waste such as those leachates from dung and uncontrolled dumping [10]. Several studies made in the WWTPs have reported that the treatment plants are capable of eliminating around 60% of hormones [11–13].

The identification of hormone residues in environment is of special interest because knowledge of these compounds is a requirement to take measures in order to regulate and minimize their environmental impact.

However, measurement of hormone residues is a very difficult task not only due to the difficulty in measuring very low concentration, but also due to a very complexity of the samples. Therefore, use of mass spectrometer (MS) as detector coupled with chromatography techniques has become a powerful method for the analysis of these types of compounds at trace levels [14–17]. Consequently, LC-MS/MS is the principally chosen technique. One of the main advantages of LC-MS/MS is its ability to analyze hormones without derivatization (necessary in GC) or the need of hydrolyze the conjugated form.

Due to low level concentration of these compounds in environmental water, it is necessary to apply an extraction and preconcentration method prior to LC analysis. The most used technique of extraction and preconcentration method for liquid samples is the solid phase extraction (SPE) [18–20].

The objective of this study is to develop a rapid and simple procedure of extraction, preconcentration, and determination of four steroid oestrogens (estrone (E1),  $17\beta$ -estradiol (E2), estriol (E3) and  $17\alpha$ -ethinylestradiol (EE)), one non-steroidal oestrogen, the diethylstilbestrol (DES), one androgen, the testosterone (TES) and two synthetic progestogens, norgestrel (NOR) and megestrol acetate (MGA) (Table 1), based on solid phase extraction and ultrahigh performance liquid chromatography-tandem mass spectrometry (SPE-UHPLC-MS/MS). The developed method was applied to the identification and quantification of these compounds in wastewater samples obtained from the influents and effluents of two wastewater treatment plants (WWTPs) of Gran Canaria (Spain). They presented different methods of wastewater treatments: WWTP 1 presented a traditional method based on activated sludge, while WWTP 2 used a membrane bioreactor technique.

## 2. Materials and Methods

**2.1. Reagents.** All of the hormonal compounds used were purchased from Sigma-Aldrich (Madrid, Spain). Stock solutions containing  $1000 \text{ mg}\cdot\text{L}^{-1}$  of each analyte were prepared by

dissolving the compound in methanol, and the solutions were stored in glass-stoppered bottles at  $4^\circ\text{C}$  prior to use. Working aqueous standard solutions were prepared daily. Ultrapure water was provided by a Milli-Q system (Millipore, Bedford, MA, USA). HPLC-grade methanol, LC-MS methanol, and LC-MS water as well as the ammonia and the ammonium acetate used to adjust the pH of the mobile phases were obtained from Panreac Química (Barcelona, Spain).

**2.2. Sample Collection.** Water samples were collected from the effluents of two wastewater treatment plants located in the northern area of Gran Canaria in May and August of 2012. WWTP 1 used a conventional activated sludge treatment system, while WWTP 2 employed a membrane bioreactor treatment system. The samples were collected in 2 L amber glass bottles that were rinsed beforehand with methanol and ultrapure water. Samples were purified through filtration with fibreglass filters and then with  $0.65 \mu\text{m}$  membrane filters (Millipore, Ireland). The samples were stored in the dark at  $4^\circ\text{C}$  and extracted within 48 hours.

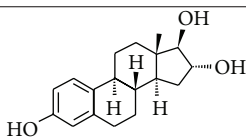
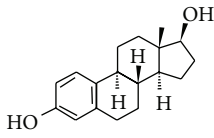
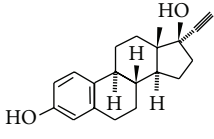
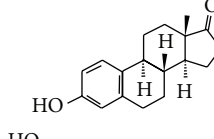
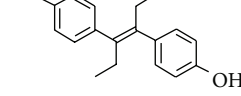
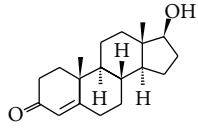
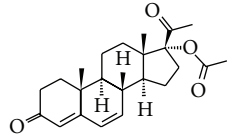
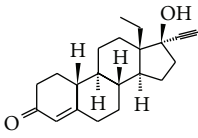
**2.3. Instrumentation.** For the SPE optimization, the instrument used was an ultrahigh performance liquid chromatography with fluorescence detector (UHPLC-FD) system consisting of an ACQUITY Quaternary Solvent Manager (QSM) used to load samples and wash and recondition the analytical column, an autosampler, a column manager, and a fluorescence detector with excitation and emission wavelengths of 280 and 310 nm, respectively, all from Waters (Madrid, Spain).

The analysis of wastewater samples was performed in a UHPLC-MS/MS system from Waters (Madrid, Spain), similar to the described above, with a 2777 autosampler equipped with a  $25 \mu\text{L}$  syringe and a tray to hold 2 mL vials, and an ACQUITY tandem triple quadrupole (TQD) mass spectrometer with an electrospray ionization (ESI) interface. All Waters components (Madrid, Spain) were controlled using the MassLynx Mass Spectrometry Software. Electrospray ionisation parameters were fixed as follows: the capillary voltage was 3 kV in positive mode, and  $-2 \text{ kV}$  in negative mode, the source temperature was  $150^\circ\text{C}$ , the desolvation temperature was  $500^\circ\text{C}$ , and the desolvation gas flow rate was  $1000 \text{ L/hr}$ . Nitrogen was used as the desolvation gas, and argon was employed as the collision gas.

The detailed MS/MS detection parameters for each hormonal compound are presented in Table 2 and were optimised by the direct injection of a  $1 \text{ mg}\cdot\text{L}^{-1}$  standard solution of each analyte into the detector at a flow rate of  $10 \mu\text{L}\cdot\text{min}^{-1}$ .

**2.4. Chromatographic Conditions.** For the SPE optimization, the analytical column was a  $50 \text{ mm} \times 2.1 \text{ mm}$ , ACQUITY UHPLC BEH Waters  $\text{C}_{18}$  column with a particle size of  $1.7 \mu\text{m}$  (Waters Chromatography, Barcelona, Spain) operating at a temperature of  $30^\circ\text{C}$ . Analytes separation was carried out employing the following gradient: starting at 55:45 (v/v) water: methanol for 1 minute. During 3 minutes, it changed to 50:50 (v/v) and stayed for 2.5 minutes more. Finally, came back to initial conditions in 1 minute, and stayed for 1.5

TABLE 1: List of hormonal compounds,  $pK_a$  values, chemical structure, and retention times.

Compound		$pK_a$ [21]	Structure	$t_R$ (min)
E3	Estriol	10.3		0.96
E2	$17\beta$ -estradiol	10.3		2.18
EE	$17\alpha$ -ethinylestradiol	10.3		2.23
E1	Estrone	10.3		2.20
DES	Diethylstilbestrol	10.2		2.35
TES	Testosterone	15.1		2.40
MGA	Megestrol acetate			3.06
NOR	Levonorgestrel	13.1		2.63

minutes. Therefore, the analysis took 9 minutes at a flow of  $0.5 \text{ mL} \cdot \text{min}^{-1}$ .

For the analysis of real samples, a UHPLC-MS/MS system was used. The analytical column was the same, and the mobile phase was water and methanol, adjusted with a buffer consisting in 0.1% v/v ammonia, and 15 mM of ammonium acetate. The analysis was performed in gradient mode at a flow rate of  $0.3 \text{ mL} \cdot \text{min}^{-1}$ . The gradient started at 50:50 (v/v) mixture of water: methanol, which changed to 25:75 (v/v) in 3 minutes, and returned to 50:50 in 1 minute more. Finally, the gradient stayed calibrating for another 1.5 minutes more. The sample volume injected was  $5 \mu\text{L}$ .

### 3. Results and Discussion

**3.1. Optimization of Solid-Phase Extraction (SPE).** There are a number of parameters that affect SPE procedure such as

type of sorbent, pH, ionic strength, sample and desorption volumes, and wash step. To optimize these parameters, it used Milli-Q water spiked with a solution of fluorescence oestrogens (estriol,  $17\beta$ -estradiol, and  $17\alpha$ -ethinylestradiol) to obtain a final concentration of  $250 \mu\text{g} \cdot \text{L}^{-1}$ .

The first parameter to optimize is the choice of sorbent, since it controls the selectivity, affinity and capacity over analytes. In this study, the SPE cartridges used were OASIS HLB, SepPak  $C_{18}$  (both from Waters, Madrid, Spain), and BondElut ENV (from Agilent, Madrid, Spain). Keeping other parameters fixed (ionic strength of 0%, sample volume of 100 mL), the cartridges were studied at three different pHs (5, 8, and 11). From the results obtained, it can be observed that the better signals are found for SepPak  $C_{18}$  cartridge (Figure 1).

After choosing the optimum cartridge, we used an initial experimental design of  $2^3$ , to study the influence of pH, ionic

TABLE 2: Mass spectrometer parameters for the determination of target analytes.

Compound	Precursor ion ( <i>m/z</i> )	Capillary voltage (Ion mode)	Quantification ion, <i>m/z</i> (collision potential, V)	Quantification ion, <i>m/z</i> (collision potential, V)
E3	287.2	−65 V (ESI −)	171.0 (37)	145.2 (39)
E2	271.2	−65 V (ESI −)	145.1 (40)	183.1 (31)
EE	295.2	−60 V (ESI −)	145.0 (37)	158.9 (33)
E1	269.2	−65 V (ESI −)	145.0 (36)	143.0 (48)
DES	267.1	−50 V (ESI −)	237.1 (29)	251.1 (25)
TES	289.2	38 V (ESI +)	187.0 (18)	104.0 (21)
MGA	385.5	30 V (ESI +)	267.3 (15)	224.2 (30)
NOR	313.2	38 V (ESI +)	109.0 (26)	245.1 (18)

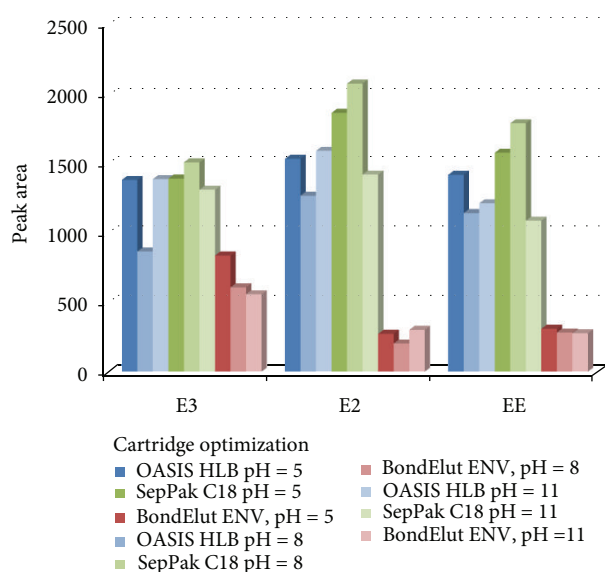


FIGURE 1: Optimization of SPE cartridges.

strength and sample volume over extraction process. The experimental design was obtained using Statgraphics Plus software 5.1 and the statistics study was done with IBM SPSS Statistics 19. We assessed two levels and three parameters: pH (3 and 8), ionic strength (0 and 30% of NaCl), and sample volume (50 and 250 mL), to obtain the influence of each parameter and the variable correlation to each other. In this study, it is observed that the ionic strength and sample volume had the major influence on the recoveries of the analytes. For that, a  $3^2$  factorial design to optimize these two variables at three levels per parameter (0, 15, and 30% of NaCl for ionic strength and 50, 100 y 250 mL for sample volume) was used. Figure 2 shows the response surface obtained for the estriol and  $17\alpha$ -ethinylestradiol. The results obtained showed that an increment of the ionic strength did not produce an increase in the response area of the compound, and the optimum volume was 250 mL. Because of that, a solution without salt addition and 250 mL of sample volume was chosen. Finally, the desorption volume (1 mL of methanol and 2 mL of methanol in one and two steps) and wash-step (5 mL

of Milli-Q water, and 5 mL of Milli-Q water with 5 and 10% of methanol v/v) were assayed to complete the optimization of the SPE process. The optimum values were 2 mL of methanol in one step and 5 mL of Milli-Q water without methanol, respectively.

In accordance with the obtained results, the optimum conditions for SPE procedure were SepPak C<sub>18</sub> cartridge, 250 mL of sample at pH = 8 and 0% of NaCl, desorption with 2 mL of methanol in one step, and wash step with 5 mL of Milli-Q water. In these conditions, we achieved a preconcentration factor of 125. In Figure 3, a chromatogram with the optimum conditions is shown, where the peaks of all compounds in their corresponding transitions can be observed.

**3.2. Analytical Parameters.** Because of the SPE optimization, that was done only with fluorescent compounds (estriol,  $17\beta$ -estradiol, and  $17\alpha$ -ethinylestradiol), it was necessary to study the recoveries of all the hormonal compounds, using the optimized SPE-UHPLC-MS/MS method. All the compounds under study showed good recoveries, over 73%, except the diethylstilbestrol, with a recovery of 50.7%.

A calibration curve was used for the quantification of the analytes by diluting the stock solution of each analyte, into the samples to concentrations ranging between 1 and  $100 \mu\text{g}\cdot\text{L}^{-1}$ . Analysis was conducted by UHPLC-MS/MS and linear calibration plots for each analyte ( $r^2 > 0.99$ ) were obtained based on their chromatographic peak areas.

The limit of detection (LOD) and the limit of quantification (LOQ) for each compound were calculated from the signal-to-noise ratio of each individual peak. The LOD was defined as the lowest concentration that gave a signal-to-noise ratio that was greater than 3. The LOQ was defined as the lowest concentration that gave a signal to noise ratio that was greater than 10. The LODs ranged from  $0.15$  to  $9.35 \text{ ng}\cdot\text{L}^{-1}$  and the LOQs ranged from  $0.49$  to  $31.18 \text{ ng}\cdot\text{L}^{-1}$ .

The performance and reliability of the process were studied by determining the repeatability of the quantification results for all target analytes under the described conditions, using six samples ( $n = 6$ ). The relative standard deviations (RSDs) were lower than 8.4% in all cases, indicating a

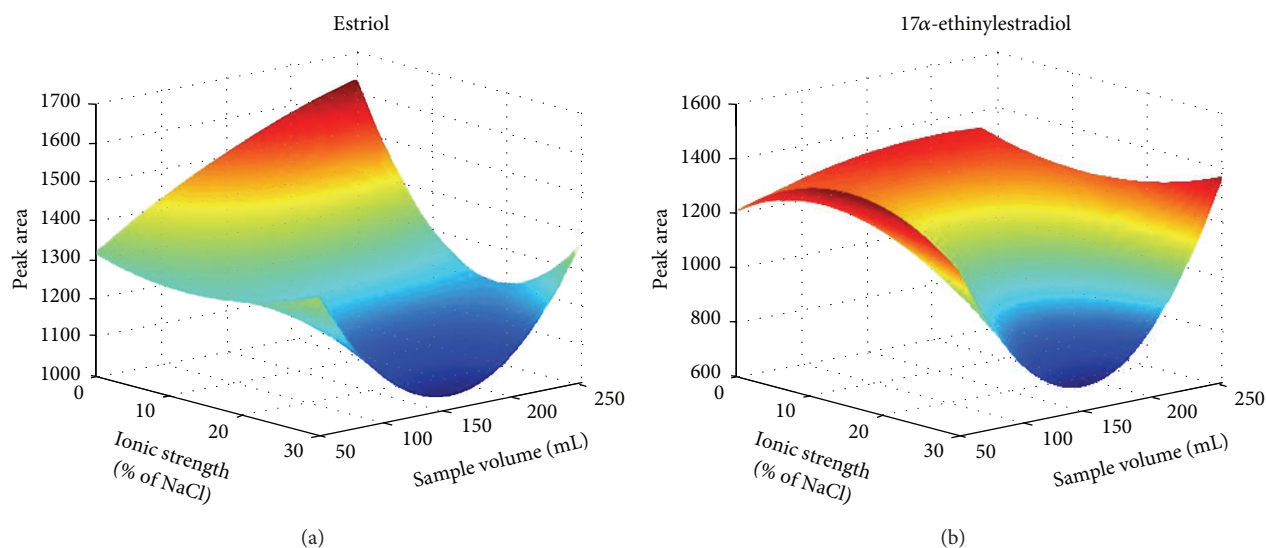


FIGURE 2: Effect of ionic strength and sample volume on the SPE extraction for estriol and 17 $\alpha$ -ethinylestradiol.

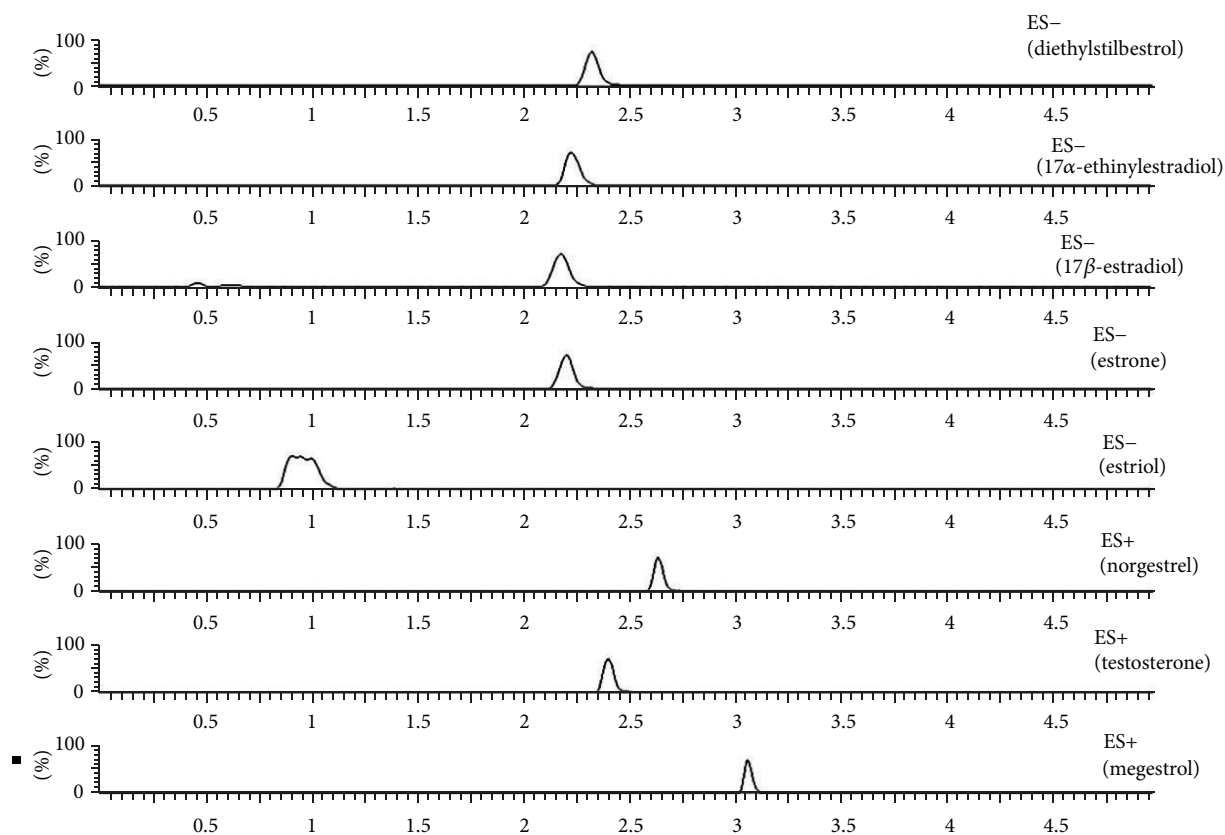


FIGURE 3: MRM chromatograms of a spiked sample ( $250 \mu\text{g}\cdot\text{L}^{-1}$ ) with all analytes after SPE process.

good repeatability. Table 3 shows the analytical parameters obtained for all compounds analysed.

