Scholars Journal of Agriculture and Veterinary Sciences
OPEN ACCESS
Abbreviated Key Title: Sch J Agric Vet Sci
ISSN 2348–8883 (Print) | ISSN 2348–1854 (Online)
Journal homepage: https://saspublishers.com

Application of Herbal Medicine in Cu^{2+} Removal from Water and Comparison of its Detection Methods

Shixian Li[1], Kunpeng Zhou[1], Xinyin Xu[1], Yangyang Zhao[1], Jing Li[1*]

[1]College of Life Science and Biotechnology, Heilongjiang Bayi Agricultural University, Daqing 163319, PR China

DOI: 10.36347/sjavs.2023.v10i05.002 | **Received:** 21.04.2023 | **Accepted:** 25.05.2023 | **Published:** 30.05.2023

***Corresponding author:** Jing Li
College of Life Science and Biotechnology, Heilongjiang Bayi Agricultural University, Daqing 163319, PR China

Abstract **Original Research Article**

In order to utilize discarded herbal medicine to treat heavy metal pollution in water, various herbal medicines were selected to explore their abilities of metal ions removal. In this experiment, copper ion was used as targeted heavy metal ion and two different treatments of several herbal medicines were compared on the effect of the copper ion removal ability. At the evaluating stage, four metal ion detection methods were employed and analyzed in this herbal medicine Cu^{2+} removal application. The results showed that directly soaking herbal medicine in the copper aqueous solution was more effective and easier to operate. Iodometry method and EDTA complexometric titration with xylenol orange as indicator were accurate and time-saving in the measurement of copper removal rate. In addition, the EDTA complexometric titration is also applicable in the detection of other metal ions. This experiment provided a basis for the implementation and application of herbal medicine in the deposition of metal ions in polluted water.

Keywords: Water pollution; Herbal medicine; Heavy metal ions; EDTA complexometric titration.

INTRODUCTION

Herbal medicines contain a variety of active ingredients. Apart from their medicinal value, there are still many uses of herbal medicine to be explored [1-3]. The resource utilization of herbal medicine is attracting more and more attention.

At present, the treatment of water polluted by heavy metal ions is a hot topic in the field of environmental protection research. Using herbal medicine to deposit heavy metal ions is an important approach [4]. Chunhua Li [5] studied the application of *Radix isatidis* residue to adsorb lead in waste water. They adopted the acid-base method to modify *Radix Isatidis* residue, which effectively improved the adsorption capacity of *Radix Isatidis*. Jing Huang *et al.*, [6] used *Notoginseng* and *Ophiopogon japonicus* residue as raw materials to prepare activated carbon for the adsorption of copper and lead in waste water. Manshu Zhou group [7] prepared extract from *Moringa* seed and used the extract protein as flocculant to purify water containing metal ions, such as Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}. Xuezhen Xie *et al.*, [8] modified Bagasse by diethylenetriamine, epichlorohydrin and triethylamine to adsorb chromium ions. Shang group [9] used the Fe^{2+}/Fe^{3+} co-precipitation method to prepare magnetic biochar with *Astragalus membranaceus*residue as Cr (VI) adsorbents.

All of the results above proved important practical significance on the treatment of water polluted by heavy metal as well as the reuse of abandoned herbal medicine.

Flame atomic absorption spectrometry (FAAS) is a common accurate method for the detection of heavy metal ions in solution. However, this method has some disadvantages such as complicated pretreatment process of samples, expensive instrument and tough operation skill requirements. Titration is a more widely used method to detect metal ions. Ethylene diamine tetraacetic acid (EDTA) complexometric titration and iodometry are commonly used methods because their reagents are widely available and their operations are simple. Lixin Fan [10] determined copper content in silver separating residue from copper smelting by iodimetry. Guoxiu Fu [11] determined lead concentration in zine oxide leaching residue by EDTA titration. Our research group used iodometry to test the ability of deposition copper ions by herbal medicine [12]. Iodometry titration was proved accurate in the detection of copper ions in the herbal solution, but it can't be applied to detect other heavy metal ions.

For the purpose of developing a metal ion detection method that can be widely applied in the process of herbal medicine-metal ions deposition, this experiment conducted a comparative study on four detection methods of copper ion removal by herbal medicine in order to demonstrate and determine the feasibility, accuracy and wide applicability of the method.

