

## RESEARCH ARTICLE

# Analysis of multiple trace elements in plasma of patients with acute diquat and paraquat poisoning using inductively coupled plasma mass spectrometry

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Diquat and paraquat are widely applied in agricultural production as herbicides. Accidental ingestion of diquat or paraquat can cause acute poisoning or even death. The poisoning mechanism may be related to the abnormal levels of trace elements such as zinc and cadmium. Elementomics can be used to identify the dynamic response of elements in the body to endogenous and exogenous stimuli, thus revealing the close relationship between the imbalance of element homeostasis and human pathological state. This study aimed to establish a qualitative and quantitative analysis method for serum trace elements in patients poisoned by paraquat or diquat using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technology to explore the relationship between serum trace elements or element ratios and poisoning, construct a characteristic spectrum of trace elements for poisoning, identify potential elemental markers reflecting disease characteristics, and provide a elementomics approach to study the patients poisoned by pesticide residues. A case-control study design was employed for selecting cases and controls matched by age and gender. A characteristic trace element spectrum for the poisoned patients was constructed, and potential trace element markers were identified through pattern recognition and multivariate statistical analysis. A total of 68 elements were identified in the characteristic trace element spectrum for paraquat and diquat poisoning. Through principal component analysis and partial least squares discriminant analysis, nine elements with significant differences were identified including Li, Mg, Ca, Zn, V, Cr, Se, Cd, and Sb. The predictive ability of these elements for herbicide poisoning was investigated by receiver operating characteristic (ROC) curve analysis. Cr, Se, and Sb were identified as potential element markers of paraquat and diquat poisoning. The identification of related element markers provided valuable insights for the clinical diagnosis and treatment of patients with herbicide poisoning.

**Keywords:** paraquat; diquat; poisoning; human plasma; inductively coupled plasma mass spectrometry; trace elementomics.

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## Introduction

Paraquat (PQ) (1,1'-dimethyl-4-4'-bipyridinium dichloride) and diquat (DQ) (1,1'-ethylene-2,2'-

dipyridylum dibromide) having similar chemical structures are the essential members of the broad-spectrum bipyridylum family. They are widely applied in agriculture as herbicides, which

are known to cause acute poisoning and even death [1]. As people who attempt suicide *via* paraquat ingestion have an extremely high mortality rate, PQ has already been banned in China. However, due to its well weeding effect, it is still widely used in rural areas. PQ results in multiple system organ damage, especially lung injury, which includes early acute pulmonary edema, pulmonary congestion, acute respiratory distress syndrome (ARDS), and progressive pulmonary fibrosis in the later stages [2, 3]. The improper application of PQ has resulted in more cases of acute PQ poisoning worldwide, which has become a serious public health event. In the past few years, with the banning of PQ in some countries, the use of DQ was on the increase [4]. Although DQ is relatively safer than PQ, it is still blamed for causing serious harm to human health. Several case reports showed that acute high-dose DQ exposure usually affected multiple organs including lung, liver, brain, and especially the kidney [5-7]. Common treatment under standard guidelines for PQ and DQ poisoning include gastric lavage, blood purification, and administration of antioxidants or glucocorticoids to reduce the absorption of toxic substances and accelerate their elimination. However, due to the complexity of clinical symptoms and insufficient understanding of the mechanism of poisoning, the overall efficacy is limited. The absorption, transportation, distribution, and excretion of trace elements in human body are closely related to human health and play an irreplaceable role in physical health, though their content does not exceed 0.01% of the body weight [8]. In recent years, studies have reported that paraquat and diquat poisoning may be related to abnormal levels of trace elements such as zinc (Zn) and cadmium (Cd) [9-11]. Most studies have focused on revealing the performance of single or several candidate elements in herbicide poisoning. However, people in real life are exposed simultaneously to a broader list of elements beyond these candidates. It is necessary to assess the mixed effects of multiple elements and investigate a broader panel of trace element exposures on patients with herbicide poisoning.