**3.3. Matrix Effect.** Despite the high sensitivity and low chemical noise in UHPLC-MS/MS systems, the sample composition has a great influence on the analyte signal [22]. To

evaluate the relative signal enhancement or suppression in the samples, the algorithm by Vieno et al. [23] was used, as following:

$$\frac{As - (Asp - Ausp)}{As} \times 100, \quad (1)$$

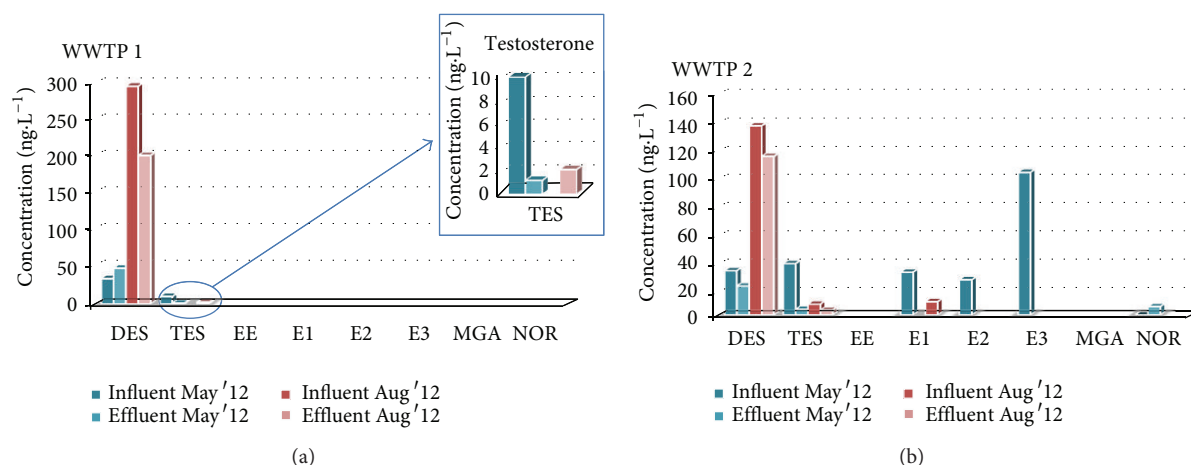


FIGURE 4: Concentrations of target compounds in sewage samples from both wastewater treatment plants (WWTPs).

TABLE 3: Analytical parameters for the SPE-UHPLC-MS/MS method.

Compound	RSD <sup>a</sup> (%) <i>n</i> = 6	LOD <sup>b</sup> (ng·L <sup>-1</sup> )	LOQ <sup>c</sup> (ng·L <sup>-1</sup> )	Recovery (%) <i>n</i> = 6	Matrix effect (%)
E3	6.53	9.35	31.2	80.5 ± 5.3	2.96
E2	8.37	2.53	8.44	89.7 ± 7.5	1.33
EE	7.25	0.51	1.71	90.6 ± 6.5	-12.6
E1	6.81	2.60	8.66	78.7 ± 5.4	15.4
DES	6.93	0.64	2.14	50.7 ± 3.5	4.48
TES	6.77	1.49	4.95	83.8 ± 5.7	17.1
MGA	7.18	0.15	0.49	73.7 ± 5.3	13.5
NOR	7.38	2.11	7.04	88.9 ± 6.5	17.2

<sup>a</sup>Relative standard derivation.<sup>b</sup>Detection limits, calculated as signal-to-noise ratio of three times.<sup>c</sup>Quantification limits, calculated as signal-to-noise ratio of ten times.

where *As* corresponds to the peak area of the analyte in pure standard solution, *Asp* to the peak area in the spiked matrix extract, and *Ausp* to the matrix extract. This procedure was applied to an effluent sample, assuming that all matrices will behave in the same way.

Suppression effect, between 13 and 17%, was observed for estrone, testosterone, norgestrel, and megestrol acetate. For estradiol, 17 $\beta$ -estradiol, and diethylstilbestrol, the signal suppressions were very low, under 5%. Only 17 $\alpha$ -ethinylestradiol showed a signal enhancement of 12.6%. The results obtained are showed in Table 3 and they are in accordance with those reported in similar studies [19, 24].

**3.4. Analysis of Selected Compounds in Wastewater Samples.** To check the efficiency of the developed method, it was applied to determination of target analytes in different wastewater samples from two WWTPs of the island of Gran Canaria (Spain). Figure 4 shows the results obtained. It can be observed that in the WWTP 1, not all compounds were detected, only diethylstilbestrol and testosterone, in concentration that ranging between 35 and 300 ng·L<sup>-1</sup> and 1.2 and 9.95 ng·L<sup>-1</sup>, respectively, for influent samples. The

concentrations of diethylstilbestrol at the effluent increased in the first sampling and diminished in the second. The behaviour of testosterone in the effluent samples was the opposite.

However, for WWTP 2 a higher number of compounds were detected. For the influents samples, the highest concentrations were between 100 and 140 ng·L<sup>-1</sup> for estradiol and diethylstilbestrol, while the rest of compounds (testosterone, estrone and 17 $\beta$ -estradiol) were detected at concentrations between 20 and 40 ng·L<sup>-1</sup>. The concentrations at the effluent for diethylstilbestrol diminished up to 110 ng·L<sup>-1</sup> and 5 ng·L<sup>-1</sup> for testosterone. The rest of compounds were not detected at the effluent samples. Only the concentration of norgestrel (about 6 ng·L<sup>-1</sup>) increased during the wastewater treatment process.

## 4. Conclusions

An analytical method for the simultaneous extraction, pre-concentration, and determination of oestrogens (estrone, 17 $\beta$ -estradiol, estradiol, 17 $\alpha$ -ethinylestradiol, and diethylstilbestrol) androgens (testosterone) and progestogens (norgestrel



and megestrol acetate) in wastewater matrices has been optimized and developed. The method used was solid phase extraction (SPE) for the extraction/preconcentration step and it was combined with UHPLC-MS/MS. The limits of detection reached were between 0.15 to 9.35 ng·L<sup>-1</sup>. In addition, the method presented high recoveries, up to 90%, for the majority of compounds and RSD lower than 9%.

The application of the method to samples from two different WWTPs showed that the concentrations of hormones found, ranged from 5 to 300 ng·L<sup>-1</sup>, and some of them (diethylstilbestrol and testosterone) were detected in all the wastewater samples, and other, like estrone or 17 $\beta$ -estradiol, only in some samples. In view of the obtained results about influent and effluent samples, it can be determined that the membrane bioreactor system is quite effective to degrade these compounds. However, it is difficult to obtain a conclusion about the activate sludge treatment effectivity owing to the small quantity of compounds detected.

## Acknowledgment

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## References

- [1] T. T. Pham and S. Proulx, "PCBs and PAHs in the Montreal urban community (Quebec, Canada) wastewater treatment plant and in the effluent plume in the St Lawrence river," *Water Research*, vol. 31, no. 8, pp. 1887–1896, 1997.
- [2] R. Rosal, A. Rodríguez, J. A. Perdigon-Melon et al., "Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation," *Water Research*, vol. 44, no. 2, pp. 578–588, 2010.
- [3] C. Baronti, R. Curini, G. D'Ascenzo, A. Di Corcia, A. Gentili, and R. Samperi, "Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water," *Environmental Science and Technology*, vol. 34, no. 24, pp. 5059–5066, 2000.
- [4] European Commission, "Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC," *Official Journal of the European Communities*, vol. L348, 2008.
- [5] D. W. Kolpin, E. T. Furlong, M. T. Meyer et al., "Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance," *Environmental Science and Technology*, vol. 36, no. 6, pp. 1202–1211, 2002.
- [6] G. G. Ying, R. S. Kookana, and Y. J. Ru, "Occurrence and fate of hormone steroids in the environment," *Environment International*, vol. 28, no. 6, pp. 545–551, 2002.
- [7] F. Stuer-Lauridsen, M. Birkved, L. P. Hansen, H. C. Holten Lützhøft, and B. Halling-Sørensen, "Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use," *Chemosphere*, vol. 40, no. 7, pp. 783–793, 2000.
- [8] D. Barcelo and M. Petrovic, *Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle*, Elsevier, 2007.
- [9] A. L. Filby, T. Neuparth, K. L. Thorpe, R. Owen, T. S. Galloway, and C. R. Tyler, "Health impacts of estrogens in the environment, considering complex mixture effects," *Environmental Health Perspectives*, vol. 115, no. 12, pp. 1704–1710, 2007.
- [10] S. K. Khanal, B. Xie, M. L. Thompson, S. Sung, S. K. Ong, and J. Van Leeuwen, "Fate, transport and biodegradation of natural estrogens in the environment and engineered systems," *Environmental Science and Technology*, vol. 40, no. 21, pp. 6537–6546, 2006.
- [11] J. W. Birkett and J. N. Lester, *Endocrine Disruptors in Wastewater and Sludge Treatment Processes*, Lewis Publishers and IWA Publishing, 2003.
- [12] J. Y. Hu, X. Chen, G. Tao, and K. Kekred, "Fate of endocrine disrupting compounds in membrane bioreactor systems," *Environmental Science and Technology*, vol. 41, no. 11, pp. 4097–4102, 2007.
- [13] T. Vega-Morales, Z. Sosa-Ferrera, and J. J. Santana-Rodríguez, "Determination of alkylphenol polyethoxylates, bisphenol-A, 17 $\alpha$ -ethynylestradiol and 17 $\beta$ -estradiol and its metabolites in sewage samples by SPE and LC/MS/MS," *Journal of Hazardous Materials*, vol. 183, no. 1–3, pp. 701–711, 2010.
- [14] M. Petrovic, E. Eljarrat, M. J. López de Alda, and D. Barceló, "Recent advances in the mass spectrometric analysis related to endocrine disrupting compounds in aquatic environmental samples," *Journal of Chromatography A*, vol. 974, no. 1–2, pp. 23–51, 2002.
- [15] D. Matějíček and V. Kubáň, "High performance liquid chromatography/ion-trap mass spectrometry for separation and simultaneous determination of ethynylestradiol, gestodene, levonorgestrel, cyproterone acetate and desogestrel," *Analytica Chimica Acta*, vol. 588, no. 2, pp. 304–315, 2007.
- [16] M. J. López De Alda and D. Barceló, "Determination of steroid sex hormones and related synthetic compounds considered as endocrine disrupters in water by liquid chromatography-diode array detection-mass spectrometry," *Journal of Chromatography A*, vol. 892, no. 1–2, pp. 391–406, 2000.
- [17] M. J. Lopez De Alda, S. Díaz-Cruz, M. Petrovic, and D. Barceló, "Liquid chromatography-(tandem) mass spectrometry of selected emerging pollutants (steroid sex hormones, drugs and alkylphenolic surfactants) in the aquatic environment," *Journal of Chromatography A*, vol. 1000, no. 1–2, pp. 503–526, 2003.
- [18] H. C. Zhang, X. J. Yu, W. C. Yang, J. F. Peng, T. Xu, and D. Q. Yin, "MCX based solid phase extraction combined with liquid chromatography tandem mass spectrometry for the simultaneous determination of 31 endocrine-disrupting compounds in surface water of Shanghai," *Journal of Chromatography B*, vol. 879, no. 28, pp. 2998–3004, 2011.
- [19] T. Vega-Morales, Z. Sosa-Ferrera, and J. J. Santana-Rodríguez, "Development and optimisation of an on-line solid phase extraction coupled to ultra-high-performance liquid chromatography-tandem mass spectrometry methodology for the simultaneous determination of endocrine disrupting compounds in wastewater samples," *Journal of Chromatography A*, vol. 1230, no. 1, pp. 66–76, 2012.
- [20] S. Masunaga, T. Itazawa, T. Furuichi et al., "Occurrence of estrogenic activity and estrogenic compounds in the Tama river, Japan," *Environmental Science*, vol. 7, no. 2, pp. 101–117, 2000.

- [21] Scifinder, Chemical Abstracts Service: Columbus, Ohio; USA, pKa, calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02, 2013, <https://scifinder.cas.org/>.
- [22] S. González, D. Barceló, and M. Petrovic, "Advanced liquid chromatography-mass spectrometry (LC-MS) methods applied to wastewater removal and the fate of surfactants in the environment," *Trends in Analytical Chemistry*, vol. 26, no. 2, pp. 116–124, 2007.
- [23] N. M. Vieno, T. Tuhkanen, and L. Kronberg, "Analysis of neutral and basic pharmaceuticals in sewage treatment plants and in recipient rivers using solid phase extraction and liquid chromatography-tandem mass spectrometry detection," *Journal of Chromatography A*, vol. 1134, no. 1-2, pp. 101–111, 2006.
- [24] V. Kumar, N. Nakada, M. Yasojima, N. Yamashita, A. C. Johnson, and H. Tanaka, "Rapid determination of free and conjugated estrogen in different water matrices by liquid chromatography-tandem mass spectrometry," *Chemosphere*, vol. 77, no. 10, pp. 1440–1446, 2009.

## Research Article

# Removal Efficiency and Mechanism of Sulfamethoxazole in Aqueous Solution by Bioflocculant MFX

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Although the treatment technology of sulfamethoxazole has been investigated widely, there are various issues such as the high cost, inefficiency, and secondary pollution which restricted its application. Bioflocculant, as a novel method, is proposed to improve the removal efficiency of PPCPs, which has an advantage over other methods. Bioflocculant MFX, composed by high polymer polysaccharide and protein, is the metabolism product generated and secreted by *Klebsiella* sp. In this paper, MFX is added to 1 mg/L sulfanilamide aqueous solution substrate, and the removal ratio is evaluated. According to literatures review, for MFX absorption of sulfanilamide, flocculant dosage, coagulant-aid dosage, pH, reaction time, and temperature are considered as influence parameters. The result shows that the optimum condition is 5 mg/L bioflocculant MFX, 0.5 mg/L coagulant aid, initial pH 5, and 1 h reaction time, and the removal efficiency could reach 67.82%. In this condition, MFX could remove 53.27% sulfamethoxazole in domestic wastewater, and the process obeys Freundlich equation.  $R^2$  value equals 0.9641. It is inferred that hydrophobic partitioning is an important factor in determining the adsorption capacity of MFX for sulfamethoxazole solutes in water; meanwhile, some chemical reaction probably occurs.

## 1. Introduction

In recent decades, the use of pharmaceutical and personal care products (PPCPs) has increased dramatically [1, 2]. They are members of a group of chemicals newly classified as organic microcontaminants in water after pesticide and endocrine disrupting compounds, which stably exist in nature, have properties of being hard-biodegraded, bioaccumulation, and long-range hazardous, posing far-reaching and unrecoverable hazard on ecosystem [3–5]. The presence of PPCPs of emerging concern as increasing evidence suggests their harmfulness [6]. Antibiotic medicine sulfamethoxazole features classic PPCPs, with very low removal ratio in water treatment and high frequency to be detected. In recent decades, although the consume of sulfamethoxazole has been reduced, it is the most popular germifuge in animal food production [7]. It is reported that SMX applied in veterinary directly discharges into the aquatic environment, which has high toxicity [8]. Therefore, there have been large amount of studies on sulfamethoxazole. However, most attention

has been focused on identification, fate, and distribution of PPCPs in municipal wastewater treatment plants [9, 10]. It is significant to develop treatment method to remove SMX. The commonly used treatment methods include advanced oxidation process, adsorption, and membrane technology [11–13]. Bioflocculation adsorption method has several advantages over other methods, such as going green, being environmentally protective, no second pollution, and being biodegradable [14]. What is more, bioflocculation has been proved to be highly effective and widely applied, and yet there is no published research on bioflocculation removal of PPCPs. Thus, it is meaningful to study the removal of PPCPs by bioflocculation. Bioflocculant MFX is a metabolized production with good flocculant activity, generated and secreted by *Klebsiella* sp. into the extracellular environment, composed of macromolecular polysaccharide and protein. In this study, based on its physical and chemical property, the effective ingredient of MFX is extracted by water abstraction and alcohol precipitation, transformed into dry powder. And then the removal efficiency and mechanism of sulfamethoxazole in



aqueous environment are researched. The study aims at developing an effective treatment method of sulfamethoxazole and expanding the applied range of bioflocculation.

## 2. Materials and Methods

### 2.1. Strains and Media

**2.1.1. Bioflocculant-Producing Bacterium, Strain J1: *Klebsiella* sp.** The strain was screened by our laboratory from activated sludge in municipal wastewater treatment plants and preserved in China General Microbiological Culture Collection Center (CGMCC number 6243).

