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Phytoremediation for antibiotics removal from aqueous solutions: A meta-analysis

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ABSTRACT

Antibiotics are widely used as drugs and enter water bodies through various routes, leading to environmental pollution. As a green in-situ remediation technology, phytoremediation has been proven to be highly effective in removing antibiotics present in the aqueous phase. However, these data are distributed in various studies and lack systematic analysis, which could provide a more comprehensive understanding of the current status and trends in the research field. Based on this, a meta-analysis was conducted from three perspectives in this study: the factors influencing antibiotics removal by phytoremediation, the effect of antibiotics on plant physiological indexes, and the accumulation and translocation of antibiotics in plants. The results showed that plants have a significant effect on antibiotics removal, which is influenced by plant species, running time, biomass, antibiotic types and antibiotic concentration. Although some physiological indexes of plants changed under stress from high antibiotic concentrations, most plant species demonstrated resistance to antibiotic concentrations below 100 μgL^{-1}. Additionally, the amount of antibiotics accumulated in plants was extremely little, so the risk of secondary pollution was minimal during phytoremediation. The results of this study reveal the main factors influencing antibiotics removal by phytoremediation and plant physiological responses to antibiotics, providing a reference for improving the rational application of phytoremediation for antibiotics removal. In addition, it will provide concepts and directions for improving the efficiency of sustainable and environmentally friendly remediation methods for treating antibiotic pollution.

1. Introduction

Antibiotics have been widely used to treat a variety of diseases, and their use has increased due to the prevalence of COVID-19 (Corona Virus Disease, 2019) in recent years (Rawson et al., 2020). However, only limited amounts of antibiotics are absorbed, and large amounts of antibiotics and their metabolites are excreted into water bodies by organisms (Hu et al., 2022). These antibiotics then enter natural water bodies through a variety of routes, and the concentrations of antibiotics currently range from ngL^{-1} to μgL^{-1} in surface water and groundwater (Liu et al., 2018). In addition, the mixtures of antibiotics present in the water are complex, Quoc Tuc et al. (2017) investigated the Orge and Chamers rivers in France, and found that a total of 15 antibiotics (most notably quinolones) were discharged into these two rivers via hospital wastewater and domestic sewage, with concentrations ranging from 18 to 12850 ngL^{-1}. Twenty antibiotics were also detected in seawater from the Mameno drainage lake in Spain with concentrations ranging from non-detectable to 168 ngL^{-1} (Moreno-Gonzalez et al., 2015). Antibiotic pollution not only has harmful effects on aquatic and terrestrial organisms, but also leads to significant public health risks (Huijbers et al., 2015). Therefore, the effective removal of antibiotics from aquatic environments is an urgent issue to be addressed.

Phytoremediation is the process of using plants to remove, decompose, or fix pollutants (heavy metals, organic pollutants) in contaminated land or water media (Awa and Hadibarata, 2020). As for heavy metals, they can be absorbed and then accumulate in the plant, which may have the risks of secondary pollution. However, plants can transform organic pollutants into less toxic or non-toxic substances (Hussain

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et al., 2018; Ali et al., 2020), leading to a greater reduction in secondary pollution risk than other methods. Even if excessive accumulation of pollutants occurs within plants, regular harvesting procedures could be implemented, and secondary pollution could be controlled by composting. Therefore, phytoremediation has long been regarded as a green in situ remediation technology, that has advantages such as low cost, high efficiency and environmental friendliness (Lee, 2013). Several studies have reported that plants can effectively remove antibiotics from water or soils medias (Gahlawat and Gauba, 2016; Guo et al., 2019; Yan et al., 2019a), and the potential of phytoremediation technique for antibiotics removal has been proven. The removal efficiency of antibiotics is affected by numerous factors (Zhang et al., 2014; Hu et al., 2021), such as plant species (Chen et al., 2021; Rocha et al., 2022; Ruan et al., 2022), antibiotic types (Brunhoferova et al., 2021) and nutrient concentrations (Gomes et al., 2018b). However, previous studies have mostly been based on the influences of single factor, and less attention has been given to the interactions between influencing factors, so it is difficult to accurately assess the influence of factors on antibiotics removal by aquatic plants in real environments. If the relevant data from previous studies can be incorporated into a unified model for analysis, the above problems may be solved to a certain extent.