As a branch of science, elementomics developed after genomics, transcriptomics, proteomics, and metabolomics, which concerns such elementome and its quantitative dynamic responses to both endogenous and exogenous stimuli to reveal element biofunctions [12]. In 2009, Zhao *et al.* first proposed the concept of serum trace elementomics and applied it to the analysis of differential elements related to clinical osteoarthritis to explore the diagnosis and related pathological mechanism of osteoarthritis and acute gouty arthritis at the overall trace element level [13]. Qin also reviewed the application research results of trace elementomics in human hair, blood, tissues, traditional Chinese medicine, genes, and proteins [12]. Inductively coupled plasma mass spectrometry (ICP-MS) is an excellent technology due to its high sensitivity, wide dynamic range, suitability for simultaneous analysis of multiple elements in multiple biological matrices, and potentials for high-throughput measurements [14-17]. Researchers detected 26 trace elements in the hair of 1,967 autistic children aged 0 to 15 years by ICP-MS and found that the lack of trace elements and excessive toxic elements in infants might play a major role in the pathogenesis of autism [15, 16]. Sargurunathan *et al.* applied ICP-MS/MS technology to analyze the presence of trace elements in urine samples of patients with urinary tract infection and healthy controls and proved, for the first time, that Mo, Sb, and V could be used as potential element markers in urine of patients with urinary tract infection [17]. However, there is few studies on the association of serum trace elementomics of patients with paraquat and diquat poisoning in China.

In this study, a qualitative and quantitative bio-analytical method of trace elements in patient's serum with paraquat and diquat poisoning was established based on ICP-MS technology. The method in terms of quantification linearity, limit of detection, precision, accuracy, and applicability were comprehensively validated. The proposed method was successfully quantified multiple elements levels in patient's plasma. The trace element characteristic

spectrum of paraquat and diquat poisoning were constructed, and the potential trace element markers were found. The results of this study would serve as a valuable reference for the detection and quantification of herbicides in clinical toxicology samples. Meanwhile, the proposed method provided clues for clarifying the relationship between herbicide poisoning and trace elements, and the basis for clinical diagnosis and treatment methods.

## Materials and methods

### Blood sample collection

The plasma samples were collected from 41 acute PQ and 65 acute DQ poisoning patients aged 3 - 88 years old with an average age of 33.1 years old. All patients were admitted to West China Hospital and West China Fourth Hospital (Chengdu, Sichuan, China) from January 2021 to April 2022. Control samples were obtained from a cohort of 65 healthy volunteers matched by gender and age (difference  $\leq 3$  years) with no previous exposure to toxicants through The Medical Examination Center (Chengdu, Sichuan, China). All procedures of this research were approved by the Medical Ethics Committee of West China Hospital (Approval No. 2022-1832) and West China Fourth Hospital (Approval No. HXSJ-EC-2022103) and were conducted complied with the Declaration of Helsinki. All participants received and signed informed consent prior to this study. 4.0 mL of peripheral blood samples were collected from poisoned patients within 30 min of hospital admission. Blood samples from healthy individuals were obtained during physical examinations. All samples were collected in EDTA anticoagulant tubes and then centrifuged at 3,000 rpm for 10 min to extract plasma. The plasma was immediately transferred to a graduated centrifuge tube and stored at  $-80^{\circ}\text{C}$ . The frozen plasma samples were thawed at  $4^{\circ}\text{C}$  and diluted 50 times using a diluent (1% nitric acid, 0.5% TBAOH, 0.3% Triton-100, 0.5% EDTA) for ICP-MS analysis.

### Establishment of the qualitative method

A mixed standard solution containing 68 elements was prepared to validate the precision, repeatability, and stability of the qualitative method. The average value (AVE), standard deviation (SD), and relative standard deviation (RSD) were calculated according to the response values (CPS) of each element in the six consecutive determinations to investigate the repeatability of the method. The mixed solution was measured at 0, 2, 4, 8, 12, and 24 h, respectively for evaluating the stability of the method. Six identical solutions of one serum sample were tested and determined, respectively.

### Establishment of the quantitative method

The standard stock solution for this measurement included 10 mg/L mixed calcium and magnesium solution, lithium and zinc solution, 100  $\mu\text{g/L}$  and 1  $\mu\text{g/L}$  mixed vanadium, cadmium, chromium, selenium, and antimony solution. 10  $\mu\text{g/L}$  scandium was selected as the internal standard for Mg, Ca, V, and Cr, while 10  $\mu\text{g/L}$  indium was the internal standard for Li, Zn, Se, Cd, and Sb according to the mass of nine elements. The internal standard working solution was prepared for six concentrations (Table 1).