**2.1.2. Inclined Plane Medium (g/L).** Peptone 10, NaCl 5, beef extract 3, agar 15~18, water 1000 mL, pH 7.0~7.2; Flocculant fermentation medium (g/L): glucose 10, yeast extract 0.5, urea 0.5,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2, NaCl 0.1,  $\text{K}_2\text{HPO}_4$  5,  $\text{KH}_2\text{PO}_4$  2,  $\text{H}_2\text{O}$  1000 mL, pH 7.2~7.5.

### 2.2. Methods

**2.2.1. Assay Method.** Flocculating rate: 5.0 g chemically pure kaolin clay, 1000 mL tap water, and 1.5 mL 10%  $\text{CaCl}_2$  liquid are added into a beaker, pH is adjusted to 7.2 by adding NaOH, then 10 mL flocculant is added, compared with control without flocculant addition. Flocculator is applied during the experiment, after 40 s fast mixing, and changed into slow mixing for 4 minutes, after 20 min settling, and the absorbance of the supernatant is measured under 550 nm by 721 UV spectrometer [15]. The flocculation efficiency is calculated as follows:

$$\text{flocculation efficiency} = \frac{(A - B)}{A} \times 100\%, \quad (1)$$

where  $A$  is turbidity of the supernatant in control (light transmittance);  $B$  is turbidity of the supernatant in sample.

The removal efficiency of sulfamethoxazole is calculated by the following equation:

$$\text{remove efficiency} = \frac{(C - D)}{C} \times 100\%, \quad (2)$$

where  $C$  is the concentration in control and  $D$  is the sulfamethoxazole concentration after treatment:

Polysaccharide measurement: Phenol-sulphuric acid method [16].

Protein measurement: Coomassie light blue [16].

**2.2.2. Bioflocculant Preparation.** Add 2x volume absolute alcohol (precooled under 4°C) to fermentation liquid, and filter and collect the white flocs after mixing. Add 1x volume absolute alcohol to filtered liquid, and then collect the white flocs again. Add small amount of DI water to collected flocs, after uniformly dissolving, freeze the flocculants in the ultralow temperature freezer for 24 h, and then put them into freeze drying to change the flocculants into dry powder.

**2.2.3. Chromatographic Condition.** Chromatographic column: C18 (250 \* 4.6 mm, 5  $\mu\text{m}$ ); mobile phase is formic acid water: Acetonitrile (60 : 40 V/V); flow rate 1.0 mL/min; sample size 10  $\mu\text{L}$ ; column temperature 30°C; wave length 265 nm [17].

**2.2.4. Impact Factor Experiment of Sulfamethoxazole Removal Efficiency.** Add flocculants into 1 mg/L sulfamethoxazole liquid with dosage 0 mL, 1 mL, 3 mL, 5 mL, 7 mL, and 9 mL; set the coagulant aids dosage as 0 mL, 0.5 mL, 1 mL, 1.5 mL, and 2 mL; adjust pH value to 4, 5, 6, 7, and 8 under 5°C, 15°C, 25°C, 35°C, 45°C, and 55°C; change the reaction time as 0 h, 0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, and 12 h; calculate the removal rate.

**2.2.5. Orthogonal Test of Sulfamethoxazole Removal by Bioflocculants.** Based on the preliminary obtained optimum condition, flocculant dosage, coagulant-aid dosage, pH, reaction time, and temperature are considered as influencing parameters. The design of experiment is shown in Table 1.

**2.2.6. Adsorption Isotherm Experiment of Sulfamethoxazole Removal by Bioflocculants.** Mix the flocculant MFX and sulfamethoxazole with initial concentration as 0.8 mg/L, 1 mg/L, 1.2 mg/L, 1.4 mg/L, 1.6 mg/L, and 1.8 mg/L separately, under different temperature condition as 15°C, 35°C, put on 140 rpm shaking table with constant temperature, conduct adsorption isotherm experiment, and adsorption time is 1 h.

## 3. Results and Discussion

**3.1. Analysis of Flocculent Active Ingredients.** The strain J1 was short rod-shaped, cream-colored, viscous, smooth, and Gram-positive. J1 was identified as *Klebsiella* sp on the basis of the morphological characteristics and 16S rDNA sequence. The J1 showed a high yield of flocculant and good flocculation activity toward kaolin suspension. The active ingredients of bioflocculant MFX produced by J1 distributed mainly in the supernatant after the first centrifugation of fermentation broth, that is to say, the flocculation active extracellular secretions remain freely in fermentation broth. (Figure 1). Flocculation ratio after first centrifugation appeared negatively, which proved that the J1 itself was not responsible for the flocculation. The addition of cell suspension leads to the increasing turbidity of raw water. After ultrasonic crushing and centrifugation, bacteria cells were broken and the intercellular content went into the supernatant; the negativity of flocculation ratio showed the fact that the intercellular content may not have flocculation effect. What is more, fermentation broth without inoculation has relatively high flocculation ratio, and it may be caused by the flocculation effect and coagulation aid effect of the phosphate or other inorganic salts. Thus, we may reach the conclusion that the flocculant active ingredient is the metabolized production; in the meantime, the growth medium also contributes to the flocculation. By the isolation and purification of flocculation active ingredients, removing disturbance of growth medium,

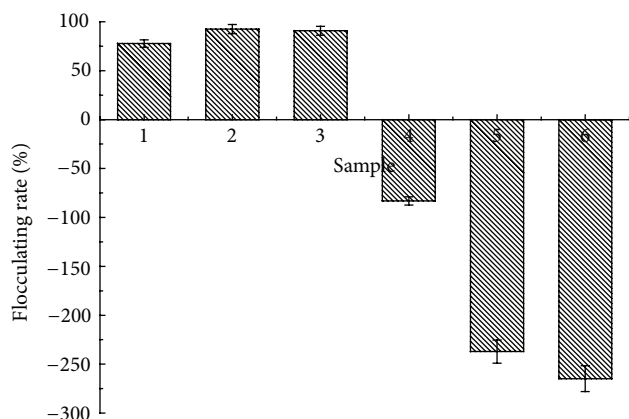


FIGURE 1: Distribution of flocculent active ingredients.

TABLE 1: Factors and levels of orthogonal test.

Levels	A pH	B Flocculant dosage (mL)	C Coagulant aid dosage (mL)	D Reaction time (h)
1	5	2	0	0.5
2	6	5	0.2	1
3	7	8	0.5	1.5

TABLE 2: Qualitative analysis of flocculent active ingredients.

Reaction type	Analytical method	Phenomenon
Polysaccharide	Molish reaction	+
	Anthrone reaction	+
	Seliwanoff reaction	-
Protein	Ninhydrin reaction	+
	Biuret reaction	+
	Xanthoprotein reaction	+

a further conclusion may be reached; that, is the active ingredient is the secondary metabolites of bacteria fermentation (extracellular polymeric substances (EPS)).

Table 2 presents MFX that has apparent results in the saccharides and protein chromogenic reactions. According to Table 2, we can conclude qualitatively that the major ingredients of the flocculant produced by J1 are polysaccharides and protein, the polysaccharides content is 0.0656 mg/mL, and the protein content is 0.1021 mg/mL.

After the enzymatic digestion of EPS, polysaccharides were removed, while proteins remained. The proteins accounted for 15.05% (cellulase), 61.9% ( $\alpha$ -amylase), and 11.4% ( $\beta$ -amylase) of the flocculation activity. On the contrary, proteins were removed, while polysaccharides remained. EPS has no flocculent activity (Table 3). Obvious decrease in flocculation activity was observed after the flocculant MFX was exposed at 70°C for 20 min, indicating that it was low thermostable. This implies that the active

TABLE 3: Enzymatic digestion of flocculent active ingredients.

Reaction type	Enzym	Flocculation rate after enzymatic digestion	Floc
Polysaccharide	Cellulase	15.05%	Small
	$\alpha$ -amylase	61.90%	Big
	$\beta$ -amylase	11.4%	Small
Protein	Trifluoroacetic acid	Negative	None
	Pepsase	Negative	None
	Trypsin	Negative	None

constituents in MFX were proteins and polysaccharides, and proteins dominant accounted for the flocculation activity.

**3.2. The Impact Factors on Removal Efficiency of Sulfamethoxazole by MFX.** Five parameters: pH value, flocculant dosage, coagulation aid ratio, flocculation time, and temperature are measured to see the effects of these factors on sulfamethoxazole removal efficiency. Along with the changes of pH value, the removal efficiency increases firstly then decrease, and changes sharply, from where we could know that pH does affect removal ratio a lot. It is shown in Figure 2(a) that, between pH 4 and 5, the removal efficiency increases along with pH increment. When pH is 5, MFX possesses the strongest flocculation capacity and the highest flocculation efficiency, which is 67.2%. Between pH 6 and 8, the removal efficiency falls steeply when pH is 8, and the removal efficiency is only 1.61%. The result demonstrated that the bioflocculant has higher removal efficiency on sulfamethoxazole in the acidic condition, while the alkali condition results in relatively poor removal efficiency. Figure 2(b) shows that along, with the increase of flocculant dosage, the removal efficiency increases firstly, then decreases, and changes acutely. When flocculant dosage varies within the range from 1 mL to 5 mL, the removal efficiency improved with the increasing dosage. When flocculant dosage is 5 mL, the optimum removal efficiency is obtained, which is 57.89%. As seen in Figure 2(c), the dosage of coagulant aid also has impact on the removal efficiency. Increasing volume ratio of coagulant aid leads to increasing removal efficiency. When coagulant aid dosage is 0.1 times of flocculation dosage, which is 0.5 mL, more than 50% removal efficiency is reached. Afterwards, the increment of coagulant aid dosage decreases flocculation capability and removal efficiency. In the meantime, the removal efficiency is around 20% without coagulant aid addition, which proves that bioflocculant could remove sulfamethoxazole without coagulant aid. Figure 2(d) shows that the removal efficiency increases firstly, then decreases with the change of temperature, but within narrow fluctuation range. When temperature is between 5°C and 25°C, the removal efficiency increases slowly. When temperature is 35°C, the strongest flocculation capability is obtained and the highest removal efficiency is reached, which is 67.20%. The removal efficiency decreases on the temperature of 45°C and 55°C. According to Figure 2(e), the removal efficiency increases exponentially within the first 30 minutes, after 30 minutes the increment slows down and

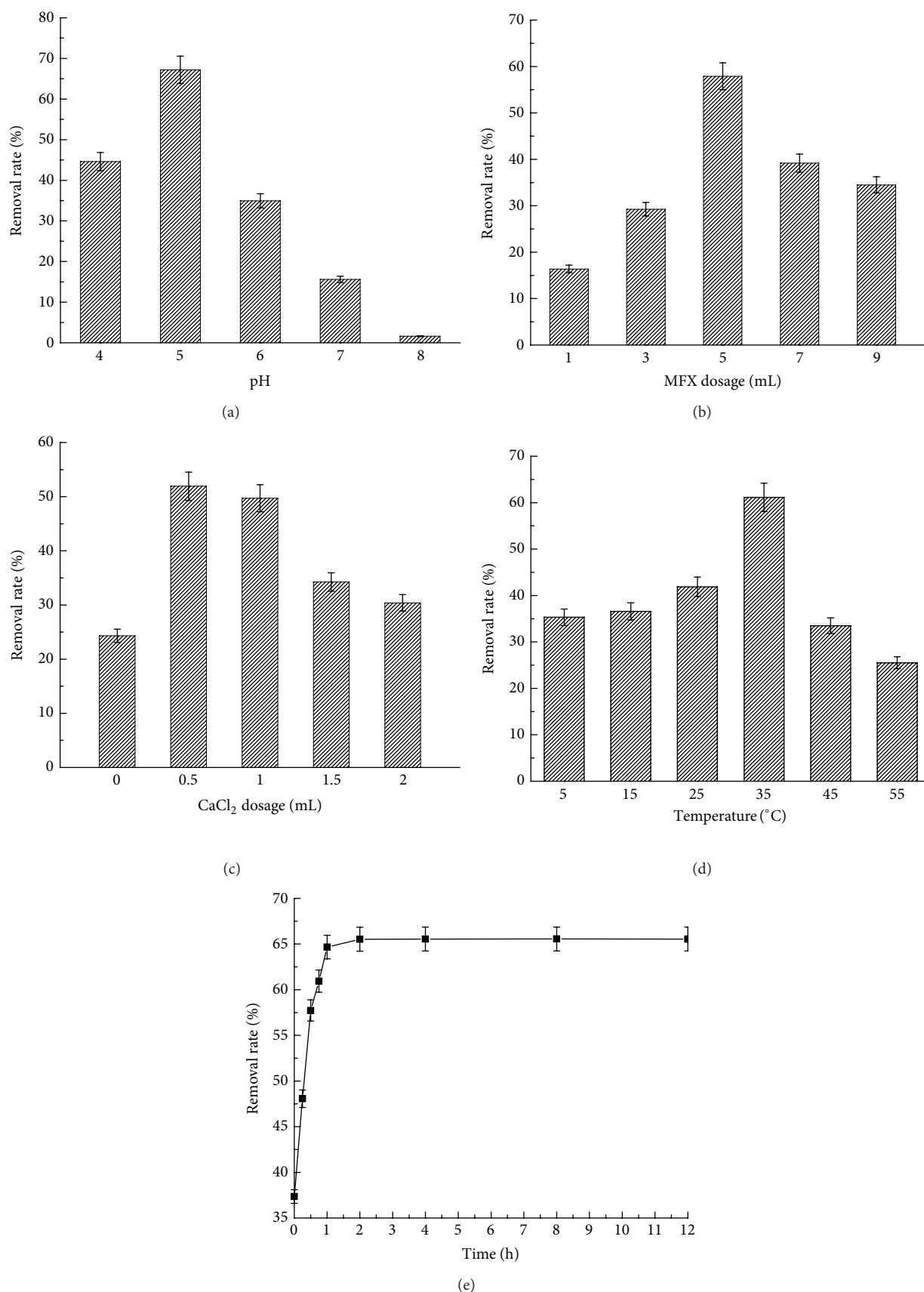


FIGURE 2: The influence of ecological factor on removal rate. (a) is the influence of pH; (b) is the influence of MFX dosage; (c) is the influence of CaCl<sub>2</sub> dosage; (d) is the influence of temperature; (e) is the influence of time on removal rate.

remains the same after 1 hour, reaching equilibrium. This is because of that a large amount of adsorbate exists in the liquid in the beginning of the reaction and is adsorbed quickly by adsorbent. With the occupation of the adsorption sites, adsorption quantity inclines slowly. After equilibrium is reached, the adsorption quantity remains constantly.

In conclusion, the optimum flocculation condition is pH 5, flocculant dosage 5 mL, coagulant aid dosage 0.5 mL, flocculant reaction time 1 h, and temperature 35°C. Under this condition, the highest removal efficiency is reached, which is 67.82%. It is reported that the removal efficiency using conventional water treatment process including preoxidation, coagulation, and sand filtration is 36% [18], and the removal efficiency by microelectrolysis Fentonis is 65.5% [19]. It can be seen that bioflocculant is obviously more efficient than normal treatment.

**3.3. The Removal Efficiency of Sulfamethoxazole in Domestic Wastewater by MFX.** In order to evaluate the removal effect of bioflocculant MFX on sulfamethoxazole in actual wastewater, domestic wastewater is used, with sulfamethoxazole concentration as 23.26  $\mu\text{g/L}$ . 9 sets of experiments are conducted according to the orthogonal design table L9 ( $3^4$ ), and results are shown in Table 4.

As is shown in Table 4, according to average value analysis, optimum flocculation condition is the combination of A1B3C2D2, which is pH 5, flocculant dosage 8 mL, coagulant aid dosage 0.2 mL, and reaction time 1 h. It has been proved that, under this condition, the removal efficiency of sulfamethoxazole is 53.27%.

By comparing the extremums, we may reach a conclusion that the effect degrees of factors obey the following order:  $RA > RB > RC > RD$ . That is to say, pH value affects mostly, followed by flocculant dosage, coagulant aid dosage, and reaction time. This is because that the dissociation of flocculant occurs within a certain pH range. Proper pH value could increase the dissociation degree, lead to a higher charge density of flocculant, benefits the spreading of the flocculant molecules, and facilitates the bridging action of the bioflocculant. Thus, pH value plays a critical role [20]. When the flocculant dosage is relatively low, early adsorption saturation may be reached, the removal efficiency of contaminants decreases. When flocculant dosage is high, extraflocculant weakens the bridging effect due to adsorption sites overlapping and finally affects the removal efficiency of specific contaminant. Thus, proper flocculant dosage plays an important role in affecting removal efficiency. Some studies show that metal ions addition could change the surface charge of colloids. sulfamethoxazole flocs in the water are negatively charged, and when approaching positively charged flocculant hydrolyzates and calcium ions in coagulant aid, charge neutralization occurs on the surface of sulfamethoxazole and makes the colloidal particles to sediment, exacerbating the collision of colloids and collision between colloids and flocculant, integrating a whole group under Van der Waals' force, finally precipitating from water by gravity [21].