In addition, the response of plants under antibiotic stress conditions should be explored to assure the long-term success of phytoremediation. Antibiotic toxicity is frequently assessed using plant growth, photosynthetic indexes, and antioxidant systems (Singh et al., 2018, 2019; Gomes et al., 2020). The results, however, are conflicting because different experimental setups are used. Some studies revealed that antibiotic stress inhibited plant photosynthesis, while others indicated that antibiotics had no effect on plants. To determine the applicability and sustainability of phytoremediation, it is essential to comprehensively evaluate the effects of antibiotic stress on plant growth and toxicity through effective means. Meanwhile, an investigation of antibiotic accumulation and translocation in plants is needed, since this is essential for determining whether secondary pollution occurs during phytoremediation.

Meta-analysis is a method to systematically and comprehensively evaluate multiple studies to gain a more comprehensive understanding of the current status and trends in a research field by collecting, integrating, analyzing, and interpreting previously published data. This method has been increasingly used in recent years for research in the environmental sciences (Huang et al., 2020; Sun et al., 2020; Zhang et al., 2023). In this study, a meta-analysis was conducted to investigate the factors influencing antibiotics removal by phytoremediation and plant physiological response to antibiotic stress in phytoremediation. Specifically, the purpose of this study was as follows: (1) to identify the main factors that affect the removal of antibiotics from aqueous solutions by phytoremediation, and to further explore the interactions between the factors; (2) to clarify the impact of antibiotics stress on plant physiological indexes, and to find a suitable concentration range of antibiotics that could be treated effectively by phytoremediation; (3) to investigate antibiotic accumulation and translocation in plants, and to analyse the potential risk of secondary pollution in phytoremediation. To the best of our knowledge, this is the first quantitative meta-analysis on the removal of antibiotics from aqueous solutions by phytoremediation. In this study, we not only discuss the effect of single factor on the removal efficiency of phytoremediation, but also explore the interactions between influencing factors. These results promote a comprehensive understanding of the influence of various factors on antibiotics removal by phytoremediation and provide a theoretical basis for their practical application in the future.

2. Materials and methods

2.1. Literature search strategy and selection criteria

The dataset was compiled from peer-reviewed scientific articles published between 1995 and 2022 using the Web of Science database (http://apps.webofknowledge.com) and the keywords (TS=(Phytoremediation)) AND (TS=(Antibiotics)). A total of 249 papers were found. We screened 38 articles based on the following criteria (Fig. 1): (1) At least one variable related to antibiotic concentration (water, root, or stem), plant physiological indexes were measured; (2) Setting up no-plant and with-plant conditions; (3) Mean, standard deviation (or standard error), and sample size (n) were provided directly or could be calculated from the study; (4) Initial antibiotic concentrations in solutions were provided; (5) The experiments were conducted in aqueous solutions.

2.2. Data extraction and classification

Data were extracted from the screened literature and classified. To explore the factors influencing the removal of antibiotics by phytoremediation, in this study, the factors were classified into four categories: plant, running time, nutrients and antibiotic. The plant category included plant life form (PLF), plant species (PS) and biomass (BM). The nutrients category included nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and chlorine (Cl). The antibiotic category included antibiotic type (AT) and antibiotic concentration (AC). The antibiotics removal efficiency was collected under different experimental conditions. Plant physiological indexes mainly included growth, photosynthetic and oxidative indexes (Fig. 1). In order to investigate the accumulation and translocation of antibiotics in plants, bioconcentration factors (BCF) and translocation factor (TF) of antibiotics in various plants were collected.

2.3. Statistical analysis

2.3.1. Meta-analysis

The natural logarithm of the response ratio (lnRR) is an index to represent the difference between the treatment group and the control group. In this study, in order to compare antibiotics removal efficiency in the treatment group (with plants) to that of the control group (without plants), lnRR was used as the effect size to merge the results from various studies, and the variance (v) associated with each lnRR was calculated.

$$ln\ RR = ln\frac{\overline{x}_e}{\overline{x}_c}$$

$$V = \frac{SD_e^2}{n_e\overline{x}_e^2} + \frac{SD_c^2}{n_c\overline{x}_c^2}$$

in which X_e and X_c represent the mean values of the experimental and control groups, respectively. n_e and n_c are the sample sizes of the experimental and control groups, and *SDe* and *SDc* are their standard deviations.