### ICP-MS analysis

The plasma trace elements in DQ and PQ poisoned patients were measured using NexION<sup>®</sup> 2000G ICP-MS (PerkinElmer, Waltham, MA, USA). The instrument was tuned using a specific tuning solution that included NexION setup solution, 1%  $\text{HNO}_3$ , 1  $\mu\text{g/L}$  of Be, Ce, Fe, In, Li, Mg, Pb, and U following the STD Performance Check method with the analyzer pressure at  $1.09 \times 10^{-7}$  mbar, RF power of 1,450 W, spray chamber temperature at  $2^{\circ}\text{C}$ , nebulizer gas flow of 0.90 L/min, dilution gas flow of 0.32 L/min, auxiliary gas flow of 0.8 L/min, cool gas flow of 15 L/min, and cell gas flow of 4 mL/min. Qualitative and quantitative modes were utilized for analysis. The serum samples of PQ, DQ, and control groups were analyzed by qualitative method for screening the differential elements followed by quantitative analysis of these elements.

**Table 1.** Concentration of each analyte in standard series ( $\mu\text{g/L}$ ).

No. of standard series	Analytes		
	Li, Zn	Ca, Mg	V, Cr, Se, Cd, Sb
1	1	100	0.01
2	5	500	0.1
3	10	1,000	1
4	50	2,000	5
5	100	5,000	10
6	200	100,000	20

**Table 2.** Linearity, LOD, and LOQ of quantitative method for the determination of nine analytes (n = 5).

Analytes	Concentration range ( $\mu\text{g/L}$ )	Calibration curve	Linearity ( $R^2$ )	LOD ( $\mu\text{g/L}$ )	LOQ ( $\mu\text{g/L}$ )
Li	1-200	$y = 419.02x - 801.72$	0.999	0.0120	0.0400
Mg	0.1-10	$y = 322156x - 20265$	0.9996	0.2133	0.7110
Ca	100-10,000	$y = 1672.7x - 239.19$	0.9992	0.5000	1.6667
Zn	1-200	$y = 1216.2x - 1873.1$	0.9991	0.1900	0.6333
V	0.01-20	$y = 4744x - 981.05$	0.9985	0.0050	0.0167
Cr	0.01-20	$y = 6377.8x - 899.75$	0.9986	0.0045	0.0150
Se	0.01-20	$y = 51.92x - 4.45$	0.9993	0.0013	0.0043
Cd	0.01-20	$y = 2346.8x - 231.97$	0.9993	0.0018	0.0060
Sb	0.01-20	$y = 4830.9x - 478.38$	0.9993	0.0038	0.0127

### Statistical analysis

SPSS 22.0 software (IBM, Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) were employed in this study. The data were represented as mean  $\pm$  standard deviation. Student's t-test was applied to conduct a comparison in two groups. One-way ANOVA was used for multiple comparisons of differences between treatments. *P* value less than 0.05 was considered a significant difference.

## Results

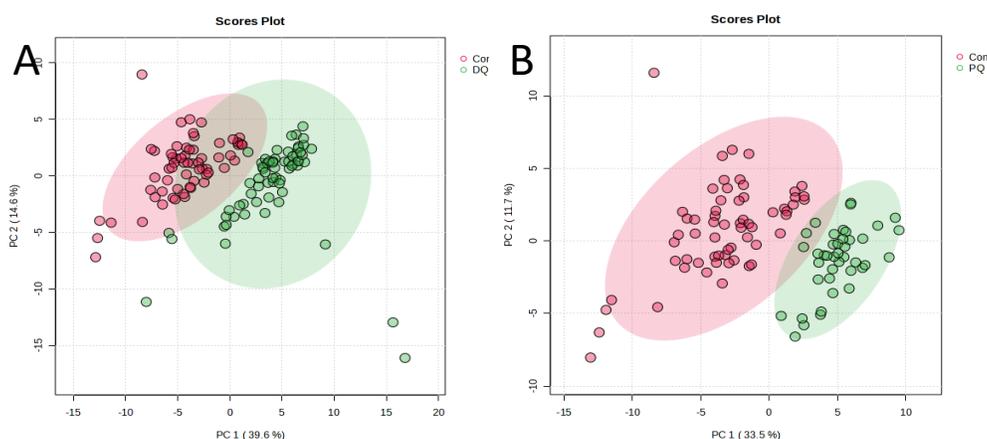
### Validation of proposed method

The standard curve was established according to the concentration and response value of each element in the standard series solution. The results confirmed that all calibration curves showed good correlation coefficients ( $R^2 > 0.999$ ) (Table 2). The limit of detection (LOD) and limit of quantification (LOQ) were determined to validate the feasibility of the proposed method for the determination of standard solution by repeating