In the research of removal mechanism of sulfamethoxazole aqueous solution, the highest removal efficiency is

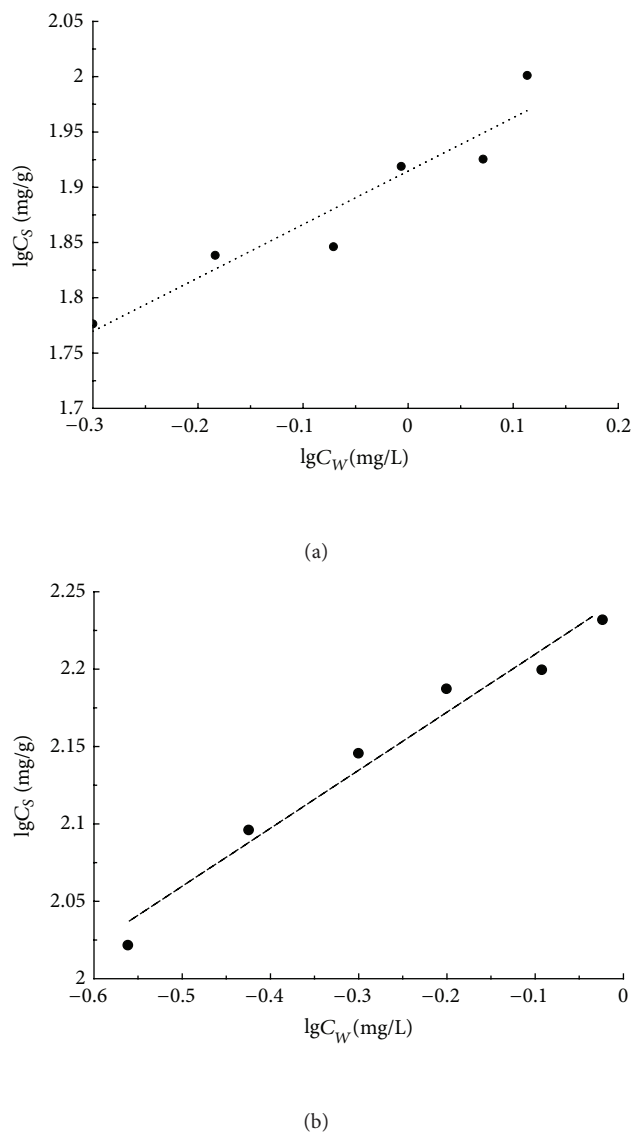


FIGURE 3: Adsorption isotherms of sulfamethoxazole by MFX adsorbent in 15°C (a) and 35°C (b).

more than 60%, but, in the removal of sulfamethoxazole in wastewater, the highest removal efficiency under optimum condition is only 53.27%. This may be caused by the relatively low concentration of sulfamethoxazole in wastewater, which is 23.26  $\mu\text{g/L}$ . The limitation of measurement methods may lead to potential systematic errors. On the other hand, domestic wastewater has complex component, and there exists reversible and irreversible competitions among substrates, limiting the combination of flocculant and sulfamethoxazole. What is more, some unknown ions and organic compounds may also decrease the removal efficiency.

**3.4. The Mechanism of Sulfamethoxazole in Aqueous Solution by MFX.** The dry power of MFX is white, sparsely and reticulate, while the aqueous solution is milky white, ropy, and turbid. The material of MFX adsorbent is glycoprotein

TABLE 4: Orthogonal test result and visual analysis.

Tests	A pH	B Flocculant dosage (mL)	C Coagulant aid dosage (mL)	D Reaction time (h)	Removal efficiency (%)
1	1	1	1	1	40.64
2	1	2	2	2	48.38
3	1	3	3	3	50.13
4	2	1	2	2	36.91
5	2	2	3	1	30.26
6	2	3	1	3	44.27
7	3	1	3	2	29.86
8	3	2	1	3	35.03
9	3	3	2	1	41.70
Average 1	46.383	35.803	39.980	37.533	
Average 2	37.147	37.890	42.330	40.837	
Average 3	35.530	45.367	36.750	40.690	
Variance	10.853	9.564	5.580	3.304	

which has hydrophobicity and displays a wide range of sorption behavior for hydrophobic organic compounds in aqueous solutions [22]. The adsorption isotherms of sulfamethoxazole on MFX adsorbents are presented in Figure 3 and Table 5. Adsorption data are fitted to Freundlich isotherm model which is the most widely used models for describing adsorption phenomena in aqueous solutions. The Freundlich isotherm model is an empirical equation accurate for describing adsorption in aqueous solutions at low solute concentrations. Expressions and interpretations are as follows. The maximum adsorption capacity of the adsorbent is represented by  $K_F$  (mg/g), while  $n$  is constant which is indicative of the adsorption energy and intensity:

$$C_S = K_F \cdot C_W^{1/n}$$

$$\lg C_S = \frac{1}{n} \lg C_W + \lg K_F, \quad (3)$$

where  $C_S$  is mass concentration in solid phase after adsorption equilibrium (mg/g);  $C_W$  is concentration in liquid phase after adsorption equilibrium (mg/L).

The results show that MFX exhibited high-adsorption capacities for sulfamethoxazole in water. A maximum adsorption capacity ( $K_F$ ) of 176.6445 mg/g was obtained with MFX in 35°C. Compared Figure 3(a) with Figure 3(b), it can be perceived that linear correlation is marked in 35°C. According to  $n$  value, it is known that, under this temperature, the adsorptive property of MFX to sulfamethoxazole is high. However, MFX has poorer adsorption efficiency in 15°C.  $R^2$  value is only 0.882, while  $n$  value is lower than the former.

This is due to the effect of temperature on chemical reaction and molecular movement, which finally promote or restrain flocculent effect. On the one hand, providing appropriate temperature, colloidal particle is bombarded intensively by molecules of flocculation to form a whole. If temperature is excessively low, molecular movement slows down, and reaction rate lessens, leading to bad adsorptive

TABLE 5: Freundlich isotherm parameters at different temperature.

$T$ (°C)	Freundlich equation	$K_F$	$R^2$	$n$
15	$y = 0.483x + 1.9146$	82.1486	0.8820	2.07
35	$y = 0.3748x + 2.2471$	176.6445	0.9641	3.18

property. On the other hand, some active groups between bioflocculant and sulfamethoxazole start the chemical reaction to separate out of aqueous solution system. What is more, when hydrophobic chain polymer-bioflocculation MFX comes into sulfamethoxazole aqueous solution system, with the help of appropriate pH and  $\text{Ca}^{2+}$ , sulfamethoxazole becomes unstable rapidly, passing into flocculation phase.

Driven by such mechanism, the adsorption on MFX does not rely on a high porosity and a resultant high specific surface area to reach a high adsorption capacity, as for most activated carbon and polymeric adsorbents. This result implies that hydrophobic partitioning is an important factor in determining the adsorption capacity of MFX for sulfamethoxazole solutes in water; meanwhile, some chemical reaction probably occurs.

## 4. Conclusion

This work demonstrates the efficient removal of sulfamethoxazole from water using bioflocculant MFX as adsorbents. The findings are summarized as follows.

- (1) The active flocculent constituents of MFX are EPS which is composed by polysaccharides and proteins fermented by J1. Proteins mainly accounted for the flocculation activity.
- (2) The MFX displays great sorption behavior for sulfamethoxazole in aqueous solutions. The optimum condition is 5 mg/L bioflocculant, 0.5 mg/L coagulant



aid, initial pH 5, and 1 h reaction time, and the removal efficiency could reach 67.82%.

- (3) Using MFX, the removal rate of sulfamethoxazole in domestic wastewater can reach 53.27%. The effect size of ecological factor is as follow: pH > flocculant dosage > coagulant aid > reaction time.
- (4) It is efficient for MFX to remove sulfamethoxazole in aqueous environment in 35°C. And the process obeys Freundlich equation.  $R^2$  value equals 0.9641. It is inferred that hydrophobic partitioning is an important factor in determining the adsorption capacity of MFX for sulfamethoxazole solutes in water.

The study shows that bioflocculation MFX can be used as an efficient alternative adsorbent for the removal of sulfamethoxazole in water, with high-adsorption capacities observed in actual wastewater. Further studies are underway to make mathematical models of the relationship between flocculant and contaminant. When many PPCPs coexist, the research on removal efficiency with bioflocculant is more significant.

## Acknowledgments

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## References

- [1] C. G. Daughton and T. A. Ternes, "Pharmaceuticals and personal care products in the environment: agents of subtle change?" *Environmental Health Perspectives*, vol. 107, Supplement 6, pp. 907–938, 1999.
- [2] J. Kagle, A. W. Porter, R. W. Murdoch, G. Rivera-Cancel, and A. G. Hay, "Biodegradation of pharmaceutical and personal care products," *Advances in Applied Microbiology*, vol. 67, pp. 65–99, 2009.
- [3] G. T. Ankley, B. W. Brooks, D. B. Huggett, and J. P. Sumpter, "Repeating history: pharmaceuticals in the environment," *Environmental Science and Technology*, vol. 41, no. 24, pp. 8211–8217, 2007.
- [4] D. W. Kolpin, E. T. Furlong, M. T. Meyer et al., "Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance," *Environmental Science and Technology*, vol. 36, no. 6, pp. 1202–1211, 2002.
- [5] A. B. A. Boxall, "The environmental side effects of medication," *EMBO Reports*, vol. 5, no. 12, pp. 1110–1116, 2004.
- [6] E. Marco-Urrea, J. Radjenović, G. Caminal, M. Petrović, T. Vicent, and D. Barceló, "Oxidation of atenolol, propranolol, carbamazepine and clofibrilic acid by a biological Fenton-like system mediated by the white-rot fungus *Trametes versicolor*," *Water Research*, vol. 44, no. 2, pp. 521–532, 2010.
- [7] J. Radjenović, C. Sirtori, M. Petrović, D. Barceló, and S. Malato, "Solar photocatalytic degradation of persistent pharmaceuticals at pilot-scale: kinetics and characterization of major intermediate products," *Applied Catalysis B*, vol. 89, no. 1–2, pp. 255–264, 2009.
- [8] C. Wu, A. L. Spongberg, J. D. Witter, M. Fang, and K. P. Czajkowski, "Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated water," *Environmental Science and Technology*, vol. 44, no. 16, pp. 6157–6161, 2010.
- [9] L. Hu, P. M. Flanders, P. L. Miller, and T. J. Strathmann, "Oxidation of sulfamethoxazole and related antimicrobial agents by  $\text{TiO}_2$  photocatalysis," *Water Research*, vol. 41, no. 12, pp. 2612–2626, 2007.
- [10] T. Polubesova, D. Zadaka, L. Groisman, and S. Nir, "Water remediation by micelle-clay system: case study for tetracycline and sulfonamide antibiotics," *Water Research*, vol. 40, no. 12, pp. 2369–2374, 2006.
- [11] S. Kaniou, K. Pitarakis, I. Barlagianni, and I. Poullos, "Photocatalytic oxidation of sulfamethazine," *Chemosphere*, vol. 60, no. 3, pp. 372–380, 2005.
- [12] K. M. Onesios, J. T. Yu, and E. J. Bouwer, "Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review," *Biodegradation*, vol. 20, pp. 441–466, 2009.
- [13] M. N. Abellan, B. Bayarri, J. Gimenez, and J. Costa, "Photocatalytic degradation of sulfamethoxazole in aqueous suspension of  $\text{TiO}_2$ ," *Applied Catalysis B*, vol. 74, no. 3–4, pp. 233–241, 2007.
- [14] L. L. Wang, F. Ma, Y. Y. Qu et al., "Characterization of a compound bioflocculant produced by mixed culture of *Rhizobium radiobacter* F2 and *Bacillus sphaericus* F6," *World Journal of Microbiology and Biotechnology*, vol. 27, no. 11, pp. 2559–2565, 2011.
- [15] J. Xing, J. Yang, F. Ma, L. Wei, and K. Liu, "Study on the optimal fermentation time and kinetics of bioflocculant produced by bacterium F2," *Advanced Materials Research*, vol. 113–114, pp. 2379–2384, 2010.
- [16] J. A. Dean, *Lange's Handbook of Chemistry*, World Publishing Corporation, Singapore, 2004.
- [17] L. C. Lin, "Simultaneous determination of sulfadiazine and sulfamethoxazole residues in muscle of European Eels (*Anguilla anguilla*) by HPLC," *Fujian Journal of Agricultural Sciences*, vol. 26, no. 5, pp. 697–700, 2011.
- [18] W. M. Shi, K. Y. Liu, and W. T. Ye, "The study on migration and transfer and removal of sulfamethoxazole in water supply system," *Technology of Water Treatment*, vol. 37, no. 6, pp. 86–89, 2011.
- [19] T. F. Wan, *The study on pretreatment of sulfadiazine in pharmaceutical wastewater by microelectrolysis—Fenton [M.S. thesis]*, Chengdu University of Technology, 2011.
- [20] L. L. Wang, L. F. Wang, and H. Q. Yu, "pH dependence of structure and surface properties of microbial EPS," *Environmental Science & Technology*, vol. 46, no. 2, pp. 737–744, 2012.
- [21] X.-M. Liu, G.-P. Sheng, H.-W. Luo et al., "Contribution of extracellular polymeric substances (EPS) to the sludge aggregation," *Environmental Science and Technology*, vol. 44, no. 11, pp. 4355–4360, 2010.

- [22] J. Han, W. Qiu, S. W. Meng, and W. Gao, "Removal of ethinylestradiol from water via adsorption on aliphatic polyamides," *Water Research*, vol. 46, no. 17, pp. 5715–5724, 2012.

## Research Article

# Pyrite Passivation by Triethylenetetramine: An Electrochemical Study

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The potential of triethylenetetramine (TETA) to inhibit the oxidation of pyrite in  $\text{H}_2\text{SO}_4$  solution had been investigated by using the open-circuit potential (OCP), cyclic voltammetry (CV), potentiodynamic polarization, and electrochemical impedance (EIS), respectively. Experimental results indicate that TETA is an efficient coating agent in preventing the oxidation of pyrite and that the inhibition efficiency is more pronounced with the increase of TETA. The data from potentiodynamic polarization show that the inhibition efficiency ( $\eta\%$ ) increases from 42.08% to 80.98% with the concentration of TETA increasing from 1% to 5%. These results are consistent with the measurement of EIS (43.09% to 82.55%). The information obtained from potentiodynamic polarization also displays that the TETA is a kind of mixed type inhibitor.

## 1. Introduction

Pyrite,  $\text{FeS}_2$ , is one of the most common sulfide minerals. It is frequently present in tailings, waste rock dumps, many valuable mineral raw materials, and coal [1]. It is easy to be oxidized under natural weathering conditions. The oxidation of pyrite results in sulfuric acid and toxic trace metals formation in acid mine drainage (AMD), which is one of the most serious environmental problems facing the mining industry [2]. That is why many studies have been carried out on the mechanism of pyrite's oxidation during the last six decades by investigators from different areas, such as metallurgy and environment science [3–9]. Now researchers have found that the oxygen and ferric iron play a very important role for the pyrite's oxidation [10, 11] and that some acidophilic microorganisms, for example, *Acidithiobacillus ferrooxidans* [12] and *Leptospirillum ferrooxidans* [13], can accelerate the oxidation of pyrite greatly. According to the knowledge of people for the mechanism of pyrite decomposition, if it is no contact between pyrite and oxidants (e.g.,  $\text{O}_2$  and  $\text{Fe}^{3+}$ ), the rate of pyrite oxidation could be suppressed. For years, several

techniques have been developed to reduce the oxidation of sulfide minerals, including bactericides [14], neutralization [15, 16], and cover treatment [17–19]. However, most of these technologies are costly, short-term solutions, and difficult to apply.

In parallel, many researchers have used certain chemical reagents that can create effective oxygen barriers to protect the surface of iron sulfide from oxidation. For example, both iron phosphate precipitates and silica precipitates have been shown to suppress pyrite oxidation efficiently. However, these treatments require initial surface oxidation with hydrogen peroxide, which is difficult to handle in a real application [20, 21]. Similarly, although some passivating agents such as acetyl acetone, humic acids, ammonium lignosulfonates, oxalic acid, and sodium silicate also have the capability to inhibit pyrite from oxidation, these treatments also need peroxidation, and the coating with oxalic acid requires a temperature control at  $65^\circ\text{C}$  [22]. In addition, Elsetinow et al. [23] have concluded that the formation of a passivating layer on the pyrite surface after exposure to the lipid could suppress pyrite oxidation by either interrupting the advection of aqueous



oxidants or the electron transfer between oxidants and the pyrite. Using the property of formation of strong insoluble chelating complex with  $\text{Fe}^{3+}$ , Lan et al. [24] have investigated the possibility of using 8-hydroxyquinoline as a passivating agent, and they demonstrated that the oxidation rates of pyrite could be reduced remarkably. In recent years, our laboratory has also developed a new passivating agent: triethylenetetramine (TETA) [25]. Compared to the coating agents mentioned above, TETA is currently used in the floatation process of sulfide minerals in Inco Limited as depressant; therefore, it does not represent any extra cost. On the other hand, TETA is a base which can neutralize protons produced in the oxidation of the sulphide minerals. TETA has already been proved that it could retard the oxidation of pyrrhotite and need not initial oxidation [25, 26]. However, there is not date on the capability of TETA to passivate pyrite. In addition, all the studies cited above were carried out by the method of extraction using hydrogen peroxide or atmospheric oxygen as oxidant to test the coating effectiveness of different passivating agents. These processes usually require long times, and, moreover, the operation is complicated as the quantity of dissolved metal ions need to be monitored by techniques such as spectrophotometer [23].

As the simplicity, efficacy, and low cost of these methods, electrochemical techniques are used extensively to investigate the corrosion of steel [27–30]. Nowadays, electrochemical techniques have been becoming essential measurements to evaluate the effect of inhibitors on the corrosion inhibition of steel. Although pyrite is not a very good electrical conductor, its oxidation is usually described in terms of electrochemical corrosion mechanisms developed for metals [31, 32]. Therefore, electrochemical methods can be chosen to study the corrosion inhibition behavior of passivating agents on pyrite.

The main aim of this study is to test the coating effectiveness of triethylenetetramine (TETA) on pyrite using the open-circuit potential (OCP), cyclic voltammetry (CV), potentiodynamic polarization, and electrochemical impedance spectroscopy (EIS).

## 2. Experimental Methods

**2.1. Mineral Samples Preparation.** Natural pyrite was obtained from the Dabaoshan sulfur-polymetallic mines in the north of Guangdong Province, China. Its chemical composition analysis by X-ray fluorescence (XRF) is listed in Table 1. The XRD pattern (Figure 1) of the crushed sample is typical that was expected for pyrite and showed that it was including trace of quartz. The material was ground with an agate mortar and then sieved to isolate particles with a diameter of less than  $75\text{ }\mu\text{m}$  and stored in a vacuum desiccator before usage.

The pyrite sample was submitted to passivation by various concentrations of TETA solution. 1 g of the pyrite powder was precisely weighed in a 50 mL glass beaker, and then 1 mL of coating solution was added. Samples were rinsed well with the coating solution and dried overnight in a vacuum desiccator. All of these reagents in this experiment were analytical grade. Milli-Q water was used to prepare all the solutions. After the

TABLE 1: Chemical composition of the studied pyrite sample.

Compound	Mass/%
$\text{FeS}_2$	93.33
$\text{SiO}_2$	3.38
$\text{Al}_2\text{O}_3$	1.45
$\text{MgO}$	0.28
$\text{Cr}_2\text{O}_3$	0.01
$\text{P}_2\text{O}_5$	0.04
$\text{K}_2\text{O}$	0.74
$\text{TiO}_2$	0.03
$\text{MnO}$	0.03
$\text{NiO}$	0.18
$\text{CuO}$	0.18
$\text{ZnO}$	0.20
$\text{WO}_3$	0.07
$\text{PbO}$	0.05
$\text{As}_2\text{O}_3$	0.04

coating step, the particles were used to construct carbon paste electrodes.

