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*Research Article*

# Extraction of Parquat from Blood by Clinoptilolite

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Paraquat is a bipyridyl herbicide and organic divalent cation which due to its high polarity and water solubility cannot be readily extracted by common organic solvents from body fluids. Dithionite color test for qualitative and quantitative determination of paraquat in urine has been proposed and used for many years. Although some methods were proposed for solvent extraction of paraquat from blood, they are less practical in clinical laboratories and lack high extraction recovery. Clinoptilolite is a highly porous natural zeolite with cation-exchange property and high surface area. In the present work, extraction of paraquat from human blood by clinoptilolite was investigated and compared with Amberlite CG-50 I, a well-known weak cation-exchanger. Blood paraquat was adsorbed by adsorbents (clinoptilolite or Amberlite) and extracted from them by saturated sodium chloride solution. Extracted paraquat was spectrophotometrically measured by means of sodium dithionite reagent at 394.5 nm. Recovery, limit of detection, considering signal-to-noise ($S/N$) ratio of 3, and limit of quantification, regarding $S/N$ of 10, of paraquat extraction by clinoptilolite and Amberlite CG-50 were 81.7% ± 3.4%, 0.58 $\mu$g, and 1.93 $\mu$g and 83.6% ± 3.2%, 0.49 $\mu$g, and 1.63 $\mu$g, respectively. Repeatabilities (within-laboratory error) of paraquat extraction by clinoptilolite and Amberlite CG-50 I were 7.1% and 6.3%, respectively.

## 1. Introduction

Paraquat, 1,1′-Dimethyl–4,4′-bipyridinium, is a bipyridyl herbicide and divalent organic cation [1] which due to its high polarity and water solubility cannot be readily extracted by common organic solvents from body fluids [2]. Dithionite color test for qualitative and quantitative determination of paraquat in urine has been proposed and used for many years [3]. Although some methods were proposed for solvent extraction of paraquat from blood [4, 5], they are less practical in clinical laboratories and lack high extraction recovery. Zeolites are microporous hydrated aluminosilicate materials which are available as naturally occurring materials or as synthetically manufactured substances [6]. They are used in industries and medicine for their high surface area/volume ratio. Clinoptilolite as a natural zeolite due to its high availability, low cost, and high surface area/volume ratio has found many applications in agriculture [7, 8], animal husbandry [9], industries [8, 10], and medicine [11–16]. It has been found to be an efficient adsorbent of cations [10, 17] and a nontoxic carrier for drugs [11]. It has high negative charge which is distributed through its internal and external surface area. In the present work, solid-phase extraction of paraquat from human blood by clinoptilolite was investigated and compared with Amberlite CG-50 I, a well-known weak cation-exchanger.

## 2. Materials and Methods

Paraquat dichloride (Riedel-deHaën, Germany), micronized (particle size ≤ 37 $\mu$m) Iranian clinoptilolite (Anzymite) powder (Afrand Tooska, Tehran, Iran), Amberlite CG-50 I (Merck, Germany), sodium dithionite (Merck, Germany), sodium hydroxide (Merck, Germany), sodium chloride (Merck, Germany), methanol (Merck, Germany), and stainless steel mesh sieves (800 and 400 mesh equivalent to 16

and 37 $\mu$m opening diameters, resp.) (Anping County Resen Screen Co., Hengshui, China) were used in the present work.

Firstly, clinoptilolite powder with particle size of 11–40 $\mu$m was prepared by suspending micronized clinoptilolite powder (particle size $\leq$ 37 $\mu$m) in distilled water (DW) followed by passing the suspension from 400 mesh (equivalent to 37 $\mu$m openings diameters) stainless steel sieve. The passed fraction of powder was passed from 800 mesh (equivalent to 16 $\mu$m openings diameters) stainless steel sieve. Retained fraction of clinoptilolite by 800 mesh sieve was used in the present study. Before use, the sieved fraction was washed several times with DW, centrifuged at 5000 rpm, and dried in an oven at 250°C for 24 hrs [17].

Particle size and zeta potential of a 0.5% (w/v) suspension of sieved clinoptilolite in DW were determined by a particle size analyzer (Mastersizer Hydro 2000S, Malvern Instruments Ltd., UK) and a Zetasizer (Nano ZS, Malvern Instruments Ltd., UK), respectively. The pH of the sieved clinoptilolite suspension was 9.5 due to inherent basicity of clinoptilolite. Zeta potential was measured to show a cation-exchange property of clinoptilolite.