1. MATERIALS AND METHODS

1.1 Materials and instruments

All the herbal medicines were from Chinese herbal medicine bases as *Sophora japonica* L and *Astragalus membranaceus* from Shandong Province, Herb *Leonurus japonicus* from Yunnan, the *Ginkgo biloba* L.from Liaoning and *Glycyrrhiza uralensis* from Gansu. All the chemical reagents were purchased from merchandise and used without further treatments.

AA-3300 atomic absorption spectrophotometer is from Shang Hai Metash instruments Co., High-speed freezing centrifuge is made by Sartorius Company, Germany. Other instruments are commonly used in laboratory, including burette, thermostat water bath, high-speed grinder, electronic balances, ultrasonic cleaner, electric thermostatic drying oven, etc.

1.2 Methods

1.2.1 Pretreatment of herbal medicine

All the herbal medicines were dried in the electric thermostatic oven at 60 ℃ until constant weight. Then the herbal medicines were pulverized by high-speed grinder and sieved through a 60-mesh sieve before use.

1.2.2 Preparation of standard solutions

Cu^{2+} standard solution: Copper sulfate pentahydrate was dissolved into distilled water to form a Cu^{2+} solution with a concentration of 3.0 mmol/L.

$Na_2S_2O_3$ standard solution: $Na_2S_2O_3·5H_2O$ (1.6 g) was added to 128 mL of newly boiled and cooled distilled water to form $Na_2S_2O_3$ solution of 50 mmol/L which was then stored in a brown reagent bottle and set in the dark place for 3-5 days. Before iodometry experiments, a certain amount of $Na_2S_2O_3$ solution of 50 mmol/L was diluted to a standard solution with concentration of 4.0 mmol/L.

EDTA standard solution: EDTA-2Na (2.69 g) was added into 1000 mL water and was heated to dissolve to prepare 8 mmol/L EDTA standard solution. Before EDTA titration experiments, a certain amount of 8 mmol/L EDTA standard solution was diluted to a standard solution with concentration of 0.4 mmol/L.

Xylenol orange indicator: Xylenol orange (0.5 g) was dissolved in 100 mL water to form the indicator solution.

Murexide indicator: Murexide (0.1 g) and sodium chloride solid (20 g) were grounded into powder form as the indicator mix.

1.2.3 Reaction method of herbal medicine and Cu^{2+}

(1) Extraction method

Appropriate amount of pretreated herbal medicine was placed in centrifuge tubes and was added distilled water at material-to-liquid ratio of 1:20. The herbal was soaked at room temperature for 6 h, treated with ultrasonic at 240 W for 30 min, and centrifuged at 3000 r/min to obtain the extract. The extract was added into 2 mL Cu^{2+} standard solution, mixed well and set for 10 min, then centrifuged at 3000 r/min at room temperature for 10 min. The supernatant was taken for the measurement of Cu^{2+} content.

(2) Soaking method

Appropriate amount of pretreated herbal medicine was added 2 mL Cu^{2+} standard solution and soaked for 2 h. The mixture was ultrasonicated for 30 min at 240 W and then was centrifuged at 3000 r/min at room temperature for 10 min. The supernatant was taken for the measurement of Cu^{2+} content.

1.2.4 Detection methods

1.2.4.1 FAAS method

Copper ion solutions were prepared in distilled water at concentrations of 0 mg/L, 0.10 mg/L, 0.25 mg/L, 0.50 mg/L, 1.00 mg/L, 2.00 mg/L, respectively. Standard curve of copper ion was established by measuring the Cu^{2+}absorbance of different copper solution by atomic absorption spectrophotometer.

Appropriate amount of *Glycyrrhiza uralensis* powder was treated with Cu^{2+} standard solution (2 mL) using soaking method. After reaction, the supernatant was taken and filtered by aqueous microporous filtration membrane to obtain test sample. Another same amount *Glycyrrhiza uralensis* powder was treated with distilled water instead of copper ion solution using soaking method. The supernatant was taken and filtered by aqueous microporous filtration membrane to obtain the calibration sample. Using atomic absorption spectrophotometer to measure the absorbance of test sample and calibration sample, the final absorption value of Cu^{2+} is the difference of the test sample and the calibration sample. The content of Cu^{2+} was calculated using the standard curve.