These electrodes were consisted of 1.0 g graphite, 0.4 mL paraffin oil, and 0.5 g pristine or coated pyrite. The method of the construction of C paste electrode was described by Arce and González [33]. A total of 1.0 g of graphite was pulverized together with 0.5 g of pristine or coated pyrite in an agate mortar, then 0.4 mL of silicon oil was added in the powder and mixed to obtain a homogeneous paste. This paste was placed in a 7 cm long and 0.5 cm diameter glass tube. The electrode surface was compacted on a plate glass to make it flat, and its apparent active area was around  $0.196\text{ cm}^2$ . From the other end of the tube, a copper wire with diameter of 1.5 mm was immersed in the paste as the conductor.

Prior to the electrochemical study, the surface of these C paste electrodes was sequentially polished with 300, 600, and 1200 grade silicon carbide paper. And then these electrodes were rinsed with distilled water and quickly transferred to the cell.

**2.2. Electrochemical Analysis.** The electrochemical measurements were performed in a typical electrochemical cell (200 mL) with three electrodes: the working electrode (Carbon paste electrode with pristine or coated pyrite), the counter electrode (a platinum foil electrode with  $1\text{ cm}^2$  area), and the reference electrode (KCl-saturated calomel electrode). The electrolyte was  $0.5\text{ mol L}^{-1}\text{ H}_2\text{SO}_4$  solution.

The electrochemical measurements were performed by an electrochemical workstation (2273, Parstart), and the experimental data was recorded on a personal computer with suitable software. Cyclic voltammetry (CV) experiments were conducted, starting from open-circuit potentials (OCPs), at a sweep rate of  $100\text{ mV s}^{-1}$ , and the scan range was from  $-0.6\text{ V/SCE}$  to  $+0.8\text{ V/SCE}$ . Polarization curves were measured over the range of OCP  $\pm 200\text{ mV}$  at a constant rate of potential change of  $1\text{ mV s}^{-1}$ . From these polarization curves,

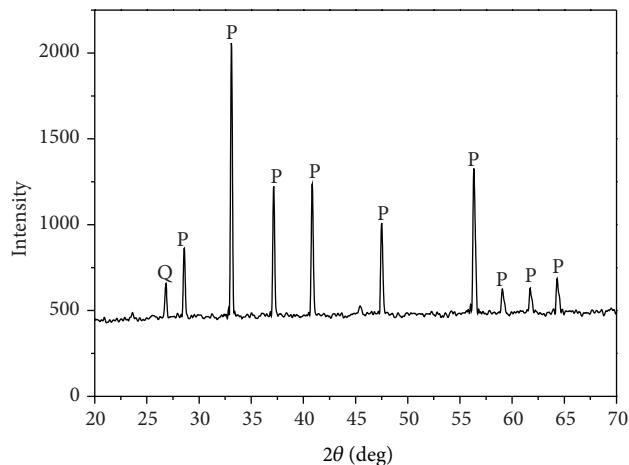


FIGURE 1: XRD spectra of the pyrite. P: Pyrite, Q: quartz.

corrosion current densities ( $j_{\text{corr}}$ ) of different pyrite electrodes were obtained. Then, the inhibition efficiency of TETA on pyrite can be calculated by using the following equation [27]:

$$\eta(\%) = \frac{j_{\text{corr}} - j_{\text{corr(inh)}}}{j_{\text{corr}}}, \quad (1a)$$

where  $j_{\text{corr}}$  and  $j_{\text{corr(inh)}}$  were the corrosion current density of pristine and coated pyrite samples, respectively.

The impedance spectra were obtained by applying a signal on the OCP with a frequency range from  $5 \times 10^5$  to  $1 \times 10^{-2}$  Hz with a sinusoidal excitation signal of 10 mV. The impedance data were analyzed using ZSimpWin software. The equivalent circuit  $R_s(Q_1(R_1Q_2))$ , as shown in Figure 2, was used to fit these impedance data. As Bevilaqua et al. [34] suggested, this equivalent circuit was simplified from the circuit of  $R_s(R_1Q_2(R_2Q_2))$ , and it described a response of the corrosion process occurring at the open-circuit potential due to parallel anodic and cathodic reactions. In the circuit of  $R_s(Q_1(R_1Q_2))$ ,  $R_s$  represented the solution resistance,  $R_1$  was the charge transfer resistance in the initial stage of pyrite oxidation,  $Q_1$  was the constant phase element which was associated with the capacitor of the double layer of the electrode/electrolyte interface with passive film, and  $Q_2$  represented the diffusion impedance component, a reaction limited by the diffusion of oxygen. According to the values of charge transfer resistance, the inhibition efficiency (IE) was obtained by using the following equation [27]:

$$IE = \frac{R_1^{-1} - R_{1(\text{inh})}^{-1}}{R_1^{-1}}, \quad (1b)$$

where  $R_1$  and  $R_{1(\text{inh})}$  were the charge transfer resistance values of pristine and coated pyrite samples, respectively.

All of the above measurements were carried out in static conditions. All potentials quoted in this paper are referenced to the saturated calomel electrode (SCE).

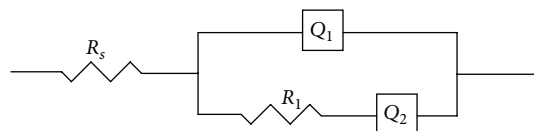


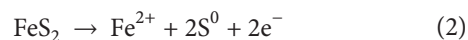
FIGURE 2: Equivalent electrical circuits proposed for fitting impedance spectra of pyrite oxidation.

### 3. Results and Discussion

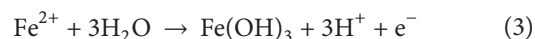
**3.1. Open-Circuit Potential Measurements.** Figure 3 shows the OCPs of the pyrite electrodes coated by different concentrations of TETA. The OCP of the uncoated sample (denoted as control) was 362.8 mV, and the OCPs of pyrite samples coated by 1% TETA, 2% TETA, 3% TETA, and 5% TETA were 304.8 mV, 279.2 mV, 261.7 mV, and 187.0 mV, respectively. It is obvious that the OCPs of coated samples were lower than that of uncoated pyrite. This phenomenon was ascribed to the relatively low redox potential of TETA [26].

**3.2. Cyclic Voltammetry Measurements.** The CV curves of the pristine and coated pyrite electrodes in  $0.5 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$  solution obtained by sweeping the potential from OCP towards negative direction are shown in Figure 4. The shape of the voltammetric curve was not significantly influenced by the presence of TETA, indicating that the inhibitor does not change the mechanism of pyrite oxidation. These curves were similar to the other reported results [35, 36], in which the reduction peaks between  $-0.4 \text{ V}$  and  $-0.2 \text{ V}$  can be interpreted as two possible reactions: (1) the reduction of S formed during the handling and preparation of samples and (2) the reduction of  $\text{FeS}_2(\text{s})$  to form  $\text{FeS}(\text{s})$  and  $\text{H}_2\text{S}$ . The reversal of potential scan produces three anodic current peaks:  $A_1$ ,  $A_2$ , and  $A_3$ .  $A_1$  is attributed to the oxidation of the  $\text{H}_2\text{S}$  formed electrochemically during the oxidation scan.  $A_2$  results from the oxidation of pyrite, via two steps [37, 38].

The first step is



The second step is:



At high potentials, the oxidation of sulfur to sulfate is expected to occur [39], contributing to the appearance of the anodic current peak  $A_3$ .

Figure 5 shows the CV curves of the pristine and coated pyrite electrode when the potentials were initially swept from OCP towards the positive direction. In addition to the anodic and cathodic current peaks mentioned above, another cathodic current peak between  $0.3 \text{ V}$  and  $0.4 \text{ V}$  appeared when the potential scan was reversed. This peak was attributed to iron oxide reduction.

The evidence of pyrite passivation by TETA can be made by measuring its electrochemical activity [40]. Comparing the CV curves of the pyrite samples coated by various concentrations of TETA, all of the anodic and cathodic current peaks

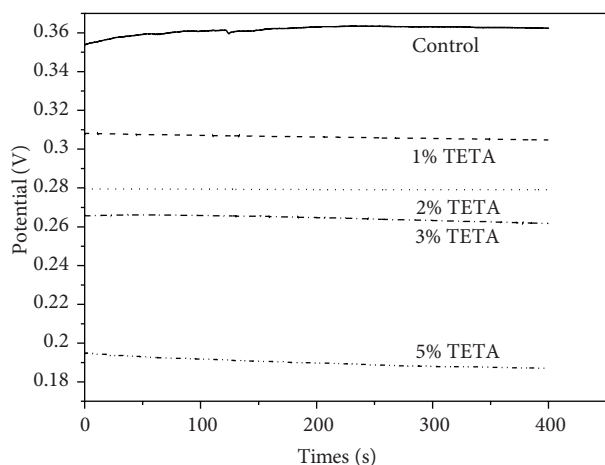


FIGURE 3: Open-circuit potentials of the pyrite electrodes coated by different concentration of TETA.

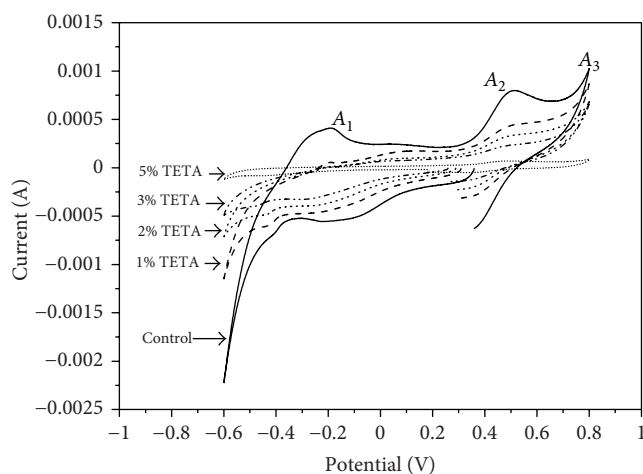


FIGURE 4: Cyclic voltammograms of pyrite electrodes coated by different concentration of TETA, initiated in the negative direction.

are decreased with an increase in TETA. When 5% of TETA was adopted in the coating treatment, the anodic and cathodic current peaks almost could not be detected. This proves that less-electrochemical activity takes place on the surface of pyrite after being coated by TETA. The decrease of electrochemical activity should be attributed to the formation of a protective layer of TETA on the surface of pyrite samples. As we know, there are several amine molecules in the structure of TETA, consequently, TETA can be absorbed on the pyrite surface through coordination bond formation between the iron in pyrite and to the electron pair on the nitrogen atom [41]. The inhibition efficiencies therefore depend on the coverage area of the adsorbed molecule. With an increase of the concentration of TETA, there is much larger surface area of pyrite coated by TETA, so the inhibition efficiency increases.

**3.3. Potentiodynamic Polarization Test.** The Tafel polarization curves for the pristine and coated pyrite electrodes in

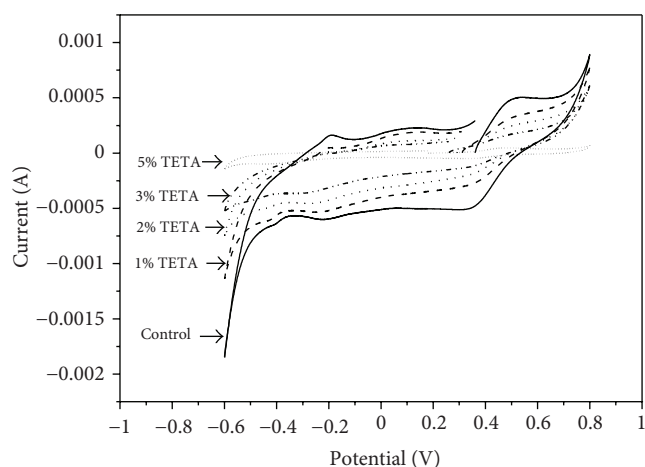


FIGURE 5: Cyclic voltammograms of pyrite electrodes coated by different concentration of TETA, initiated in the positive direction.

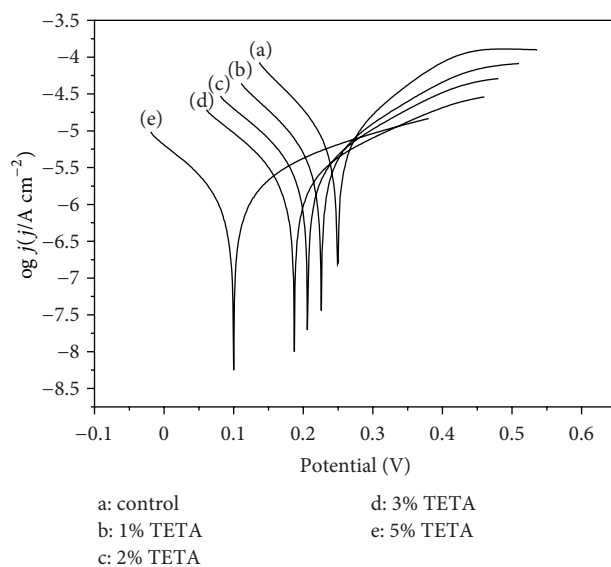


FIGURE 6: Polarization curves of the pyrite electrodes coated by different concentration of TETA.

TABLE 2: Electrochemical polarization parameters and the corresponding inhibition efficiencies for pyrite coated with different concentrations of TETA.

Concentration of TETA (%)	$E_{\text{corr}}$ (mV/SCE)	$\beta_c$ (decade $V^{-1}$ )	$\beta_a$ (decade $V^{-1}$ )	$j_{\text{corr}}$ (mA $\text{cm}^{-2}$ )	$\eta$ (%)
0%	253	9.59	7.44	0.0426	—
1%	226	8.82	6.73	0.0247	42.08
2%	206	7.91	5.96	0.0183	57.04
3%	187	7.72	5.23	0.0131	69.24
5%	100	7.70	4.53	0.0081	80.98

0.5 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> solution are shown in Figure 6. It was clear that the addition of TETA caused more negative shift in corrosion potential ( $E_{\text{corr}}$ ) especially in high concentrations.

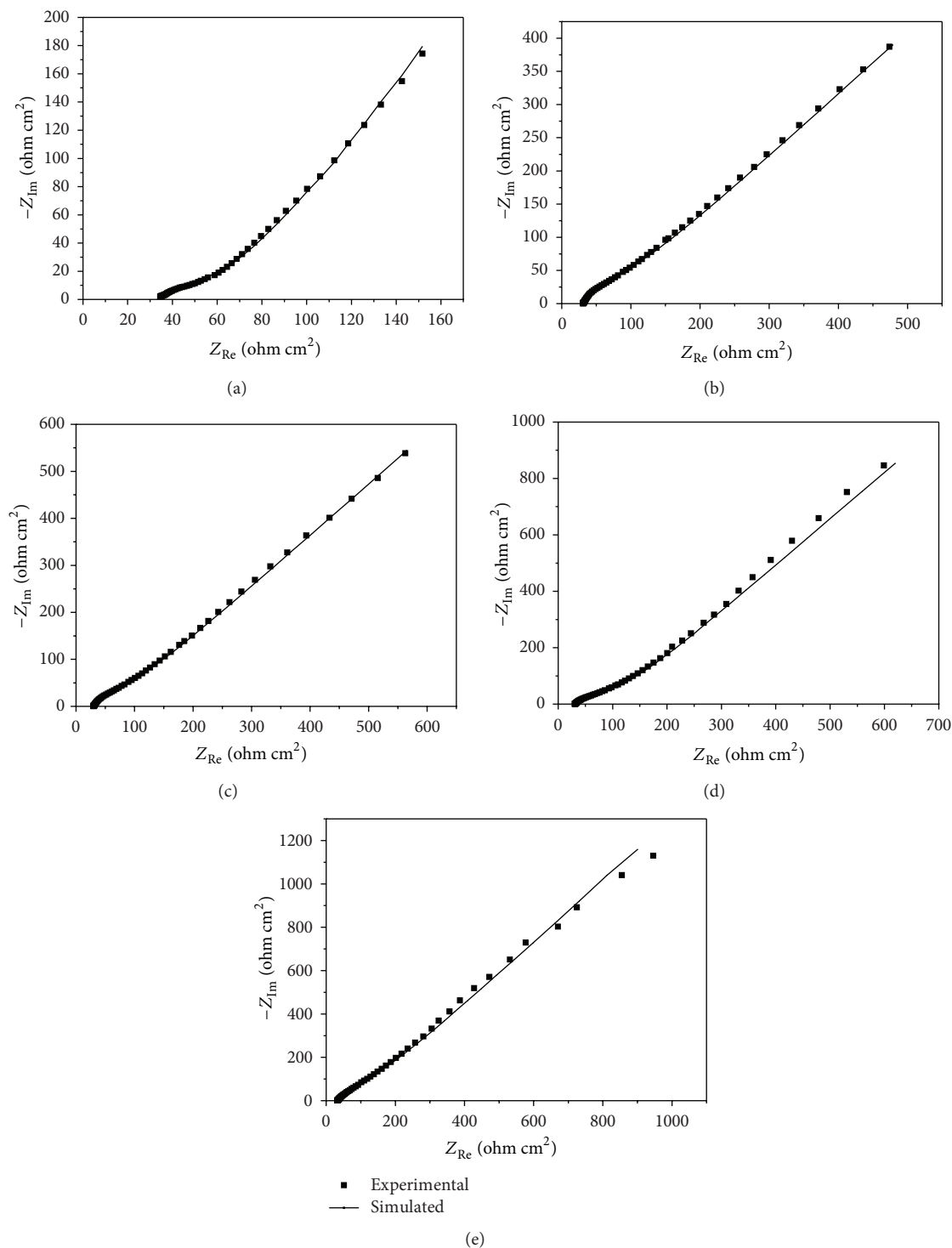


FIGURE 7: Experimental and simulated Nyquist plots of pyrite samples coated by different concentrations of TETA. (a) Uncoated; (b) coated by 1% TETA; (c) coated by 2% TETA; (d) coated by 3% TETA; (e) coated by 5% TETA.