The random effects model in the meta-analysis software Metawin 2.1 was used to calculate the overall mean effect values or weighted means and to generate 95% confidence intervals. When 95% CI of lnRR did not overlap with zero, the treatment had significant effects on the variables (Vetter, 2014). To assess the reliability of the meta-analysis, we calculated Rosenberg's fail-safe numbers, and fail-safe numbers greater than 5n+10 were considered robust to publication bias (n is the number of original studies) (Table S1).

The bioconcentration factors (BCF) and translocation factor (TF) were used to assess the accumulation of antibiotics in plants and their migration from roots to aboveground parts. The formula for calculating BCF and TF is as follows:

$$BCF = \frac{C_{plant}}{C_{water}}$$

$$TF = \frac{C_{shoot}}{C_{root}}$$

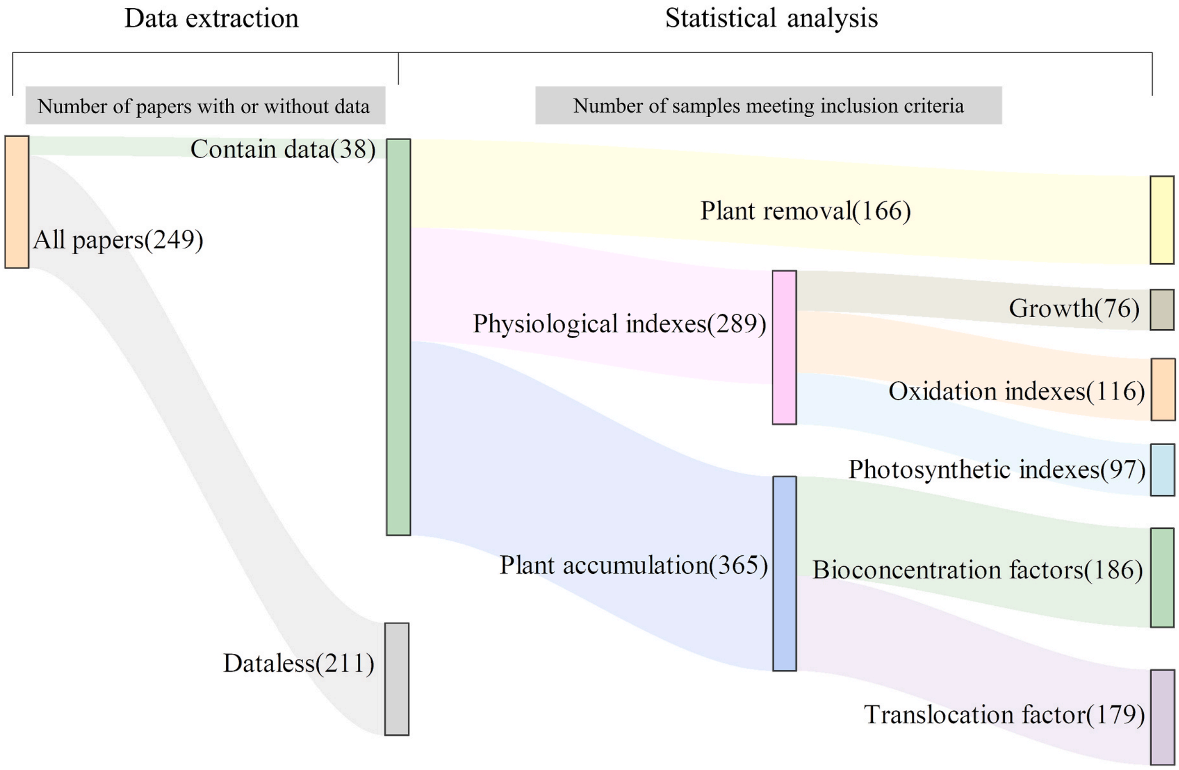

Fig. 1. Framework of literature screening, data extraction and statistical analysis model of phytoremediation for antibiotics removal from aqueous solutions.