1.2.4.2 Titration method

Iodometry

Excess KI (0.02 g) was added to 2 mL of test solution obtained from methods above. After KI dissolved, the mixture was titrated with $Na_2S_2O_3$ standard solution. When the solution color turned into light yellow, 0.1% starch solution (0.2 mL) was added and test solution changed to dark blue color. $Na_2S_2O_3$ was then added to make the tested solution light blue;

then KSCN (0.25 g/mL, 0.2 mL) was added to the mixture and the solution turned blue. $Na_2S_2O_3$ was added again until the solution color turned into nearly white, which was regarded as the titration end point. Record the total amount of $Na_2S_2O_3$ standard solution. 2 mL of Cu^{2+} standard solution was titrated asblank test at the same time.

EDTA-Xylenol orange: 2 mL of solution to be tested was added with hexamethylenetetramine buffer solution (0.6 mL, pH 5.4) and 4 drops of xylenol orange indicator. The solution was titrated with 0.4 mmol/L EDTA standard solution until the solution changed color from purplish red to yellowish green, which was the end point of titration. The dosage of EDTA was recorded and blank test (2 mL of Cu^{2+} standard solution) was performed at the same time.

EDTA-Murexide: 2 mL of solution to be tested was added NH_3-NH_4Cl buffer solution (0.6 mL, pH = 8) and murexide indicator (0.1 g). The solution was titrated with 0.4 mol/L EDTA standard solution until the solution changed color from yellow-green to purple red, which was the end point of titration. Record the amount of EDTA. A blank test (2 mL of Cu^{2+} standard solution) was completed at the same time.

$$\text{Removal rates} = \left(1-\frac{C_2 \times V_2}{C_1 \times V_1}\right) \times 100\% \qquad (1)$$

1.2.5 Calculation of Cu^{2+} removal rate

As shown in equation (1), C_1 and C_2 represent the concentration (mol/L) of copper ion standard solution and titration reagents, respectively. V_1 and V_2 represent the volume (L) of reaction amount of Cu^{2+}standard solution and amount of titration reagent, respectively.

2. RESULTS

2.1 Comparisons of reaction methods between herbal medicine and Cu^{2+}

Five kinds of flavonoid-rich herbal medicine, including *Sophora Japonica* L, *Astragalus membranaceus*, *Leonurus japonicus*, *Ginkgo biloba* L, *Glycyrrhiza uralensis* were selected to explore the efficient reaction method between herbal medicine and Cu^{2+}. Each herbal medicine with dosage of 20 mg was treated with 2 mL of copper standard solution. Extraction and soaking methods were carried out on the herbal crumbs. The removal rate of Cu^{2+} was measured by iodometry method based on the study [12].

The reaction results are shown in Fig 1, four of the herbal medicines, *Sophora Japonica* L, *Astragalus membranaceus*, *Leonurus japonicus* and *Glycyrrhiza uralensis*, presented higher copper removal rate when treated with soaking method than extraction method.

In the extraction method, the extract contained soluble substances which could coordinate with metal ions and form precipitates. In the soaking method, in addition to the soluble active components, a variety of tissue structures in herbal medicine can enhance the deposition of metal ions, which leads to a slightly higher removal rate. *Astragalus membranaceus*has higher content of flavones and has better ion removal effect. The removal effect of *ginkgo biloba* leaves is poor, and cannot effectively remove copper ions in water via soaking method. This is because that there is a layer of wax on the surface of *Ginkgo biloba* L, which prevents the internal components from contacting with copper ions in water. In the extraction method, *Ginkgo biloba* L were fully exposed to water, and soluble substances were increased in the extract, so the deposition of copper ion occurred.

The experiment showed that both the type of medicine and treatment method have impacts on the removal effect during the interaction of metal ions and herbal medicine. For the herbal medicine easily infiltrated by water, the soaking method could achieve better results.

2.2Comparison of Cu^{2+} detection methods

After *Glycyrrhiza uralensis* reacted with copper standard solution, the color of the solution to be tested is the lightest, which has little influence on the judgment of titration color change point. Therefore, *Glycyrrhiza uralensis* was selected as the object in this experiment to explore the accuracy of different detection methods in determining the Cu^{2+} removal rate.

In the experiment, 14~30 mg of *Glycyrrhiza uralensis* powder was soaked in 2 mL of copper ion standard solution. After the reaction, the removal rate of copper ion was tested by four methods. The results were shown in Fig 2.