Sieved clinoptilolite was photographed by a field-emission scanning electron microscope (FE-SEM) (Hitachi, Model S-4160, Japan) to study the morphology and crystallinity of clinoptilolite particles. Specific surface of sieved clinoptilolite was also determined by Nano SORD (Toseye Hesgarsazan Asia Co., Iran). A BET (Brunauer, Emmett, Teller) isotherm was used to measure specific surface of sieved clinoptilolite. Sieved clinoptilolite was firstly degassed at 350°C for 3 hours and then specific surface was determined at 298.00 K (24.85°C) by nitrogen gas as adsorbate.

Calibration curve of paraquat dichloride in distilled water (DW) was constructed as follows: different concentrations (working solutions) of paraquat dichloride (0 (blank), 2, 5, 10, 15, and 20 $\mu$g/mL) in DW were prepared from a 1000 $\mu$g/mL stock solution of paraquat dichloride in DW. To one mL of these solutions, one mL of dithionite reagent (a freshly prepared 0.1% (w/v) solution of sodium dithionite in 1 M sodium hydroxide) was separately added and their ultraviolet (UV) absorbances at 394.5 nm were read versus blank. UV absorbances were then depicted versus respective concentrations. This experiment was repeated 3 times and the best fitted curve was passed through data points. This calibration curve was used to determine the concentration of extracted paraquat from aqueous solution by clinoptilolite and Amberlite CG-50 I.

Calibration curve of paraquat dichloride in human blood was constructed as follows: 6 mL fresh heparinized human blood was divided into 1 mL aliquots. Appropriate amounts of a 1000 $\mu$g/mL solution of paraquat dichloride in DW were added to them to produce 0 (blank), 2, 5, 10, 15, and 20 $\mu$g/mL of paraquat dichloride, respectively. To one mL of each sample, one mL of methanol was added to precipitate protein content of blood. Samples were then centrifuged at 5000 rpm for 5 min. To one mL of each supernatant, one mL of dithionite reagent (a freshly prepared 0.1% (w/v) solution of sodium dithionite in 1 M sodium hydroxide) was added except blank tube to which one mL of 1 M sodium hydroxide was added. Supernatant of the sample containing 2 $\mu$g/mL of paraquat dichloride was spectrophotometrically scanned between 400 and 800 nm. The UV absorbance maximum at 394.5 nm was used for quantitative measurements. UV absorbances were depicted versus respective concentrations. This experiment was repeated 3 times and the best fitted curve was passed through data points. This calibration curve was used to determine the concentration of extracted paraquat from blood by clinoptilolite and Amberlite CG-50 I. It was also used for determination of extraction recovery of paraquat from blood by division of absorbances of respective concentrations in this calibration curve into those of aqueous solutions. The results were then multiplied by 2 (dilution factor) and mean and standard deviation of them were calculated.

Extraction of paraquat from aqueous solution by Amberlite CG-50 I was done as follows: one gram of Amberlite CG-50 I was washed three times with DW. 5 mL of DW containing 20 $\mu$g paraquat dichloride was added to washed Amberlite, vortexed for 15 min, and centrifuged at 5000 rpm for 5 min. To one mL of supernatant, one mL of dithionite reagent was added and UV absorbance at 394.5 nm was recorded versus dithionite reagent as blank. Amount of adsorbed paraquat by Amberlite particles was determined by the interpolation of UV absorption of supernatant at 394.5 nm over paraquat tabulated concentrations on respective calibration curve followed by multiplication of resulting concentration by respective volume of supernatant and subtraction from original paraquat amount (20 $\mu$g). For extraction (desorption) of adsorbed paraquat, supernatant was discarded and one mL of saturated sodium chloride solution in DW was added to the precipitate followed by 15 min vortex and later centrifugation at 5000 rpm for 5 min. To one mL of supernatant, one mL of dithionite reagent was added and UV absorbance at 394.5 nm was recorded versus dithionite reagent as blank. Amount of extracted (desorbed) paraquat from Amberlite particles was determined by interpolation of UV absorption of supernatant at 394.5 nm over paraquat tabulated concentrations on respective calibration curve followed by multiplication of resulting concentration by respective volume of supernatant. This experiment was repeated 5 times. Recovery of extraction was calculated by division of extracted paraquat value into initial paraquat amount (20 $\mu$g). Limit of detection (LOD), considering signal-to-noise ($S/N$) ratio of 3, limit of quantification (LOQ), regarding $S/N$ of 10, and repeatability (within-laboratory error, WLE) of paraquat extraction were also calculated as previously described [18].