It was consistent with the tendency of OPCs shown in Figure 3. It should be pointed out that the value of the corrosion potential of the same electrode in the same electrolyte was different from the value of the open-circuit potential. This phenomenon was due to the concentrations of reductive

products at the interface of the electrode being higher than in a real solution when the applied potential was swept from negative to positive potentials, so the corrosion potential obtained by the polarization curve is lower than the open-circuit potential [42].

TABLE 3: Parameters using the circuit  $R_s(Q_1(R_1Q_2))$  and the corresponding inhibition efficiencies for pyrite coated with different concentrations of TETA.

Concentration of TETA (%)	$R_s/\Omega\text{ cm}^2$	$Y_{0,1}/10^{-3}$ $S\text{ s}^n\text{ cm}^{-2}$	$n$	$R_1/\Omega\text{ cm}^2$	$Y_{0,2}/10^{-3}$ $S\text{ s}^n\text{ cm}^{-2}$	$n$	$x^2/10^{-4}$	IE (%)
0	33.63	4.21	0.8	43.77	9.15	0.5	5.36	—
1	31.04	1.24	0.8	76.91	5.70	0.5	2.14	43.09
2	30.01	1.21	0.8	96.21	4.69	0.5	1.65	54.50
3	30.56	1.41	0.8	154.3	3.89	0.6	4.02	71.63
5	32.64	1.34	0.8	250.8	1.97	0.6	2.60	82.55

From the Tafel polarization curves, some electrochemical corrosion kinetic parameters can be obtained, such as the corrosion potential ( $E_{\text{corr}}$ ), cathodic and anodic Tafel slopes ( $\beta_c, \beta_a$ ), and corrosion current density ( $j_{\text{corr}}$ ), which are listed in Table 2.

From the values of cathodic and anodic Tafel slopes, both cathodic and anodic processes were found that are inhibited by the coating of TETA. The cathodic Tafel slopes were slightly decreased from 9.59 dec/V to 7.70 dec/V when the concentration of TETA increasing from 0% to 5%. This indicated that the cathodic reaction (the reduction of  $\text{FeS}_2$ ) is slightly inhibited by TETA. When the pyrite samples were coated by TETA, the anodic slopes decreased remarkably, which indicates that the inhibition of pyrite dissolution by TETA is mainly controlled by the anodic process. Therefore, TETA can be classified as inhibitors of relatively mixed effect (anodic/cathodic inhibition) in acid solution.

The inhibition efficiencies ( $\eta\%$ ) have been calculated by using (1a), which shows that the inhibition efficiency increases and the corrosion current density decreases with the increase of the TETA concentration. An increase from 42.08% to 80.98% was found when the concentration of TETA increased from 1% to 5%. This could be explained that there is a larger surface area of pyrite sample coated by TETA with the increase of TETA concentration.

**3.4. Electrochemical Impedance Spectroscopy.** The experimental and simulated impedance diagrams for pyrite samples coated by different concentrations of TETA are presented in Figure 7. Table 3 shows the quantitative results for impedance which fitted using the equivalent circuit  $R_s(Q_1(R_1Q_2))$  as mentioned above. The fact of a low value of  $x^2$ , which represents the sum of quadratic deviations between experimental and calculated data suggested that the proposed circuit is suitable for explaining the EIS spectra. Because all of the experiments were carried out in the same electrolyte, the values of the solution resistance ( $R_s$ ) were almost no change.

The value of charge transfer resistance " $R_1$ " is inversely proportional to corrosion rate. The charge transfer resistance increases with the increase in concentration of TETA.  $R_1$  increased from the value of  $43.7\ \Omega\text{ cm}^2$  for the pristine pyrite to  $283.6\ \Omega\text{ cm}^2$  for the pyrite coated by highest concentration of TETA, which indicates that the corrosion of pyrite is obviously inhibited in the presence of TETA.

According to (1b), the inhibition efficiencies (IE) have been calculated. The obtained results show that the inhibition

efficiency increases, while the charge transfer resistance increased when the concentration of the TETA increased. The results obtained from the EIS method were in good agreement with those obtained from the polarization measurements.

## 4. Conclusion

The feasibility of using TETA as a protecting agent to reduce the oxidation of pyrite had been studied using electrochemical technique. The results show that TETA is an efficient coating agent in preventing the oxidation of pyrite, and the inhibition efficiency was more pronounced with TETA concentration. The CV measurements reveal that TETA possessed strong capability to be used as passivation agent for AMD control. The potentiodynamic polarization curves indicate that TETA inhibited both anodic pyrite dissolution and also cathodic hydrogen evolution reactions, and it acted as mixed type inhibitor in acid solution. The values of inhibition efficiency obtained from the EIS method are in good agreement with the results of polarization measurement.

## Acknowledgments

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## References

- [1] A. N. Thorpe, F. E. Senftle, C. C. Alexander, and F. T. Dulong, "Oxidation of pyrite in coal to magnetite," *Fuel*, vol. 63, no. 5, pp. 662–668, 1984.
- [2] M. Perdicakis, S. Geoffroy, N. Grosselin, and J. Bessière, "Application of the scanning reference electrode technique to evidence the corrosion of a natural conducting mineral: pyrite. Inhibiting role of thymol," *Electrochimica Acta*, vol. 47, no. 1, pp. 211–216, 2001.



- [3] R. Garrels and M. Thompson, "Oxidation of pyrite by iron sulfate solutions," *American Journal of Science*, vol. 258, pp. 57–67, 1960.
- [4] M. P. Silverman, "Mechanism of bacterial pyrite oxidation," *Journal of Bacteriology*, vol. 94, no. 4, pp. 1046–1051, 1967.
- [5] J. C. Bennett and H. Tributsch, "Bacterial leaching patterns on pyrite crystal surfaces," *Journal of Bacteriology*, vol. 134, no. 1, pp. 310–317, 1978.
- [6] R. T. Lowson, "Aqueous oxidation of pyrite by molecular oxygen," *Chemical Reviews*, vol. 82, no. 5, pp. 461–497, 1982.
- [7] C. L. Wiersma and J. D. Rimstidt, "Rates of reaction of pyrite and marcasite with ferric iron at pH 2," *Geochimica et Cosmochimica Acta*, vol. 48, no. 1, pp. 85–92, 1984.
- [8] V. Nicholson, R. W. Gillham, and E. J. Reardon, "Pyrite oxidation in carbonate-buffered solution: 2. Rate control by oxide coatings," *Geochimica et Cosmochimica Acta*, vol. 54, no. 2, pp. 395–402, 1990.
- [9] R. Murphy and D. R. Strongin, "Surface reactivity of pyrite and related sulfides," *Surface Science Reports*, vol. 64, no. 1, pp. 1–45, 2009.
- [10] T. Xu, S. P. White, K. Pruess, and G. H. Brimhall, "Modeling of pyrite oxidation in saturated and unsaturated subsurface flow systems," *Transport in Porous Media*, vol. 39, no. 1, pp. 25–56, 2000.
- [11] P. R. Holmes and F. K. Crundwell, "The kinetics of the oxidation of pyrite by ferric ions and dissolved oxygen: an electrochemical study," *Geochimica et Cosmochimica Acta*, vol. 64, no. 2, pp. 263–274, 2000.
- [12] M. Gleisner, R. B. Herbert, and P. C. Frogner Kockum, "Pyrite oxidation by *Acidithiobacillus ferrooxidans* at various concentrations of dissolved oxygen," *Chemical Geology*, vol. 225, no. 1–2, pp. 16–29, 2006.
- [13] K. J. Edwards, M. O. Schrenk, R. Hamers, and J. F. Banfield, "Microbial oxidation of pyrite: experiments using microorganisms from an extreme acidic environment," *American Mineralogist*, vol. 83, no. 11–12, pp. 1444–1453, 1998.
- [14] A. A. Sobek, V. Rastogi, and D. A. Benedetti, "Prevention of water pollution problems in mining: the bactericide technology," *International Journal of Mine Water*, vol. 9, no. 1–4, pp. 133–148, 1990.
- [15] I. Doye and J. Duchesne, "Neutralisation of acid mine drainage with alkaline industrial residues: laboratory investigation using batch-leaching tests," *Applied Geochemistry*, vol. 18, no. 8, pp. 1197–1213, 2003.
- [16] M. Benzaazoua, P. Marion, I. Picquet, and B. Bussière, "The use of pastefill as a solidification and stabilization process for the control of acid mine drainage," *Minerals Engineering*, vol. 17, no. 2, pp. 233–243, 2004.
- [17] C. G. Romano, K. U. Mayer, D. R. Jones, D. A. Ellerbroek, and D. W. Blowes, "Effectiveness of various cover scenarios on the rate of sulfide oxidation of mine tailings," *Journal of Hydrology*, vol. 271, no. 1–4, pp. 171–187, 2003.
- [18] A. Peppas, K. Komnitsas, and I. Halikia, "Use of organic covers for acid mine drainage control," *Minerals Engineering*, vol. 13, no. 5, pp. 563–574, 2000.
- [19] G. M. Mudd, S. Chakrabarti, and J. Kodikara, "Evaluation of engineering properties for the use of leached brown coal ash in soil covers," *Journal of Hazardous Materials*, vol. 139, no. 3, pp. 409–412, 2007.
- [20] K. Nyavor and N. O. Egiebor, "Control of pyrite oxidation by phosphate coating," *Science of the Total Environment*, vol. 162, no. 2–3, pp. 225–237, 1995.
- [21] Y. L. Zhang and V. P. Evangelou, "Formation of ferric hydroxide-silica coatings on pyrite and its oxidation behavior," *Soil Science*, vol. 163, no. 1, pp. 53–62, 1998.
- [22] N. Belzile, S. Maki, Y. W. Chen, and D. Goldsack, "Inhibition of pyrite oxidation by surface treatment," *Science of the Total Environment*, vol. 196, no. 2, pp. 177–186, 1997.
- [23] A. R. Elsetinow, M. J. Borda, M. A. A. Schoonen, and D. R. Strongin, "Suppression of pyrite oxidation in acidic aqueous environments using lipids having two hydrophobic tails," *Advances in Environmental Research*, vol. 7, no. 4, pp. 969–974, 2003.
- [24] Y. Lan, X. Huang, and B. Deng, "Suppression of pyrite oxidation by iron 8-hydroxyquinoline," *Archives of Environmental Contamination and Toxicology*, vol. 43, no. 2, pp. 168–174, 2002.
- [25] M. F. Cai, Z. Dang, Y. W. Chen, and N. Belzile, "The passivation of pyrrhotite by surface coating," *Chemosphere*, vol. 61, no. 5, pp. 659–667, 2005.
- [26] Y. W. Chen, Y. Li, M. F. Cai, N. Belzile, and Z. Dang, "Preventing oxidation of iron sulfide minerals by polyethylene polyamines," *Minerals Engineering*, vol. 19, no. 1, pp. 19–27, 2006.
- [27] Q. B. Zhang and Y. X. Hua, "Corrosion inhibition of mild steel by alkylimidazolium ionic liquids in hydrochloric acid," *Electrochimica Acta*, vol. 54, no. 6, pp. 1881–1887, 2009.
- [28] A. Aytac, U. Ozmen, and M. Kabasakaloglu, "Investigation of some Schiff bases as acidic corrosion of alloy AA3102," *Materials Chemistry and Physics*, vol. 89, no. 1, pp. 176–181, 2005.
- [29] M. Lebrini, M. Lagrenee, H. Vezin, L. Gengembre, and F. Bentiss, "Electrochemical and quantum chemical studies of new thiadiazole derivatives adsorption on mild steel in normal hydrochloric acid medium," *Corrosion Science*, vol. 47, no. 2, pp. 485–505, 2005.
- [30] F. Bentiss, F. Gassama, D. Barbry et al., "Enhanced corrosion resistance of mild steel in molar hydrochloric acid solution by 1,4-bis(2-pyridyl)-5H-pyridazino[4,5-b]indole: electrochemical, theoretical and XPS studies," *Applied Surface Science*, vol. 252, no. 8, pp. 2684–2691, 2006.
- [31] B. F. Giannetti, S. H. Bonilla, C. F. Zinola, and T. Rabóczkay, "Study of the main oxidation products of natural pyrite by voltammetric and photoelectrochemical responses," *Hydrometallurgy*, vol. 60, no. 1, pp. 41–53, 2001.
- [32] D. P. Tao, P. E. Richardson, G. H. Luttrell, and R. H. Yoon, "Electrochemical studies of pyrite oxidation and reduction using freshly-fractured electrodes and rotating ring-disc electrodes," *Electrochimica Acta*, vol. 48, no. 24, pp. 3615–3623, 2003.
- [33] E. M. Arce and I. González, "A comparative study of electrochemical behavior of chalcopyrite, chalcocite and bornite in sulfuric acid solution," *International Journal of Mineral Processing*, vol. 67, no. 1–4, pp. 17–28, 2002.
- [34] D. Bevilacqua, H. A. Acciari, F. A. Arena et al., "Utilization of electrochemical impedance spectroscopy for monitoring bornite ( $\text{Cu}_5\text{FeS}_4$ ) oxidation by *Acidithiobacillus ferrooxidans*," *Minerals Engineering*, vol. 22, no. 3, pp. 254–262, 2009.
- [35] R. Liu, A. L. Wolfe, D. A. Dzombak, C. P. Horwitz, B. W. Stewart, and R. C. Capo, "Electrochemical study of hydrothermal and sedimentary pyrite dissolution," *Applied Geochemistry*, vol. 23, no. 9, pp. 2724–2734, 2008.
- [36] H. K. Lin and W. C. Say, "Study of pyrite oxidation by cyclic voltammetric, impedance spectroscopic and potential step techniques," *Journal of Applied Electrochemistry*, vol. 29, no. 8, pp. 987–994, 1999.

- [37] C. M. V. B. Almeida and B. F. Giannetti, "Comparative study of electrochemical and thermal oxidation of pyrite," *Journal of Solid State Electrochemistry*, vol. 6, no. 2, pp. 111–118, 2002.
- [38] Y. Liu, Z. Dang, P. Wu, J. Lu, X. Shu, and L. Zheng, "Influence of ferric iron on the electrochemical behavior of pyrite," *Ionics*, vol. 17, no. 2, pp. 169–176, 2011.
- [39] R. Cruz, V. Bertrand, M. Monroy, and I. Gonzalez, "Effect of sulfide impurities on the reactivity of pyrite and pyritic concentrates: a multi-tool approach," *Applied Geochemistry*, vol. 16, no. 7-8, pp. 803–819, 2001.
- [40] P. Ačai, E. Sorrenti, T. Gerner, M. Polakovič, M. Kongolo, and P. de Donato, "Pyrite passivation by humic acid investigated by inverse liquid chromatography," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 337, no. 1–3, pp. 39–46, 2009.
- [41] S. Sathiyarayanan, C. Marikkannu, and N. Palaniswamy, "Corrosion inhibition effect of tetramines for mild steel in 1M HCl," *Applied Surface Science*, vol. 241, no. 3-4, pp. 477–484, 2005.
- [42] S. Y. Shi, Z. H. Fang, and J. R. Ni, "Electrochemistry of marmatite—carbon paste electrode in the presence of bacterial strains," *Bioelectrochemistry*, vol. 68, no. 1, pp. 113–118, 2006.

## Research Article

# A Green Preconcentration Method for Determination of Cobalt and Lead in Fresh Surface and Waste Water Samples Prior to Flame Atomic Absorption Spectrometry

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Cloud point extraction (CPE) has been used for the preconcentration and simultaneous determination of cobalt (Co) and lead (Pb) in fresh and wastewater samples. The extraction of analytes from aqueous samples was performed in the presence of 8-hydroxyquinoline (oxine) as a chelating agent and Triton X-114 as a nonionic surfactant. Experiments were conducted to assess the effect of different chemical variables such as pH, amounts of reagents (oxine and Triton X-114), temperature, incubation time, and sample volume. After phase separation, based on the cloud point, the surfactant-rich phase was diluted with acidic ethanol prior to its analysis by the flame atomic absorption spectrometry (FAAS). The enhancement factors 70 and 50 with detection limits of  $0.26 \mu\text{g L}^{-1}$  and  $0.44 \mu\text{g L}^{-1}$  were obtained for Co and Pb, respectively. In order to validate the developed method, a certified reference material (SRM 1643e) was analyzed and the determined values obtained were in a good agreement with the certified values. The proposed method was applied successfully to the determination of Co and Pb in a fresh surface and waste water sample.

## 1. Introduction

Release of large quantities of metals into the environment (especially in natural water) is responsible for a number of environmental problems [1]. Metals are major pollutants in marine, ground, industrial, and even treated waste waters [2]. Industrial wastes are the major source of various kinds of toxic metals which have nonbiodegradability and persistence properties resulted in a number of public health problems [3]. Metals of interest, cobalt (Co) and lead (Pb), were chosen based on their industrial applications and potential pollution impact on the environment [4].

Pb is a toxic metal and widely distributed in the environment. It is an accumulative toxic metal, which is responsible for a number of health problems [5].

Pb reaches humans from natural as well as anthropogenic sources, for example, drinking water, soils, industrial emissions, car exhaust, and contaminated food and beverages. Therefore, highly sensitive and selective methods have

needed to be developed to determine the trace level of Pb in water samples. The maximum contaminant levels of Pb in drinking water allowed by environmental protection agency (EPA) is  $15.0 \mu\text{g L}^{-1}$ , while the world health organization (WHO) for drinking water quality containing the guideline value of  $10 \mu\text{g L}^{-1}$  [6, 7].

Co is known to be an essential micronutrient for metabolic processes in both plants and animals [8]. It is mainly found in rocks, soil, water, plants, and animals. The determination of trace level of Co in natural waters is very important because Co is important for living species and it is part of vitamin B12 [9]. Exposures to a high level of Co lead to serious public health problems and are responsible for several diseases in human such as in lung, heart, and skin [10].

Flame atomic absorption spectrometry (FAAS) is a widely used technique for quantification of metal species. The determination of metals in water samples is usually associated with a step of preconcentration of the analyte



before detection [11]. The determination of trace levels of Pb and Co in water samples is particularly difficult because of the usually low concentration; on the bases of these facts a great effort is needed to develop highly sensitive and selective methods to simultaneously determine trace level of these metals in water samples [12, 13].