C_{plant} represents the concentration of antibiotics in plants (μgkg^{-1}), C_{water} represents the concentration of antibiotics in the hydroponic solution (μgL^{-1}); C_{root} and C_{shoot} represent the concentration of antibiotics in the root and aboveground parts (μgkg^{-1}).

2.3.2. *Geographical detector model*

The geographical detector model (http://www.geodetector.cn/) was used to statistically analyse the eligible data to obtain the main factors influencing antibiotics removal. It is a statistical tool that quantifies the driving forces and interactions of each independent variable on the dependent variable without the need to make linear assumptions. In this study, the effect of explanatory variables (plant, running time, nutrient, and antibiotic) on the response variable (antibiotics removal efficiency) was quantified using a factor detector. The explanatory force of different factors on antibiotics removal efficiency was measured quantitatively by Q values, with higher Q values indicating greater factor explanation (Zhang et al., 2023).

2.3.3. *Data processing*

Data and statistical analyses were conducted using Excel 2019 (Microsoft Corporation, USA) and IBM SPSS Statistics 25 (IBM Inc., USA) software. Images were created using Origin 2022. Logarithmic transformations were used on the data as necessary to obtain a normal distribution. In the study, linear regression analysis was employed to assess correlations between dependent variables (the antibiotics removal effect size, plant accumulation factor, and transfer factor) and independent factors (antibiotic concentration and time). Non-parametric Spearman's correlation analysis was used to assess the relationship between plant physiological indexes and environmental factors. Values with P less than 0.05 in correlation tests were considered statistically significant.

3. Results and discussion

3.1. *Status of the research on phytoremediation for antibiotics removal*

The meta-analysis included 38 studies on phytoremediation for antibiotics removal published from 2002 to 2022. In recent years, there has been a dramatic rise in research on this topic (Fig. S1), and these studies were conducted across Asia, Europe, and America (Fig. S2). Antibiotic types investigated in these studies were mainly quinolones, tetracyclines and sulfonamides antibiotics (Fig. S3a). Plant species primarily consisted of wetland plants, which could be classified into three life forms as follows: floating plants, emergent plants and submerged plants (Fig. S3b). Almost all studies showed that the presence of plants significantly increased antibiotics removal rates (Table 1).

3.2. *The factors influencing antibiotics removal by phytoremediation*

3.2.1. *Screening of influencing factors*

To identify the major factors that affect antibiotics removal, before conducting a subgroup analysis, it was necessary to evaluate the roles of each factor. The Geodetector analysis model was used to investigate the effect of each factor on antibiotics removal, which was classified into four major categories as follows: plant, running time, nutrient and antibiotic. The results showed that plant species, biomass, running time, the concentration of N, P and Cl, antibiotic type, and antibiotic concentration all exhibited a strong explanatory force (with larger Q values) for antibiotics removal (Fig. S4). Therefore, they were considered as the main factors and were analysed in the subgroup below.

3.2.2. *Plant species*

The factor analysis of Geodetector in this study revealed that plant species had a significant effect on antibiotics removal (Fig. S4). Therefore, subgroup analysis was used to explore the differences in antibiotics removal efficiency among each plant species. The results showed that plants with the three life forms, namely, floating, submerged, and

Table 1
Selected antibiotics removal rate with plants and without plants.