Extraction of paraquat from aqueous solution by clinoptilolite was exactly done as for Amberlite CG-50 I.

Extraction of paraquat from blood by Amberlite CG-50 I was done as follows: one gram of Amberlite CG-50 I was separately added to two test tubes labeled as test and blank. Content of each tube was washed three times with DW followed by centrifugation at 5000 rpm for 5 min; supernatants were discarded. 5 mL of fresh heparinized human blood, containing 20 $\mu$g paraquat, was separately added to two test tubes labeled as test and blank. 5 mL methanol was separately added to each tube and mixed gently followed by centrifugation at 5000 rpm for 5 min.

Supernatant of each tube was added to respective tube containing washed Amberlite followed by 15 min vortex and later by centrifugation at 5000 rpm for 5 min. 1 mL dithionite reagent was added to 1 mL of supernatant of test tube while to 1 mL of supernatant of blank tube 1 mL of 1 M sodium hydroxide solution in DW was added. UV absorbance of test solution was read versus blank solution at 394.5 nm. Amount of adsorbed paraquat by Amberlite particles was determined by interpolation of UV absorption of supernatant at 394.5 nm over paraquat tabulated concentrations on respective calibration curve followed by multiplication of resulting concentration by respective volume of supernatant and subtraction from original paraquat amount (20 $\mu$g). For extraction (desorption) of adsorbed paraquat, supernatant was discarded and 1 mL of saturated sodium chloride solution in DW was added to precipitate of each tube followed by 15 min vortex and later centrifugation at 5000 rpm for 5 min. 1 mL dithionite reagent was added to 1 mL of supernatant of test tube while to 1 mL of supernatant of blank tube was added 1 mL of 1 M sodium hydroxide solution in DW. UV absorbance of test solution was read versus blank solution at 394.5 nm. Amount of extracted (desorbed) paraquat from Amberlite particles was determined by interpolation of UV absorption of supernatant at 394.5 nm over paraquat tabulated concentrations on respective calibration curve followed by multiplication of resulting concentration by respective volume of supernatant. This experiment was repeated 5 times. Recovery of extraction was calculated by division of extracted paraquat value into initial paraquat amount (20 $\mu$g) followed by multiplication of resulting value by extraction recovery of paraquat from blood by methanol. LOD, considering signal-to-noise ($S/N$) ratio of 3, LOQ, regarding $S/N$ of 10, and WLE of paraquat extraction were also calculated as previously described [18].

Extraction of paraquat from blood by clinoptilolite was exactly done as for Amberlite CG-50 I.

Difference between percentages of extraction recovery of paraquat from aqueous solutions by Amberlite and clinoptilolite was statistically analyzed by Student $t$-test. Also, differences of percentages of extraction recovery of paraquat from blood by methanol, Amberlite, and clinoptilolite from 100 (maximum extraction recovery) were statistically analyzed by separate one-sample $t$-tests. One-way analysis of variance (ANOVA) with Scheffe post hoc was used to test statistical differences between extraction recovery values of paraquat from blood by methanol, Amberlite, and clinoptilolite. The statistical analyses were done on SPSS 19 statistical package. $P$ values less than 0.05 were statistically considered significant.

## 3. Results

Particle size (11–40 $\mu$m) and zeta potential (−27.9 mV) of sieved clinoptilolite were shown in Figures 1 and 2, respectively.

BET of sieved clinoptilolite was 33.79 $m^2/g$. FE-SEM photomicrograph of sieved clinoptilolite was shown in Figure 3. Sieved clinoptilolite shows some degrees of crystallinity. In fact, it shows a semicrystalline morphology.