A variety of procedures for preconcentration of metals, such as solid phase extraction (SPE) [14], liquid-liquid extraction (LLE) [15], and coprecipitation and cloud point extraction (CPE) [16] have been developed. Among them, CPE is one of the most reliable and sophisticated separation methods for the enrichment of trace metals from different types of samples. While other methods such as LLE are usually time consuming and labor intensive and require relatively large volumes of solvents, which are not only responsible for public health problems but also a major cause of environmental pollution [17–22]. It was reported in literature that Pb and Co had been preconcentrated by CPE method after the formation of sparingly water-soluble complexes with different chelating agents such as ammonium pyrrolidine dithiocarbamate (APDC) [23, 24] 1-(2-thiazolylazo)-2-naphthol (TAN) [25], 1-(2-pyridylazo)-2-naphthol (PAN) [26, 27], and diethyldithiocarbamate (DDTC) [28–30].

In the present work, we introduce a simple, sensitive, selective, and low-cost procedure for simultaneous preconcentration of Co and Pb after the formation of complex with oxine, using Triton X-114 as surfactant and later analysis by flame atomic absorption spectrometry. Several experimental variables affecting the sensitivity and stability of separation/preconcentration method were investigated in detail. The proposed method was applied for the determination of trace amount of both metals in fresh surface and waste water samples.

## 2. Experimental

**2.1. Chemical Reagents and Glassware.** Ultrapure water, obtained from ELGA lab water system (Bucks, UK), was used throughout the work. The nonionic surfactant Triton X-114 was obtained from Sigma (St. Louis, MO, USA) and was used without further purification. Stock standard solution of Pb and Co at a concentration of  $1000 \mu\text{g L}^{-1}$  was obtained from the Fluka Kamica (Bush, Switzerland). Working standard solutions were obtained by appropriate dilution of the stock standard solutions before analysis. Concentrated nitric acid and hydrochloric acid were analytical reagent grade from Merck (Darmstadt, Germany) and were checked for possible trace Pb and Co contamination by preparing blanks for each procedure. The 8-hydroxyquinoline (oxine) was obtained from Merck, prepared by dissolving appropriate amount of reagent in 10 mL ethanol and diluting to 100 mL with 0.01 M acetic acid, and were kept in a refrigerator  $4^\circ\text{C}$  for one week. The 0.1 M acetate and phosphate buffer were used to control the pH of the solutions. The pH of the samples was adjusted to the desired pH by the addition of  $0.1 \text{ mol L}^{-1}$  HCl/NaOH solution in the buffers. For the accuracy of methodology, a certified reference material of water SRM-1643e, National

Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) was used. The glass and plastic wares were soaked in 10% nitric acid overnight and rinsed many times with deionized water prior to use to avoid contamination.

**2.2. Instrumentation.** A centrifuge of WIROWKA Laboratorium type WE-1, nr-6933 (speed range 0–6000 rpm, timer 0–60 min, 220/50 Hz, Mechanika Pheczyzyna, Poland) was used for centrifugation. The pH was measured by pH meter (720-pH meter, Metrohm). Global positioning system (iFinder GPS, Lowrance, Mexico) was used for sampling locations.

A Perkin Elmer Model 700 (Norwalk, CT, USA) atomic absorption spectrometer, equipped with hollow cathode lamps and an air-acetylene burner. The instrumental parameters were as follows: wavelength 240.7 and 283.3 nm and slit widths: 0.2 and 0.7 nm for Co and Pb. Deuterium lamp background correction was also used.

**2.3. Sample Collection and Preparation Procedure.** The fresh surface water samples (canals) and waste water were collected on alternate month in 2011 from twenty (20) different sampling sites of Jamshoro, Sindh (southern part of Pakistan) with the help of the global positioning system (GPS). The understudy district positioned between  $25^\circ 19' 26''$  N and  $67^\circ 12' 68''$  E. The sampling network was designed to cover a wide range of the whole district. The industrial waste water samples of understudy areas were also collected. All water samples were filtered through a 0.45 micropore size membrane filter to remove suspended particulate matter and were stored at  $4^\circ\text{C}$ .

**2.4. General Procedure for CPE.** For Co and Pb determination, aliquot of 25 mL of the standard or sample solution containing both analytes ( $20\text{--}100 \mu\text{g/L}$ ), oxine  $5 \times 10^{-3} \text{ mol L}^{-1}$  and Triton X-114 0.5% (v/v), were added. To reach the cloud point temperature, the system was allowed to stand for about 30 min into an ultrasonic bath at  $50^\circ\text{C}$  for 10 min. Separation of the two phases was achieved by centrifuging for 10 min at 3500 rpm. The contents of tubes were cooled down in an ice bath for 10 min. The supernatant was then decanted by inverting the tube. The surfactant-rich phase was treated with  $200 \mu\text{L}$  of  $0.1 \text{ mol L}^{-1} \text{HNO}_3$  in ethanol (1:1, v/v) in order to reduce its viscosity and facilitate sample handling. The final solution was introduced into the flame by conventional aspiration. Blank solution was submitted to the same procedure and measured in parallel to the standards and real samples.

## 3. Result and Discussion

**3.1. Optimization of CPE.** The preconcentration of Pb and Co was based on the formation of a neutral, hydrophobic complex with oxine, which is subsequently trapped in the micellar phase of a nonionic surfactant (Triton X-114). Utilizing the thermally induced phase extraction separation process known as CPE, the analyte is highly preconcentrated and free of interferences in a very small micellar phase. Several parameters play a significant role in the performance of

the surfactant system that is used and its ability to aggregate, thus entrapping the analyte species. The pH, complexing reagent and surfactant concentration, temperature, and time were studied for optimum analytical signals.

**3.2. Effect of pH.** The effect of pH on the CPE of Co and Pb was investigated because this parameter plays an important role in metal-chelate formation. The effect of pH upon the extraction of Co and Pb ions from the six replicate standard solutions  $20.0 \mu\text{g L}^{-1}$  was studied within the pH range of 3–10, while each operational desired pH value was obtained by the addition of  $0.1 \text{ mol L}^{-1}$  of  $\text{HNO}_3/\text{NaOH}$  in the presence of acetate/borate buffer. The maximum extraction efficiency of understudy metals was obtained at pH range of 6.5–7.5 as shown in Figure 1, for subsequent work pH 7.0 was chosen as the optimum for subsequent work.

**3.3. Effect of Triton X-114 Concentration.** Separation of metal ions by a cloud point method involves the prior formation of a complex with sufficient hydrophobicity to be extracted in a small volume of surfactant-rich phase. The temperature corresponding to cloud point is correlated with the hydrophilic property of surfactants. The nonionic surfactant Triton X-114 was chosen as surfactant due to its low cloud point temperature and high density of the surfactant-rich phase, which facilitates phase separation by centrifugation. The effect of Triton X-114 concentrations on the extraction efficiencies of Co and Pb were examined at the range of 0.1 to 1.0% (v/v). Figure 2 shows that quantitative extraction was observed when surfactant concentration was  $> 0.5\%$  (v/v). At lower concentrations, the extraction efficiency of complexes was low probably because of the inadequacy of the assemblies to entrap the hydrophobic complex quantitatively. A Triton X-114 concentration of 0.5% (v/v) was selected for subsequent studies.

**3.4. Effect of Oxine Concentration.** The oxine is a relatively very stable and selective hydrophobic complexing reagent which reacts with both selected cations. Replicate 10 mL of standard, SRM, and real sample solution in 0.5% (w/v) Triton X-114 at a buffer of pH 7.0 and complexed with oxine solutions in the range of  $1.0\text{--}10.0 \times 10^{-3} \text{ mol L}^{-1}$ . The results revealed in Figure 3 that extraction efficiency of both metals increases up to  $5 \times 10^{-3} \text{ mol L}^{-1}$ . This value was, therefore, selected as the optimal chelating agent concentration. The concentrations above this value have no significant effect on the efficiency of CPE.

**3.5. Effects of Sample Volume on Preconcentration Factor.** The preconcentration factor (PCF) is defined as the concentration ratio of the analyte in the final diluted surfactant-rich extract ready for its determination and in the initial solution. Among the other factors, this depends on the phase relationship, on the distribution constant of the analyte between the phases, and on sample volume. The sample volume is one of the most important parameters in the development of the preconcentration method, since it determines the sensitivity and enhancement of the technique. The phase

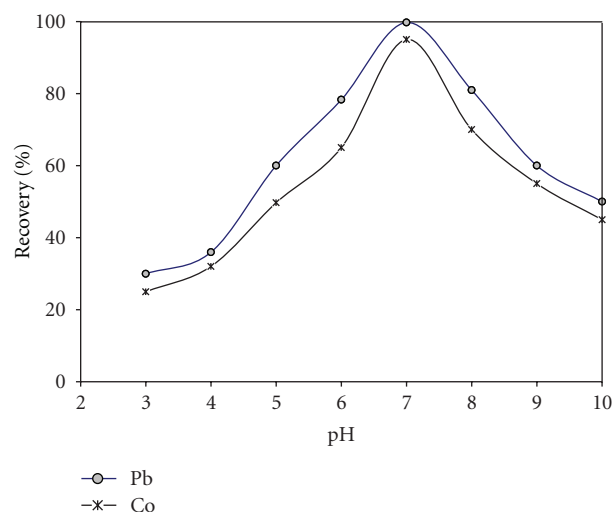


FIGURE 1: Effect of pH on the percentage of recovery:  $20 \mu\text{g L}^{-1}$  of Pb and Co,  $5.0 \times 10^{-3} \text{ mol L}^{-1}$  oxine, 0.5% (v/v) Triton X-114, temperature  $50^\circ\text{C}$ , and centrifugation time 10 min (3500 rpm).

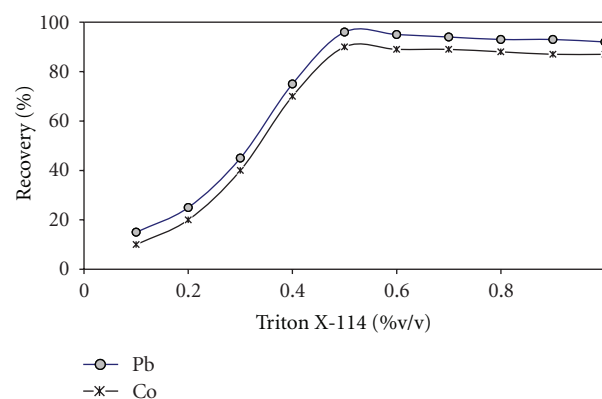


FIGURE 2: Effect of Triton X-114 on the percentage of recovery:  $20 \mu\text{g L}^{-1}$  of Pb and Co,  $5.0 \times 10^{-3} \text{ mol L}^{-1}$  oxine, pH 7.0, temperature  $50^\circ\text{C}$ , and centrifugation time 10 min (3500 rpm).

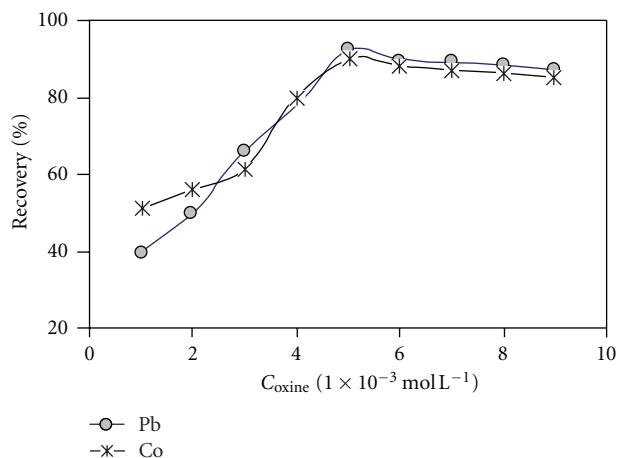


FIGURE 3: Effect of oxine concentration on the percentage of recovery:  $20 \mu\text{g L}^{-1}$  of Pb and Co, 0.5% (v/v) Triton X-114, pH 7.0, temperature  $50^\circ\text{C}$ , and centrifugation time 10 min (3500 rpm).

TABLE 1: Influences of some foreign ions on the recoveries of cobalt and lead ( $20 \mu\text{g L}^{-1}$ ) determination by applied CPE method.

Ion	Concentration ( $\mu\text{g L}^{-1}$ )	Pb recovery (%)	Co recovery (%)
$\text{Na}^+$	20000	$97 \pm 2.14$	$98 \pm 2.22$
$\text{K}^+$	5000	$98 \pm 2.21$	$99 \pm 3.02$
$\text{Ca}^{2+}$	5000	$98 \pm 2.12$	$97 \pm 1.12$
$\text{Mg}^{2+}$	5000	$97 \pm 1.04$	$98 \pm 3.08$
$\text{Cl}^-$	30000	$99 \pm 2.05$	$98 \pm 3.04$
$\text{F}^-$	1000	$96 \pm 3.01$	$97 \pm 1.12$
$\text{NO}_3^-$	3000	$97 \pm 1.04$	$98 \pm 3.06$
$\text{HCO}_3^-$	1000	$98 \pm 3.12$	$97 \pm 2.04$
$\text{Al}^{3+}$	500	$97 \pm 2.21$	$99 \pm 3.05$
$\text{Fe}^{3+}$	50	$96 \pm 2.23$	$97 \pm 3.02$
$\text{Zn}^{2+}$	100	$97 \pm 3.05$	$96 \pm 2.32$
$\text{Cr}^{3+}$	100	$96 \pm 2.08$	$98 \pm 2.25$
$\text{Cd}^{2+}$	100	$97 \pm 3.12$	$98 \pm 3.22$
$\text{Ni}^{2+}$	100	$96 \pm 2.23$	$97 \pm 3.01$

TABLE 2: Analytical characteristics of the proposed method.

Element condition	Concentration range ( $\mu\text{g L}^{-1}$ )	Slope	Intercept	$R^2$	R.S.D. ( $n = 5$ ) <sup>a</sup>	LOD <sup>b</sup> ( $\mu\text{g L}^{-1}$ )
Co without preconcentration	250–5000	$3.97 \times 10^{-3}$	-0.013	0.9871	1.45 (500)	32.0
Co with preconcentration	20.0–100	0.279	+0.008	0.9997	2.22 (20)	0.26
Pb without preconcentration	250–5000	$5.03 \times 10^{-3}$	-0.034	0.9972	0.88 (600)	46.0
Pb with preconcentration	20.0–100	0.256	-0.012	0.9989	1.88 (30)	0.44

<sup>a</sup>Values in parentheses are the Co and Pb concentrations ( $\mu\text{g L}^{-1}$ ) for which the RSD was obtained.<sup>b</sup>Limit of detection, calculated as three times the standard deviation of the blank signal.

TABLE 3: Determination of cobalt and lead in certified reference material and water samples.

(a)						
Certified reference material	Certified values ( $\mu\text{g L}^{-1}$ )		Measured values ( $\mu\text{g L}^{-1}$ )		Percentage of recovery (RSD %)	
	Co	Pb	Co	Pb	Co	Pb
SRM 1643e	$27.06 \pm 0.3$	$19.63 \pm 0.2$	$26.8 \pm 0.82$	$19.24 \pm 0.5$	99.0% (3.06%)	98.0% (2.60%)
(b)						
Samples	Added ( $\mu\text{g L}^{-1}$ )		Measured ( $\mu\text{g L}^{-1}$ )		Recovery (%)	
	Co	Pb	Co	Pb	Co	Pb
Canal water	0	0	$3.34 \pm 0.962$	$6.08 \pm 0.781$	—	—
	2	2	$5.32 \pm 0.384$	$8.06 \pm 0.822$	99.8	99
	5	5	$8.33 \pm 0.432$	$11.0 \pm 0.784$	100	98.4
	10	10	$13.3 \pm 0.642$	$15.9 \pm 0.828$	99.6	98.2

Mean  $\pm$  SD ( $n = 3$ ).

TABLE 4: Determination of lead and cobalt in water samples.

Sample	Co ( $\mu\text{g L}^{-1}$ )	Pb ( $\mu\text{g L}^{-1}$ )
Canal water	$3.34 \pm 0.962$	$6.08 \pm 0.781$
Waste water	$14.6 \pm 1.20$	$17.3 \pm 1.52$

Mean  $\pm$  SD ( $n = 3$ ).

ratio is an important factor, which has an effect on the extraction recovery of cations. A low phases ratio improves the recovery of analytes, but decreases the preconcentration factor. However, to determine the optimum amount of the

phase ratio, different volumes of a water sample 10–1000 mL and a constant volume of surfactant solution 0.5% were chosen. The obtained results show that with increasing the sample volume  $>100$  mL, the extracted understudy analytes were decreased as compared to those obtained with 25–50 mL. A successful cloud point extraction should maximize the extraction efficiency by minimizing the phase volume ratio, thus improving its concentration factor. In the present work, the initial sample volume was 25 mL and the final volume of surfactant rich phase after diluted with acidic ethanol was 0.5 mL, hence the PCF achieved in this work was 50 for both understudy analytes.

TABLE 5: Comparative table for determination of cobalt and lead in different types of samples applying CPE before analysis by atomic spectrometric technique.