The FAAS method has high sensitivity, so it can get stable experimental results and good linearity.Iodometry and EDTA-Xylenol orange methods have also showed stable and linear results similar as that of FAAS method. For EDTA-Murexide method, the results were not accurate and the deviation of test results was large, which was mainly because that the color change point of indicator was easily missed which leads to a large reading error in the determination of titration ending point.

Thus, iodometry and EDTA-Xylenol orange method have stronger applicability in the titration of copper ion concentration, demonstrating reliable testing results with much simpler operation process compared to FAAS method, especially in the system of removing copper ions by herbal medicine.

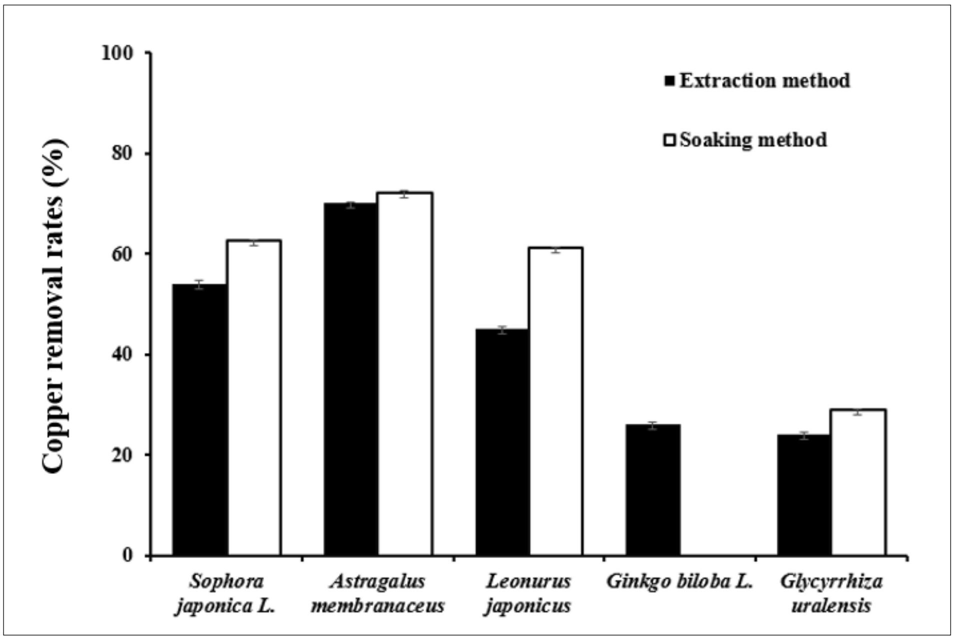

Fig-1: The effect by different ways of reaction

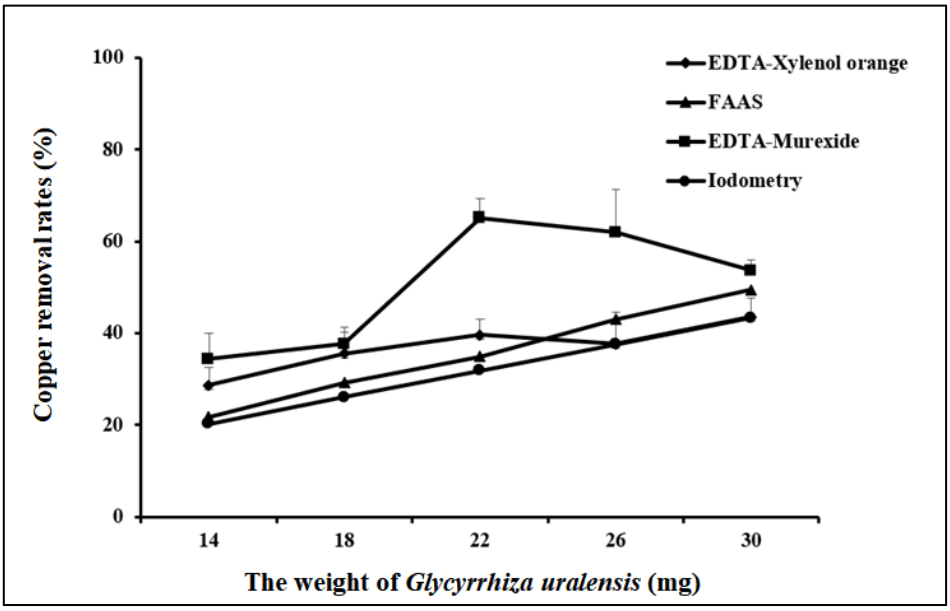

Fig-2: The comparison between four detection methods

2.3 Further comparison of iodometry and EDTA-Xylenol orange methods in determining the Cu^{2+} removal rates with other 4 herbal medicines

The application of iodometry and EDTA-Xylenol orange in determining the copper removal rates was further compared in the reaction of copper standard solution (2 mL) with 30 mg of *Sophora japonica* L, *Astragalus membranaceus*, *Leonurus japonicus* and *Glycyrrhiza uralensis*, respectively, via soaking method.