Author, Year	Concentration (μgL^{-1})	Temperature (°C)	Plant	Antibiotics	removal rate without plant	removal rate with plant
Forni et al., (2002)	50000, 150000, 300000, 450000	25 ± 3	*Azolla filiculoides*	Sulfonamides	5%–30%	56.34%–88.49%
Thi Thanh Thuy et al., 2012	5000, 10000	27.6 ± 5	*Ceratophyllum demersum, Chrysopogon zizanioides*	4-quinolones	17%–24%	34%–44%
Lu et al., (2014)	5000	25 ± 2	*Eichhornia crassipes*	Tetracyclines	53.33%–81.79%	79.84%–100%
Iatrou et al., (2017)	250	24 ± 0.5	*Lemna minor*	β-lactams, Nitroimidazoles, Sulfonamides	24.7%–33%	59.02%–95.41%
Singh et al., (2018)	0.1, 1, 10, 100, 1000	23 ± 2	*Spirodela polyrhiza*	β-lactams	62.1%–73%	84.6%–93.8%
Gomes et al., (2019)	0.75, 1.5, 2.25	20 ± 2	*Elodea canadensis*	4-quinolones	8%–19%	10.17%–17.55%
Guo et al., (2019)	50000	25	*Myriophyllum aquaticum*	Tetracyclines	0–1%	50.96%–54.52%
Kurade et al., (2019)	50, 200, 500, 1000	25 ± 5	*Ipomoea aquatica*	Sulfonamides	0.6%–1.8%	71.8%–100%
Panja et al., (2019)	50, 100, 1000, 10000	25 ± 3	*Chrysopogon zizanioides*	4-quinolones	0–1%	80.93%–100%
Tai et al., (2019)	20, 400	25 ± 2	*Canna indica, Iris pseudacorus*	Sulfonamides	15.28%–40%	12.45–97.04%
Singh et al., (2019)	10, 50, 100, 500, 1000	23 ± 2	*Spirodela polyrhiza*	4-quinolones	54.76%–75.53%	93.73%–98.36%
Yan et al., (2019a)	10, 100, 1000	25.1	*Eichhornia crassipes*	Sulfonamides	8.79%–71.98%	25.28%–83.41%
Bianchi et al., (2020)	1	20 ± 4	*Azolla filiculoides, Lemna minor*	4-quinolones	11.34%–18.21%	50.6%–60.3%
Gomes et al., (2020)	1, 2	24 ± 2	*Lemna minor*	4-quinolones, Tetracyclines, β-lactams	21.1%–77.23%	38.07%–100%
Panja et al., (2020)	50, 100, 1000, 10000	25 ± 3	*Chrysopogon zizanioides*	4-quinolones, Tetracyclines	5.64%–20.45%	72.88%–100%
Huang et al., (2022)	100, 500, 1000, 5000, 10000	23 ± 2	*Lemna minor*	Aminoglycosides	15.8%–44.6%	33.6%–71.4%
Rocha et al., (2022)	1.7	20 ± 3	*Salvinia molesta, Lemna minor, Rotala rotundifolia, Myriophyllum aquaticum*	Macrolides	11.04%–29.19%	30.59%–64.12%
Ruan et al., (2022)	50, 500	33 ± 5	*Canna indica, Acorus calamus*	Sulfonamides	8%–69.3%	12.8%–97.8%
Akiyama Kitamura et al., (2023)	1, 10, 100	23 ± 2	*Salvinia molesta, Egeria densa Planch*	4-quinolones	3.96%–35.96%	58.99%–100%

emergent, all significantly contributed to antibiotics removal compared to the controls (without plants). However, *Chrysopogon zizanioides*, *Ipomoea aquatica*, *Salvinia moleata*, *Lemna minor*, *Azolla filliculoides*, *Eichhornia crassipes*, *Egeria densa Planch* and *Canna indica* showed higher antibiotics removal rates, ranging from 60 to 95% (Fig. S5), while *Myriophyllum aquaticum*, *Ceratophyllum demersum*, *Iris pseudacorus*, and *Acorus calamus* had no significant effect (Fig. 2). This suggested that antibiotics removal efficiency varies by plant species. Ruan et al. (2022) found that *Canna indica* had a higher antibiotics removal efficiency than *Acorus calamus*, which could be attributed to the fact that it has a larger root area and supports more inter-rooted microorganisms for greater biodegradation. Chen et al. (2021) also found that *Cyperus papyrus* had significantly higher sulfonamides removal rates than other plants, through the best effect on antibiotic uptake and rhizosphere