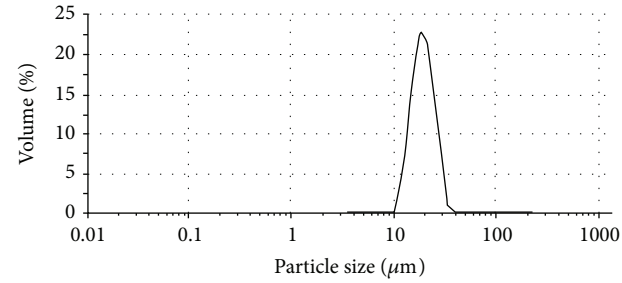


Figure 1: Particle size distribution of sieved clinoptilolite.

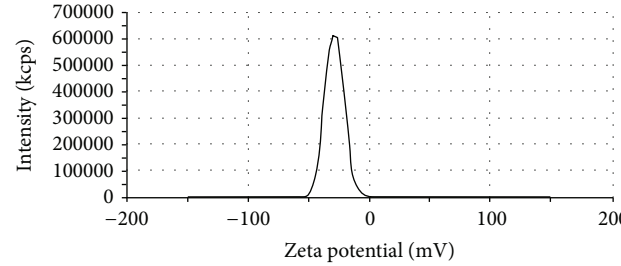


Figure 2: Zeta potential curve of sieved clinoptilolite.

UV spectrum of a 10 $\mu$g/mL aqueous solution of paraquat was shown in Figure 4.

UV-visible spectrum of this solution when reacted with dithionite reagent in a 1 : 1 ratio was shown in Figure 5.

UV-visible spectrum of a methanolic extract of blood containing 2 $\mu$g/mL paraquat when reacted with dithionite reagent was shown in Figure 6.

Paraquat calibration curves in aqueous solution and blood when solutions were reacted with dithionite reagent were shown in Figures 7 and 8, respectively.

Recovery of extraction, LOD, LOQ, and WLE of paraquat extraction by methanol, Amberlite, and clinoptilolite from different matrices were shown in Table 1.

As it is shown in Table 1, no statistical difference ($P$ value = 0.994) was seen between percentages of recovery of paraquat extraction by Amberlite and clinoptilolite from aqueous solutions. Recovery of paraquat extraction from blood by methanol ($P$ value = 0.007), Amberlite ($P$ value = 0.012), and clinoptilolite ($P$ value = 0.011) was significantly lower than maximum extraction recovery of 100. Extraction recovery of paraquat from blood by Amberlite ($P$ value = 0.700) and clinoptilolite ($P$ value = 0.323) was not statistically different from that by methanol. No statistical difference ($P$ value = 0.745) was also seen between percentages of recovery of paraquat extraction by Amberlite and clinoptilolite from blood samples.

## 4. Discussion

High extraction recovery of paraquat from blood, regardless of the analytical method used, has been a concern in analytical toxicology. Although qualitative as well as quantitative determination of paraquat in urine by dithionite reagent has been routinely used in hospital toxicology for many years

Table 1: Recovery, limit of detection, limit of quantification, and within-laboratory error of paraquat extraction by Amberlite and clinoptilolite from different matrices.

| Experiment number | Experimental group | % recovery (mean ± sd) | LOD ($\mu$g/mL) | LOQ ($\mu$g/mL) | Repeatability (%RSD) |
|---|---|---|---|---|---|
| 1 | Extraction of paraquat from blood by methanol | 85.7 ± 2.1* | 0.91 | 3.03 | 2.4 |
| 2 | Extraction of paraquat from aqueous solution by Amberlite | 98.3 ± 2.7 | 0.34 | 1.13 | 3.5 |
| 3 | Extraction of paraquat from aqueous solution by clinoptilolite | 97.2 ± 3.1 | 0.43 | 1.43 | 4.3 |
| 4 | Extraction of paraquat from blood by Amberlite | 83.6 ± 3.2* | 0.49 | 1.63 | 6.3 |
| 5 | Extraction of paraquat from blood by clinoptilolite | 81.7 ± 3.4* | 0.58 | 1.93 | 7.1 |

LOD: limit of detection; LOQ: limit of quantification; WRE: within-laboratory error; RSD: relative standard deviation. *Significantly ($P$ value < 0.05) lower than maximum extraction recovery of 100.

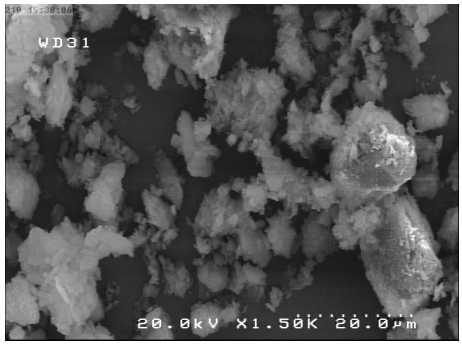


Figure 3: FE-SEM photomicrograph of sieved clinoptilolite.