As shown in Fig 3, two titration methods showed give similar value, and the error of the titration results were small. Therefore, both of them could be used to detect the removal rates of copper ions in this herbal medicine experiment. Additionally, EDTA-Xylenol orange method has a wider range of application since it can be used in the detection other metal ions.

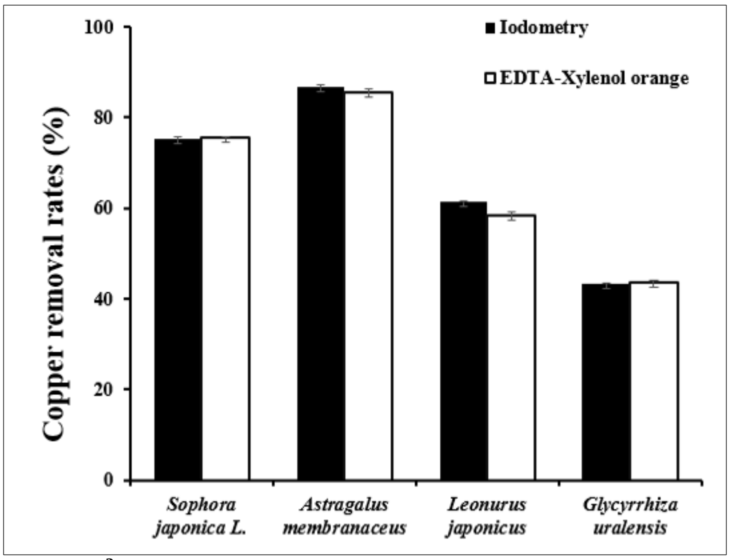

Fig-3: The removal rates of Cu^{2+} of four medicine was determined by iodometry and EDTA-Xylenol orange

3. DISCUSSION

Herbal medicine contains a variety of active ingredients, including flavonoids, polysaccharides, alkaloids, cellulose and other components. These compounds can coordinate metal ions through the functional groups of carbonyl, carboxyl, amino and hydroxyl to form complexes which are usually poorly soluble in water, adhesive to solid substances, low biologically active, and less harmful to the environment.

Herbal medicine is a good resource to remove heavy metal ions from aqueous and inert metal ions. In existing research studies, it was mainly the extract of herbal medicine that used to react with metal ions to achieve the effect of removing heavy metal ions. In order to explore a more economical and simplified way of removing heavy metal ions from water, in this study, herbal crumbs were directly put into the solution containing copper ions, soaked for 2 h and ultrasonicated for 30 min to achieve the purpose of removing copper ions. Compared with the extraction method, the results indicated that the soaking method has a better removal rates of copper ions. The water-soluble components of herbal medicine contact and deposit copper ions. The insoluble residue of herbal medicine combine with copper ions through adsorption [13], thus increasing the removal efficiency. However, this method also has some limitations. In the case where herbal medicine materials like *Ginkgo biloba L.* carrying hydrophobic characteristics, the contact between herbal medicine and aqueous solution is hindered, and the removal effect of copper ions is not ideal.

In order to apply titration method to the study of metal removal by herbal medicine, four detection methods of *Glycyrrhiza uralensis* samples were compared in details in this experiment: FAAS, iodometry, EDTA-Xylenol orange and EDTA-Murexide. FAAS is considered to be of high accuracy and is therefore used as the standard for this experiment. In previous studies, iodometry had a good effect on the detection of copper ions [12], but this method cannot detect other metal ions. EDTA can complex a variety of metal ions, promising a wider range of use, and the selection of indicators has an important impact on the judgment of experimental results. The experimental results show that EDTA-Xylenol orange and iodometry have good stability, and the judgment of the end point is clear, but EDTA-Murexide is not suitable for this application because of the major error of the end point determination. In the follow-up experiments, the detection results of EDTA-Xylenol orange and iodometry were proved accurate and stable for the detection of a variety of herbal medicine. EDTA-Xylenol orange can be adopted to detect the content of other metal ions, which is beneficial for the research team to carry out study on other metal ions removal with herbal medicine in the future.