Reagent and surfactant	Matrix and technique	PF <sup>a</sup> and EF <sup>b</sup>	LOD <sup>c</sup> ( $\mu\text{g L}^{-1}$ )	Reference
Cobalt				
TAN/Triton X-114	Water/(FAAS)	—/57 <sup>b</sup>	0.24	[25]
PAN/TX-100	Water samples/(GFAAS)	—/100 <sup>b</sup>	0.003	[31]
PAN/TX-114	Urine/(FAAS)	—/115 <sup>b</sup>	0.38	[32]
5-Br-PADAP/TX-100-SDS	Pharmaceutical samples/(FAAS)	—/29 <sup>b</sup>	1.1	[14]
TAN/Triton X-100	Water/(GFAAS)	—/100 <sup>b</sup>	0.003	[33]
APDC/Triton X-114	Biological tissues/(TS-FF-FAAS)	—/130 <sup>b</sup>	2.1	[34]
APDC/Triton X-114	Water/(FAAS)	—/20 <sup>b</sup>	5.0	[23]
1,2-N,N /PONPE 7.5	Water sample/(FAAS)	—/27 <sup>b</sup>	1.22	[35]
Me-BTABr/Triton X-114	Water sample/(FAAS)	—/28 <sup>b</sup>	0.9	[36]
Oxine/Triton X-114	Water sample/(FAAS)	50/50	0.44	Present work
Lead				
DDTP/Triton X-114	Human hair/(FAAS)	—/43 <sup>b</sup>	2.86	[28]
PONPE 7.5/—	Human saliva/(FAAS)	—/10 <sup>b</sup>	—	[37]
APDC/Triton X-114	Certified biological reference materials/(ETAAS)	—/22.5 <sup>b</sup>	0.04 <sup>c</sup> /—	[24]
DDTP/Triton X-114	Certified blood reference samples/(ETAAS)	—/34 <sup>b</sup>	0.08	[38]
PONPE 7.5/—	Tap water certified reference material/(ICP-OES)	—/300 <sup>b</sup>	0.07	[39]
DDTP/Triton X-114	Riverine and sea water enriched water reference materials/(ICP-MS)	—/—	40.0	[40]
5-Br-PADAP/Triton X-114	Water/(GFAAS)	50 <sup>a</sup> /—	0.08 <sup>c</sup> /—	[41]
PAN/Triton X-114	Water/(FAAS)	—/55.6 <sup>b</sup>	1.1	[27]
—/Tween 80	Environmental sample/FAAS	10 <sup>a</sup> /—	7.2	[42]
TAN/Triton X-114	Water sample/(FAAS)	15.1 <sup>a</sup> /—	4.5	[43]
Pyrogallol/Triton X-114	Water sample/(FAAS)	72 <sup>a</sup> /—	0.4	[44]
Oxine/Triton X-114	Water sample/(FAAS)	50/70	0.26	Present work

<sup>a</sup>preconcentration factor, <sup>b</sup>enhancement factor, and <sup>c</sup>limit of detection.

**3.6. Interferences.** The interference is those relating to the preconcentration step, which may react with oxine and decrease the extraction. To perform this study, 25 mL solution containing  $20 \mu\text{g/L}^{-1}$  of both metals at different interference to analyte ratio were subjected to the developed procedure. Table 1 shows the tolerance limits of the interfering ions error <5%. The tolerance limit of coexisting ions is defined as the largest amount making variation of less than 5% in the recovery of analytes. The effects of representative potential interfering species were tested. Commonly encountered matrix components such as alkali and alkaline earth elements generally do not form stable complexes under the experimental conditions. A high concentration of oxine reagent was used, for the complete chelation of the selected ions even in the presence of interferent ions.

**3.7. Analytical Figures of Merit.** The calibration graph using the preconcentration step for Co and Pb were linear with a concentration range of  $5.0\text{--}20 \mu\text{g L}^{-1}$  of standards and subjecting to CPE methods at optimum levels of all understudy variables. The extracted analytes in diluted micellar media were introduced into the flame by conventional aspiration. Table 2 gives the calibration parameters for the proposed CPE method including the linear ranges, relative standard deviation RSD, and limit

of detection LOD. The experimental enhancement factors calculated as the ratio of the slopes of calibration graphs with and without preconcentration. The enhancement factors of Co and Pb subjected to CPE method were found to be 70 and 50, respectively. The limits of detection LOD were calculated as the ratio between three times the standard deviation of ten blank readings and the slope of the calibration curve after preconcentration were calculated as 0.26 and  $0.44 \mu\text{g L}^{-1}$ , respectively, for Co and Pb. The obtained LOD was sufficiently low for detecting trace levels of Co and Pb in different types of fresh and waste water samples.

The accuracy of the proposed method was evaluated by analyzing a standard reference material of water SRM-1643e with certified values of Co and Pb content. It was found that there is no significant difference between results obtained by the proposed method and the certified results of both metals. Reliability of the proposed method was also checked by spiking both metals at three concentration levels ( $2.0\text{--}10.0 \mu\text{g L}^{-1}$ ) in a real water sample. The results are presented in Tables 3(a) and 3(b). The percentage of recoveries (*R*) of spike standards were calculated as follows:

$$R (\%) = \frac{(C_m - C_o)}{m} \times 100, \quad (1)$$

where  $C_m$  is a value of metal in a spiked sample,  $C_o$  the value of metal in a sample, and  $m$  is the amount of metal spiked.



These results demonstrate the applicability of developed procedure for Co and Pb determination in different water samples.

**3.8. Application to Real Samples.** The CPE procedure was applied to determine Co and Pb in fresh surface and waste water samples. The results are shown in Table 4. The Co and Pb concentrations in fresh surface water were found in the range of  $2.12\text{--}5.12\ \mu\text{g L}^{-1}$  and  $1.49\text{--}8.56\ \mu\text{g L}^{-1}$ , respectively. In waste water, the levels of both analyte were high, found in the range of  $13.6\text{--}16.8$  and  $15.1\text{--}19.4\ \mu\text{g L}^{-1}$  for Co and Pb, respectively.

#### 4. Conclusion

In this study, Triton X-114 was chosen for the formation of the surfactant-rich phase due to its excellent physicochemical characteristics, low cloud point temperature, high density of the surfactant-rich phase, which facilitates phase separation easily by centrifugation, and commercial availability and relatively low price and low toxicity. This method is a promising alternative for the determination of Co and Pb linked with FAAS. From the results obtained, it can be considered that oxine is an efficient ligand for cloud point extraction of Co and Pb. The simple accessibility, the formation of stable complexes, and consistency with the cloud point extraction method are the major advantages of the use of oxine in cloud point extraction of Co and Pb. CPE has been shown to be a practicable and versatile method, being adequate for environmental studies. Cloud point extraction is an easy, safe, rapid, inexpensive, and environmentally friendly methodology for preconcentration and separation of trace metals in aqueous solutions. The surfactant-rich phase can be directly introduced into flame atomic absorption spectrometer FAAS after dilution with acidic ethanol. The proposed CPE method incorporating oxine as chelating agent permits effective separation and preconcentration of Co and Pb and final determination by FAAS provides a novel route for trace determination of these metals in water samples of different ecosystem. A low-cost surfactant was used, thus toxic organic solvent extraction generating waste disposal problems was avoided. The comparison of the results found in the presented study and some works in the literature was given in [31–44]. The proposed cloud point extraction method is superior for having lower detection limits when compared to other methods as shown in Table 5.

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#### References

- [1] M. Soylak, L. Elci, and M. Dogan, "Determination of some trace metals in dialysis solutions by atomic absorption spectrometry after preconcentration," *Analytical Letters*, vol. 26, pp. 1997–2007, 1993.
- [2] Y. Bayrak, Y. Yesiloglu, and U. Gecgel, "Adsorption behavior of Cr(VI) on activated hazelnut shell ash and activated bentonite," *Microporous and Mesoporous Materials*, vol. 91, no. 1–3, pp. 107–110, 2006.
- [3] M. Kobya, E. Demirbas, E. Senturk, and M. Ince, "Adsorption of heavy metal ions from aqueous solutions by activated carbon prepared from apricot stone," *Bioresource Technology*, vol. 96, no. 13, pp. 1518–1521, 2005.
- [4] M. Kazemipour, M. Ansari, S. Tajrobehkar, M. Majdzadeh, and H. R. Kermani, "Removal of lead, cadmium, zinc, and copper from industrial wastewater by carbon developed from walnut, hazelnut, almond, pistachio shell, and apricot stone," *Journal of Hazardous Materials*, vol. 150, no. 2, pp. 322–327, 2008.
- [5] F. Shah, T. G. Kazi, H. I. Afridi et al., "Environmental exposure of lead and iron deficit anemia in children age ranged 1–5 years: a cross sectional study," *Science of the Total Environment*, vol. 408, no. 22, pp. 5325–5330, 2010.
- [6] D. L. Tsalev and Z. K. Zaprianov, *Atomic Absorption in Occupational and Environmental Health Practice*, Analytical Aspects and Health Significance, CRC Press, Boca Raton, Fla, USA, 1983.
- [7] H. G. Seiler, A. Siegel, and H. Siegel, *Handbook on Metals in Clinical and Analytical Chemistry*, Marcel Dekker, New York, NY, USA, 1994.
- [8] A. Sasmaz and M. Yaman, "Distribution of chromium, nickel, and cobalt in different parts of plant species and soil in mining area of Keban, Turkey," *Communications in Soil Science and Plant Analysis*, vol. 37, pp. 1845–1857, 2006.
- [9] M. Soylak, L. Elci, and M. Dogan, "Determination of trace amounts of cobalt in natural water samples as 4-(2-Thiazolylazo) resorcinol complex after adsorptive preconcentration," *Analytical Letters*, vol. 30, no. 3, pp. 623–631, 1997.
- [10] M. Soylak, L. Elci, I. Narin, and M. Dogan, "Application of solid phase extraction for the preconcentration and separation of trace amounts of cobalt from urine," *Trace Elements and Electrolytes*, vol. 18, pp. 26–29, 2001.
- [11] V. A. Lemos and G. T. David, "An on-line cloud point extraction system for flame atomic absorption spectrometric determination of trace manganese in food samples," *Microchemical Journal*, vol. 94, no. 1, pp. 42–47, 2010.
- [12] M. Ghaedi, M. R. Fathi, F. Marahel, and F. Ahmadi, "Simultaneous preconcentration and determination of copper, nickel, cobalt and lead ions content by flame atomic absorption spectrometry," *Fresenius Environmental Bulletin*, vol. 14, no. 12 B, pp. 1158–1163, 2005.
- [13] M. D. G. Pereira and M. A. Z. Arruda, "Trends in preconcentration procedures for metal determination using atomic spectrometry techniques," *Mikrochimica Acta*, vol. 141, no. 3–4, pp. 115–131, 2003.
- [14] C. C. Nascentes and M. A. Z. Arruda, "Cloud point formation based on mixed micelles in the presence of electrolytes for cobalt extraction and preconcentration," *Talanta*, vol. 61, no. 6, pp. 759–768, 2003.
- [15] A. Shokrollahi, M. Ghaedi, S. Gharaghani, M. R. Fathi, and M. Soylak, "Cloud point extraction for the determination of copper in environmental samples by flame atomic absorption spectrometry," *Quimica Nova*, vol. 31, no. 1, pp. 70–74, 2008.

- [16] J. A. Baig, T. G. Kazi, A. Q. Shah et al., "Optimization of cloud point extraction and solid phase extraction methods for speciation of arsenic in natural water using multivariate technique," *Analytica Chimica Acta*, vol. 651, no. 1, pp. 57–63, 2009.
- [17] P. Liu, Q. Pu, and Z. Su, "Synthesis of silica gel immobilized thiourea and its application to the on-line preconcentration and separation of silver, gold and palladium," *Analyst*, vol. 125, no. 1, pp. 147–150, 2000.
- [18] C. D. Stalikas, "Micelle-mediated extraction as a tool for separation and preconcentration in metal analysis," *TrAC Trends in Analytical Chemistry*, vol. 21, no. 5, pp. 343–355, 2002.
- [19] Z. Wang, M. Jing, F. S. Lee, and X. Wang, "Synthesis of 8-hydroxyquinoline Bonded Silica (SHQ) and its application in flow injection-inductively coupled plasma mass spectrometry analysis of trace metals in seawater," *Chinese Journal of Analytical Chemistry*, vol. 34, no. 4, pp. 459–462, 2006.
- [20] X.-J. Sun, B. Welz, and M. Sperling, "Determination of lead in wine by the FIAS-FAAS combination on-line preconcentration system," *Chemical Journal of Chinese Universities*, vol. 17, no. 8, pp. 1219–1221, 1996.
- [21] M. Soylak, "Determination of trace amounts of copper in high-purity aluminum samples after preconcentration on an activated carbon column," *Fresenius Environmental Bulletin*, vol. 7, no. 7-8, pp. 383–387, 1998.
- [22] J. L. Manzoori and G. Karim-Nezhad, "Development of a cloud point extraction and preconcentration method for Cd and Ni prior to flame atomic absorption spectrometric determination," *Analytica Chimica Acta*, vol. 521, no. 2, pp. 173–177, 2004.
- [23] D. L. Giokas, E. K. Paleologos, S. M. Tzouwara-Karayanni, and M. I. Karayannis, "Single-sample cloud point determination of iron, cobalt and nickel by flow injection analysis flame atomic absorption spectrometry—application to real samples and certified reference materials," *Journal of Analytical Atomic Spectrometry*, vol. 16, no. 5, pp. 521–526, 2001.
- [24] J. L. Manzoori and A. Bavili-Tabrizi, "The application of cloud point preconcentration for the determination of Cu in real samples by flame atomic absorption spectrometry," *Microchemical Journal*, vol. 72, no. 1, pp. 1–7, 2002.
- [25] J. Chen and K. C. Teo, "Determination of cobalt and nickel in water samples by flame atomic absorption spectrometry after cloud point extraction," *Analytica Chimica Acta*, vol. 434, no. 2, pp. 325–330, 2001.
- [26] J. L. Manzoori and A. Bavili-Tabrizi, "Cloud point preconcentration and flame atomic absorption spectrometric determination of cobalt and nickel in water samples," *Mikrochimica Acta*, vol. 141, no. 3-4, pp. 201–207, 2003.
- [27] J. Chen and K. C. Teo, "Determination of cadmium, copper, lead and zinc in water samples by flame atomic absorption spectrometry after cloud point extraction," *Analytica Chimica Acta*, vol. 450, no. 1-2, pp. 215–222, 2001.
- [28] J. L. Manzoori and A. Bavili-Tabrizi, "Cloud point preconcentration and flame atomic absorption spectrometric determination of Cd and Pb in human hair," *Analytica Chimica Acta*, vol. 470, no. 2, pp. 215–221, 2002.
- [29] A. Ohashi, H. Ito, C. Kanai, H. Imura, and K. Ohashi, "Cloud point extraction of iron(III) and vanadium(V) using 8-quinolinol derivatives and Triton X-100 and determination of 10<sup>-7</sup> mol dm<sup>-3</sup> level iron(III) in riverine water reference by a graphite furnace atomic absorption spectroscopy," *Talanta*, vol. 65, no. 2, pp. 525–530, 2005.
- [30] D. Zhao, R. Bian, Y. Ding, and L. Li, "Determination of lead and cadmium in water samples by cloud point extraction prior to flame atomic absorption spectrometry determination," *Journal of the Iranian Chemical Research*, vol. 6, no. 2, pp. 87–94, 2009.
- [31] Y. Zhang, W. H. Luo, and H. Li, "Determination of trace cobalt in water samples by graphite furnace atomic absorption spectrometry after cloud point," *Spectroscopy Spectral Analysis*, vol. 25, pp. 576–578, 2005.
- [32] J. L. Manzoori and G. Karim-Nezhad, "Sensitive and simple cloud-point preconcentration atomic absorption spectrometry: application to the determination of cobalt in urine samples," *Analytical Sciences*, vol. 19, no. 4, pp. 579–583, 2003.
- [33] Ma. C. C. Oliveros, O. J. De Blas, J. L. P. Pavón, and B. M. Cordero, "Cloud point preconcentration and flame atomic absorption spectrometry: application to the determination of nickel and zinc," *Journal of Analytical Atomic Spectrometry*, vol. 13, no. 6, pp. 547–550, 1998.
- [34] G. L. Donati, C. C. Nascentes, A. R. A. Nogueira, M. A. Z. Arruda, and J. A. Nóbrega, "Acid extraction and cloud point preconcentration as sample preparation strategies for cobalt determination in biological materials by thermospray flame furnace atomic absorption spectrometry," *Microchemical Journal*, vol. 82, no. 2, pp. 189–195, 2006.
- [35] J. L. Manzoori and A. Bavili-Tabrizi, "Cloud point preconcentration and flame atomic absorption spectrometric determination of cobalt and nickel in water samples," *Mikrochimica Acta*, vol. 141, no. 3-4, pp. 201–207, 2003.
- [36] V. A. Lemos, R. d. França, and B. O. Moreira, "Cloud point extraction for Co and Ni determination in water samples by flame atomic absorption spectrometry," *Separation and Purification Technology*, vol. 54, no. 3, pp. 349–354, 2007.
- [37] M. O. Luconi, M. F. Silva, R. A. Olsina, and L. P. Fernández, "Cloud point extraction of lead in saliva via use of nonionic PONPE 7.5 without added chelating agents," *Talanta*, vol. 51, no. 1, pp. 123–129, 2000.
- [38] F. Shemirani, M. Baghdadi, M. Ramezani, and M. R. Jamali, "Determination of ultra trace amounts of bismuth in biological and water samples by electrothermal atomic absorption spectrometry (ET-AAS) after cloud point extraction," *Analytica Chimica Acta*, vol. 534, no. 1, pp. 163–169, 2005.
- [39] M. O. Luconi, L. L. Sombra, M. F. Silva, L. D. Martinez, R. O. Olsina, and L. P. Fernandez, "Determination of lead by flow injection—inductively coupled plasma optical emission spectrometry after cloud point enrichment without chelating agents," *Chemia Analityczna*, vol. 48, p. 749, 2003.
- [40] M. A. M. Da Silva, V. L. A. Frescura, and A. J. Curtius, "Determination of trace elements in water samples by ultrasonic nebulization inductively coupled plasma mass spectrometry after cloud point extraction," *Spectrochimica acta, Part B*, vol. 55, no. 7, pp. 803–813, 2000.
- [41] J. Chen, S. Xiao, X. Wu, K. Fang, and W. Liu, "Determination of lead in water samples by graphite furnace atomic absorption spectrometry after cloud point extraction," *Talanta*, vol. 67, no. 5, pp. 992–996, 2005.
- [42] S. Candir, I. Narin, and M. Soylak, "Ligandless cloud point extraction of Cr(III), Pb(II), Cu(II), Ni(II), Bi(III), and Cd(II) ions in environmental samples with Tween 80 and flame atomic absorption spectrometric determination," *Talanta*, vol. 77, no. 1, pp. 289–293, 2008.
- [43] H. Sang, P. Liang, and D. Du, "Determination of trace aluminum in biological and water samples by cloud point extraction preconcentration and graphite furnace atomic



- absorption spectrometry detection,” *Journal of Hazardous Materials*, vol. 154, no. 1–3, pp. 1127–1132, 2008.
- [44] F. Shemirani, S. D. Abkenar, and A. Khatouni, “Determination of trace amounts of lead and copper in water samples by flame atomic absorption spectrometry after cloud point extraction,” *Bulletin of the Korean Chemical Society*, vol. 25, no. 8, pp. 1133–1136, 2004.