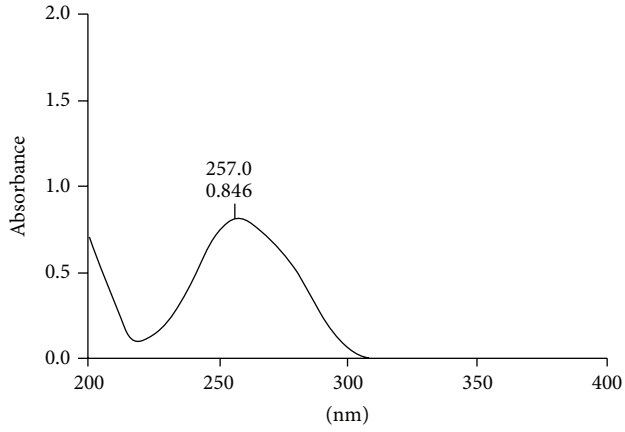


Figure 4: UV spectrum of a 10 $\mu$g/mL aqueous solution of paraquat.

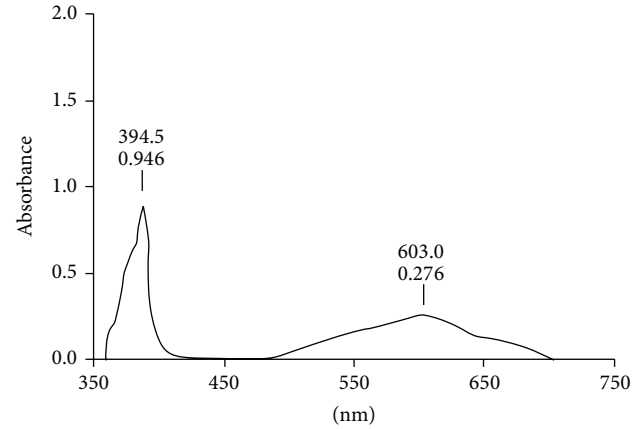


Figure 5: UV-visible spectrum of a 10 $\mu$g/mL aqueous solution of paraquat when reacted in a 1 : 1 ratio with dithionite reagent.

[3], due to a relationship between paraquat blood level and severity of its toxicity, quantitative determination of paraquat in blood has still kept its importance in clinic [19–21].

Liquid-liquid extraction (LLE) methods have been proposed for extraction of paraquat from urine and blood, but they lack high recovery due to high polarity and water solubility of paraquat [4, 5]. Solid-phase extraction (SPE) is gradually replacing the traditional LLE method in the extraction of drugs and poisons from biological and nonbiological materials. It can be used for three important purposes in up-to-date analyses, including concentrating of the analyte, removing interfering substances, and changing the matrix of the analyte as needed for subsequent analyses. SPE has several advantages over LLE such as low solvent consumption, enormous saving of time, increased extraction efficiency, decreased evaporation volumes, higher selectivity, cleaner extracts, greater reproducibility, avoidance of emulsion formation, and easier automation [22]. SPE has been successfully used for the extraction of paraquat from biological materials [2, 23, 24].

It has been shown that clinoptilolite has a cation-exchange capacity of 1.4–1.6 mEq/g [25]. So, it is considered a weak cation-exchange substance. Zeta potential is a measure of particle surface charge. A negative zeta potential indicates a negative surface charge of particles. The measured zeta potential of sieved clinoptilolite (−27.9 mV) in DW showed a slight cation-exchange property of clinoptilolite. Amberlite CG-50 I, a well-known weak cation-exchanger, has a cation-exchange capacity of 10 mEq/g. Thus, it is estimated that each