the determination of blank diluent solution for three and ten times, respectively, and calculating the SDs as the LOD and LOQ of quantitative method, respectively. The AVE, SD, and RSD values were calculated according to the concentrations of each element to investigate the precision of the method by six-time determination of the mixed standard solution. Six identical solutions of one serum sample were tested to investigate the repeatability of the method and were measured at 0, 2, 4, 8, 12, and 24 hours, respectively, for investigating the stability of the method (Table 3). The accuracy of the method was investigated on the recovery of each element, which was calculated according to the results of twice determinations. The results showed that the extraction recoveries ranged from 86.77 % and 109.49 % for nine elements and the precision of quantitative method ranged from 3.54 % and 18.66 %. All results suggested that the developed ICP-MS method could meet the requirements of trace element quantification.

**Table 3.** Intra- and Inter-day precision and accuracy for analytes in samples.

Analytes	Intra-day (n = 6)		Inter-day (n = 6, 24 hours)		Accuracy (n = 6)		Recovery
	Mean $\pm$ SD	RSD	Mean $\pm$ SD	RSD	Mean $\pm$ SD	RSD	
Li	99.70 $\pm$ 4.50	4.51%	100.73 $\pm$ 5.21	5.20%	10,710.90 $\pm$ 994.57	9.30%	93.57%
Mg	2.03 $\pm$ 0.08	4.00%	2.07 $\pm$ 0.05	2.20%	22.93 $\pm$ 1.57	6.80%	89.41%
Ca	2.05 $\pm$ 0.09	4.21%	2.09 $\pm$ 0.06	3.00%	113.18 $\pm$ 8.99	7.90%	99.23%
V	0.66 $\pm$ 0.03	4.46%	0.67 $\pm$ 0.02	2.90%	15.60 $\pm$ 3.99	25.60%	109.49%
Cr	0.41 $\pm$ 0.06	15.31%	0.39 $\pm$ 0.06	14.10%	10.37 $\pm$ 0.39	3.70%	103.50%
Zn	33.66 $\pm$ 1.19	3.54%	33.95 $\pm$ 0.75	2.20%	1,092.24 $\pm$ 79.36	7.30%	94.68%
Se	2.19 $\pm$ 0.41	18.66%	2.13 $\pm$ 0.27	12.40%	197.33 $\pm$ 38.69	19.60%	89.11%
Cd	0.77 $\pm$ 0.06	7.55%	0.79 $\pm$ 0.06	7.30%	6.86 $\pm$ 1.18	17.20%	106.58%
Sb	0.44 $\pm$ 0.04	10.14%	0.45 $\pm$ 0.05	10.60%	4.89 $\pm$ 0.68	14.00%	86.77%