4. CONCLUSION

It is of great practical significance to study the treatment of heavy metals in water by wasted herbal medicine. In this experiment, the removal abilities of Cu^{2+} from water by a variety of herbal medicine were verified, and a simple and effective direct soaking operation was proposed to achieve the optimal removal effect. The experiment also proved that the texture of herbal medicine itself and the effective ingredients in herbal medicine have impacts on the removal effect. Additionally, the detection methods were compared on copper ion removal effect. Iodometry and EDTA-Xylenol orange are both suitable for the detection of copper ions while the latter has a wider application range and can be used in the comprehensive detection of other metal ions. This experiment provides technical support for the utilization of herbal medicine resources in heavy metal polluted water treatment areas.

ACKNOWLEDGEMENT

National Innovation and Entrepreneurship Training Program for college students (202110223037X); Heilongjiang Provincial Natural Science Foundation (SS2022B001); Heilongjiang "Double First Class" Construction Projects (LJGXCG2022-006); Heilongjiang Bayi Agriculture University Sanhengsanzong Foundation (ZRCPY202112).

REFERENCE

1. Mohammadi, A., Mansoori, B., & Baradaran, B. (2017). Regulation of miRNAs by herbal medicine: An emerging field in cancer therapies. *Biomedicine & Pharmacotherapy*, *86*, 262-270.
2. Ruiz-Noa, Y., Ibarra-Reynoso, L. D. R., Ruiz-Padilla, A. J., Alonso-Castro, A. J., Ramírez-Morales, M. A., Zapata-Morales, J. R., ... & Lara-Morales, A. (2021). Use of herbal medicine for diabetes mellitus in adults from the central–western region of Mexico. *Primary Care Diabetes*, *15*(6), 1095-1099.
3. Wiśniewska, M., Marciniak, M., Gęca, M., Herda, K., Pietrzak, R., & Nowicki, P. (2022). Activated Biocarbons Obtained from Plant Biomass as Adsorbents of Heavy Metal Ions. *Materials*, *15*(17), 5856.
4. Tao, W., Jin, J., Zheng, Y., & Li, S. (2021). Current advances of resource utilization of herbal extraction residues in China. *Waste and Biomass Valorization*, 12(11), 5853-5862.
5. Li, C. (2012). Research on the adsorption properties of the containing lead wastewater with Radix Isatidis [D]. Jiling University.
6. Huang, J. (2012). Preparation of activated carbon from pyrolusite-added herb residues and its adsorption of heavy metals in the waste water [D]. *Chengdu University of Information Technology*.
7. Zhou, M., Luo, B., Yan, M. (2017). Study on the removal of four heavy metals with moringa seeds extract [J]. *Technology of Water Treatment*, 43(5), 68-71.
8. Xie, X., Ye, Y., & Hua, X. (2021). Preparation of modified bagasse-based adsorbent and its adsorption of chromium in wastewater [J]. *Mining and Metallurgical Engineering*, 41(4), 166-169.
9. Shang, J., Pi, J., Zong, M., Wang, Y., Li, W., & Liao, Q. (2016). Chromium removal using magnetic biochar derived from herb-residue. *Journal of the Taiwan Institute of Chemical Engineers*, *68*, 289-294.
10. Fan, L., & Lu, Q. (2021). Determination of copper in silver separating residue from copper smelting by iodometry [J]. *Metallurgical Analysis*, 41(2), 60-65.
11. Fu, G., Niu, Q., & Dang, X. (2014). Determination of lead in zine oxide leaching residue by EDTA titrmetry [J]. *Metallurgical Analysis*, 34(2), 70-73.
12. Li, S. X., Zhou, K., & Zhang, Q. (2022). Study on the removal of Cu^{2+} from water by *Sophora japonica* L. Extract [J]. *Biological Chemical Engineering*, 8(3), 90-92+96.
13. Sulyman, M., Namiesnik, J., & Gierak, A. (2017). Low-cost Adsorbents Derived from Agricultural By-products/Wastes for Enhancing Contaminant Uptakes from Wastewater: A Review. *Polish Journal of Environmental Studies*, *26*(3), 479-510.