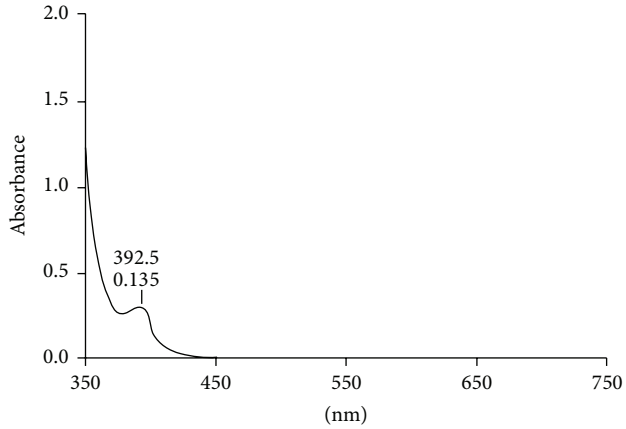


Figure 6: UV-visible spectrum of a methanolic extract of blood containing 2 $\mu$g/mL paraquat, when reacted in a 1:1 ratio with dithionite reagent.

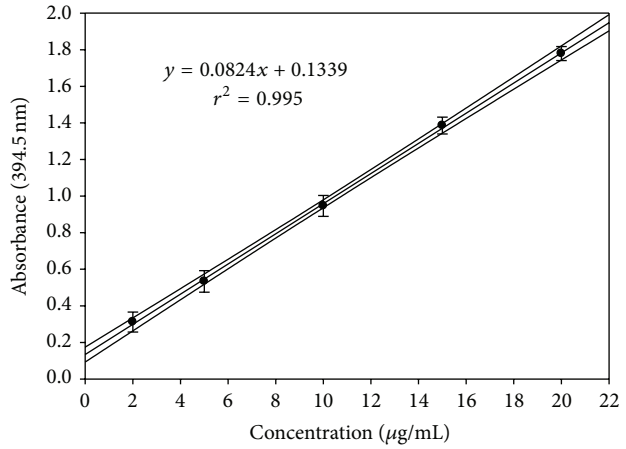


Figure 7: Paraquat calibration curve in aqueous solution when reacted with dithionite reagent.

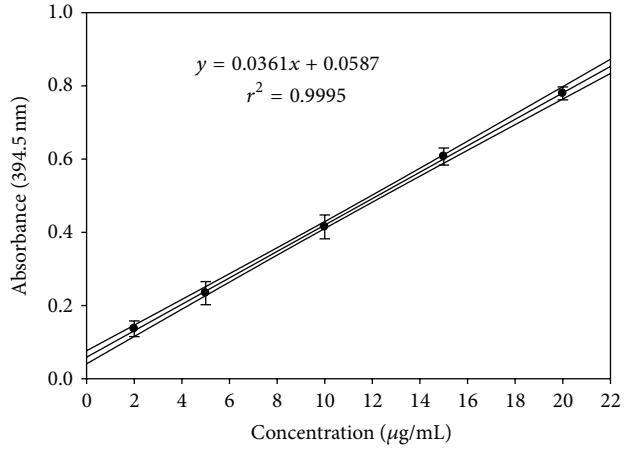


Figure 8: Paraquat calibration curve in blood when reacted with dithionite reagent.

gram of Amberlite CG-50 I and clinoptilolite can extract about 931.5 mg and 130.4–149.0 mg of paraquat, respectively. Paraquat blood concentrations greater than about 2 mg/L and 0.1 mg/L at 4 h and 24 h postingestion, respectively, are lethal in most subjects. Thus, it seems that both Amberlite CG-50 I and clinoptilolite are good extractors of paraquat from blood.

The present work shows no statistical difference between percentages of extraction recovery of paraquat by Amberlite and clinoptilolite from blood samples. On the other hand, Amberlite has been used for extraction of paraquat [26]. Thus, clinoptilolite as a high available and low cost natural substance with high specific surface area is proposed as an alternative for the extraction of paraquat from blood. Moreover, the present work shows that paraquat extraction from blood by methanol has a high recovery rate (85.7±2.1%). Thus, extraction of paraquat from blood by methanol as well as further spectrophotometric determination by dithionite reagent is proposed for routine quantitative determination of paraquat in blood. If lower LOD is needed, Amberlite or clinoptilolite is proposed for this purpose.

## 5. Conclusion

The obtained results show that natural clinoptilolite should be used effectively for the extraction of paraquat from water solution and blood. The high extraction efficiency of paraquat from water solution and blood (98% and 82%, resp.) by availability and low cost clinoptilolite is close to the extraction efficiency obtained for Amberlite (98% and 82%, resp.). It seems that clinoptilolite could also be useful for gastrointestinal decontamination of paraquat.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Acknowledgment

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