**Figure 1.** PCA scores of Con, DQ (A), and PQ (B) group.

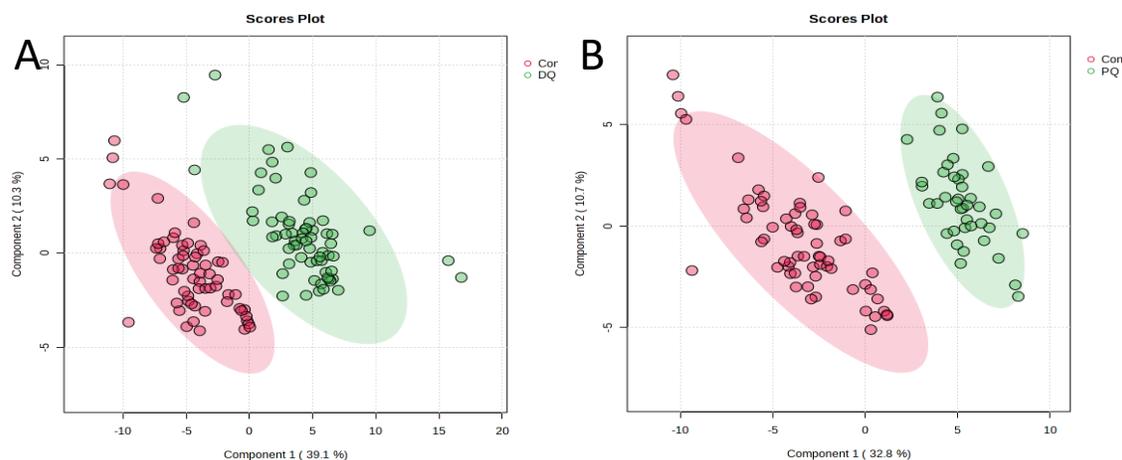
### Data analysis of qualitative method

The qualitative analysis results showed that there were significant differences in the present levels of trace elements among the three groups of serum samples. The processed data were further processed to conduct the principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) to reveal whether DQ and PQ had an obvious influence on human plasma trace element. The results of the PCA analysis displayed that the points in the control, DQ, and PQ groups were separated from each other (Figure 1). To further identify the component separation between poisoned patients and healthy volunteers, the PLS-DA model was applied in the follow-up study. The plasma samples from the control and poisoned groups could be completely separated,

suggesting that the plasma trace elements were significantly influenced by DQ and PQ (Figure 2).

### Selection of differential elements

The variable influence on projection (VIP) values acquired from the PLS-DA model between the control and poisoned groups and the *P* values determined by the Mann-Whitney U test were used to screen the potential candidates responsible for DQ and PQ poisoning. The metabolites with VIP > 1.0 and *P* < 0.01 were initially selected as the differential elements. There were 35 types of elements with VIP value greater than 1. Among them, Mg, Ca, Zn, V, Cr, Se, Cd, and Sb were essential trace elements for human body, which was worth further analyzing the different presentation levels in poisoning patients and healthy volunteer. The contents of



**Figure 2.** PLS-DA scores of Con, DQ (A), and PQ (B) group.

**Table 4.** U test results of response values of nine elements in DQ, PQ, and control groups.

Analytes	Mean $\pm$ SD			P value	
	Con	DQ	PQ	DQ	PQ
Li	7,608.35 $\pm$ 972.64	1,779,426.64 $\pm$ 183,205.55	3,233,893.93 $\pm$ 2,117,765.86	< 0.01	< 0.01
Mg	23,654,710.10 $\pm$ 3,891,140.00	19,954,500.16 $\pm$ 3,799,836.69	17,821,084.10 $\pm$ 4,341,883.09	< 0.01	< 0.01
Ca	253,091,759.50 $\pm$ 40,898,284.10	221,730,689.90 $\pm$ 33,886,243.30	200,867,419.17 $\pm$ 38,126,378.17	< 0.01	< 0.01
V	104,937.31 $\pm$ 18,177.16	93,000.43 $\pm$ 15,867.59	112,687.86 $\pm$ 22,301.34	< 0.01	< 0.01
Cr	788,556.47 $\pm$ 136,922.76	675,107.75 $\pm$ 89,737.56	624,766.70 $\pm$ 134,246.50	< 0.01	< 0.01
Zn	872,775.10 $\pm$ 29,102.74	744,997.27 $\pm$ 180,732.86	670,699.06 $\pm$ 292,728.73	< 0.01	< 0.01
Se	37,241 $\pm$ 9,753.62	57,669 $\pm$ 3,138.65	25,652.80 $\pm$ 815.11	< 0.01	< 0.01
Cd	797.03 $\pm$ 373.05	116,104.72 $\pm$ 32,836.57	1,355.20 $\pm$ 2,574.18	< 0.01	< 0.01
Sb	17,578.48 $\pm$ 6,523.00	13,848.47 $\pm$ 2,511.54	13,510.31 $\pm$ 4,465.36	< 0.01	< 0.01

lanthanide elements such as Pr, Sm, Eu, Ga, La, Dy, Ho, Er, Tm, Yb and actinide element uranium (U) were very low in human body. In addition, the physiological functions of most rare elements including Au, Bi, Re, Th, Re, Ir, Pt with micro-amount in the human body had not been clarified yet. Nine elements including Li, Mg, Ca, Zn, V, Cr, Se, Cd, and Sb were selected for Mann Whitney U-test to further verify whether the presentations of differential elements were statistically significant (Table 4).

#### Quantitative analysis of serum samples

Quantitative analysis of the nine selected elements across the three groups showed statistically significant differences in their concentrations with PQ group showing higher levels of V, Cd, Sb and lower levels of Mg, Ca, Cr, Zn, Se compared to the control group ( $P < 0.05$ ). Similarly, the DQ group exhibited higher levels of

Se, Cd and significantly lower levels of Mg, Ca, V, Cr, Zn, Sb ( $P < 0.05$ ) (Table 5).

#### ROC curve analysis for quantitative data

Receiver operating characteristic (ROC) analysis was conducted, and the areas under the ROC curve (AUC) were used to assess the diagnostic power of the nine differential elements. The results revealed that Cr, Se, Sb had high AUC values, suggesting their potentials as markers for distinguishing paraquat and diquat poisoning (Figure 3).

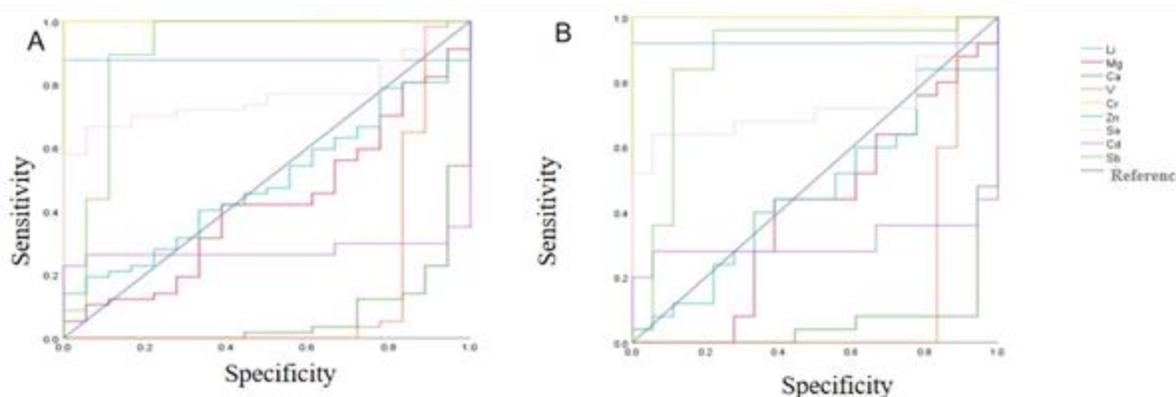
#### Discussion

Elemental fluctuations within organisms are intricately linked to their internal environments and metabolic processes. Disorders in metal metabolism, precipitated by various diseases,

**Table 5.** The amount of nine elements in DQ, PQ, and control groups detected by quantitative method.

Analytes	Mean $\pm$ SD			P value	
	Con	DQ	PQ	DQ	PQ
Li	416.94 $\pm$ 28.52	9,294.47 $\pm$ 426.22	5,661.00 $\pm$ 264.03	< 0.01	< 0.01
Mg	21.97 $\pm$ 2.57*	15.64 $\pm$ 3.60*	12.30 $\pm$ 1.51*	< 0.05	< 0.01
Ca	97.51 $\pm$ 8.67*	75.17 $\pm$ 9.66*	85.35 $\pm$ 8.72*	< 0.01	< 0.01
V	7.15 $\pm$ 1.59	10.01 $\pm$ 0.83	3.43 $\pm$ 1.05	< 0.01	< 0.01
Cr	2.06 $\pm$ 0.38	0.97 $\pm$ 0.38	1.44 $\pm$ 0.24	< 0.01	< 0.01
Zn	636.16 $\pm$ 141.91	324.99 $\pm$ 27.86	527.59 $\pm$ 26.77	< 0.01	< 0.01
Se	113.91 $\pm$ 29.22	105.83 $\pm$ 16.17	274.49 $\pm$ 28.78	< 0.01	< 0.05
Cd	21.77 $\pm$ 13.94	43.80 $\pm$ 7.09	126.77 $\pm$ 38.34	< 0.01	< 0.01
Sb	2.35 $\pm$ 1.15	2.98 $\pm$ 0.34	2.14 $\pm$ 1.85	< 0.01	< 0.05

Note: \* the unit was mg/L, otherwise  $\mu$ g/L.

**Figure 3.** ROC curve of differential elements in Con, DQ (A), and PQ (B) group.

can disrupt the elemental equilibrium in the body. The prevailing research suggested that toxicological mechanisms of paraquat and diquat poisoning predominantly involved oxidative stress, the induction of apoptosis, and the impairment of numerous enzymatic activities within the body [18-21]. Trace elements, serving as foundational components of organisms, are pivotal in maintaining systemic homeostasis through their synergistic effects. The emergence and progression of diseases within the body are intricately linked to variations in the concentration of these elements. ICP-MS was utilized in this study for qualitative and quantitative analysis of serum samples from individuals poisoned by paraquat and diquat. The levels of nine elements including Li, Mg, Ca, Zn, V, Cr, Se, Cd, and Sb were identified, while Cr, Se,

and Sb were found to have important diagnostic potential for identifying poisoning.

Notably, the study observed a reduction in serum calcium levels among the poisoned groups compared to controls, which potentially attributed to oxidative stress-induced modifications in cell membrane lipid peroxidation and permeability, suggesting a pivotal role for calcium homeostasis in the pathophysiology of lung damage caused by paraquat exposure [22]. The results aligned with Yang's findings [23], which identified abnormal extracellular calcium as a crucial precursor to PQ toxicity. The STIM1-TRPC1 complex, which played an important role in pulmonary epithelial cells, might be the key target of PQ by promoting extracellular calcium influx, increasing intracellular calcium level, and aggravating

oxidative stress. Consequently, inhibiting this PQ-induced calcium influx could mitigate the rise in calcium ions and aid in reducing abnormal ROS levels in cells. Treatments, such as activated charcoal infusion and anti-inflammatory protocols, could effectively address PQ poisoning in clinical practice. This approach underscored the significance of the glutathione and thioredoxin systems in cellular redox maintenance. Selenium played a crucial role in the activity expression of glutathione peroxidase and thioredoxin reductase, which highlighted selenium's critical role in cellular defense mechanisms [24]. Studies have shown that PQ exposure can deactivate the key antioxidant enzymes, glutathione peroxidase, and thioredoxin reductase in pulmonary microvascular endothelial cells, which led to an accumulation of hydrogen peroxide, reduction in glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, and the creation of glutathione-protein mixed disulfide bonds to significantly accelerate oxidative stress and culminate in cell apoptosis. This mechanism emphasized the toxic effects of PQ on cellular antioxidant defense, highlighting its role in the pathogenesis of PQ induced lung injury [25].

Tamura *et al.* found that intracellular  $H_2O_2$  produced by PQ was taken up through dopamine transporters, then retrogradely transported to presynaptic glutamatergic terminals and triggered the activation of TRPM2 cation channels, causing a swift influx of extracellular zinc into dopaminergic terminals, which had significant implications for cellular health and function [26]. Long term exposure to high concentrations of zinc in the substantia nigra striatum tissue could consume NADPH oxidase and glutathione, thereby activate the apoptotic pathway and accelerate the decline of dopaminergic neurons. This process significantly promoted the acceleration of age-related neurodegenerative diseases, emphasizing the key impact of zinc imbalance on neuronal health and aging [27, 28]. Neurons enduring prolonged exposure to high zinc concentrations experienced detrimental effects.

Currently, it is widely believed that the main way cadmium exerted toxic effects in mammalian cells is by inducing oxidative stress and inhibiting DNA damage repair. Cadmium can disrupt the blood-brain barrier, enter the central nervous system, cause morphological changes in the brain, and affect content of neurotransmitter and enzyme activity [29]. Therefore, the elevated concentration of cadmium in the serum of PQ and DQ poisoning patients can cause damage to the central nervous system, and result in mental disorders. In addition, elevated cadmium concentrations are associated with an increased risk of many chronic diseases including reproductive dysfunction. LV *et al.* demonstrated that cadmium had potential toxicity to ovarian granulosa cells and ovaries, which was closely related to the increased risk of female infertility [30].

This research established qualitative and quantitative methods for trace elements in the serum of paraquat and diquat poisoned patients. Differential elements between groups were identified and validated through multivariate analysis. The findings suggested that trace elementomics could effectively identify potential markers in poisoned patients, offering insights into the pathological characteristics of poisoning and providing scientific guidance for clinical diagnosis. There was a definite relationship between paraquat and diquat poisoning and various trace elements in serum. However, due to the limited sample size, the applicability of these results was restricted. Current trace elementomics focused on the changes in element content within different organisms, overlooking the distribution, function, and interaction of different valence state ions. Future research will expand sample sizes, analyze trace element levels in poisoned patients at different stages, optimize sample preprocessing to prevent changes in trace element valence states. The changes of trace elements in different forms and valence states will also be analyzed using chromatography-mass spectrometry to verify and supplement these findings.

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