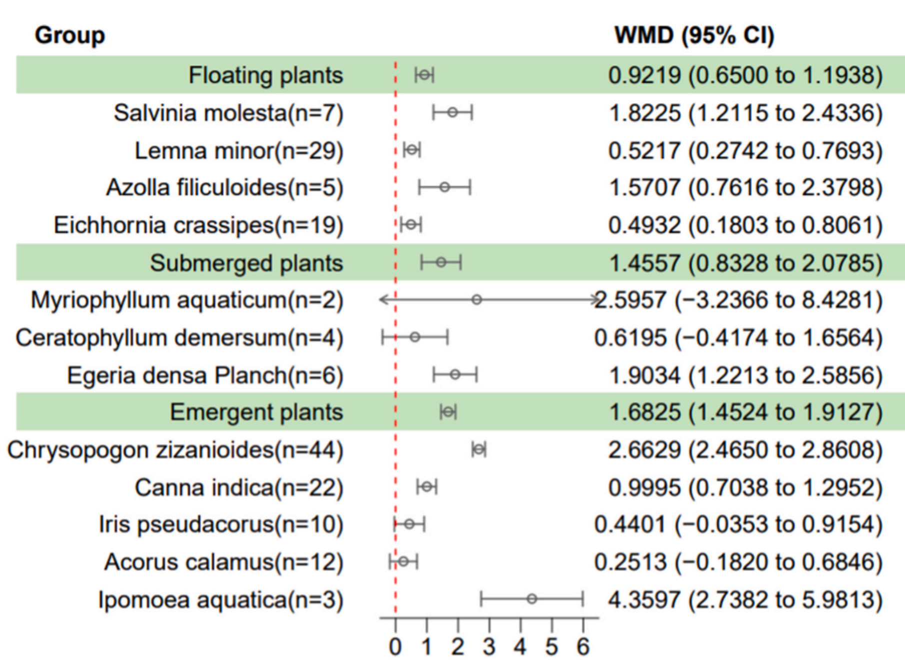

Fig. 2. The removal rate of antibiotics varies with plant species, as indicated by the mean effect size and the 95% confidence interval. The numbers following the name of each variable indicate the number of observations conducted.

biodegradation. Therefore, the variation in the capacity of rhizosphere microorganisms to degrade antibiotics and the ability of plants to absorb antibiotics may be important reasons for the differences in antibiotics removal efficiency among each plant species (Gujarathi et al., 2005; Chen et al., 2021; Ruan et al., 2022).

The different plant species were highly variable, which made it difficult to use a unified unit to compare their biomass, so a subgroup analysis of biomass was not conducted. In the same plant species, the larger the biomass is, especially the root biomass, the higher the antibiotics removal efficiency. Plants with more developed roots have a stronger ability to absorb antibiotics because they have higher specific surface area. (Guo et al., 2019; Yan et al., 2020).

3.2.3. Antibiotic type

The antibiotic type had a strong explanatory force for the removal of antibiotics (Fig. S4), and the results of the subgroup analysis also revealed differences in the removal efficiency between the different types of antibiotics (Fig. 3). Phytoremediation had a significant removal efficiency for quinolones, tetracyclines and sulfonamides antibiotics, with median removal rates of 83.17%, 68.25% and 99.19%, respectively (Fig. S6). In contrast, the removal efficiency of macrolides, β-lactams and aminoglycosides antibiotics was not significant, presumably due to the limited number of studies conducted on these classes of antibiotics in the selected literature. In terms of specific antibiotic type, CIP, NOR, TC, SDZ, SM2 and SMX removal efficiency increased significantly in the presence of plants (Fig. 3). Possible reasons for these results were that different antibiotics have different physicochemical properties (e.g., hydrophobic properties, chemical structure, water solubility, and molecular weight), which affects their adsorption and uptake by plants (Briggs et al., 1982; Xu et al., 2018; Adesanya et al., 2020). Antibiotics with log Kow values ranging from 0.5 to 3, and molecular masses less than 1000 gmol^{-1} are expected to be easily absorbed by plant roots because they are lipophilic enough to cross the lipid bilayer of the membrane and are water-soluble enough to enter the cell fluid (Zhang et al., 2014). Therefore, antibiotic type affected the antibiotics removal efficiency.

3.2.4. Running time, antibiotic concentration and nutrient concentration

Antibiotics removal efficiency of phytoremediation was significantly different at different running time and antibiotic concentrations (Fig. 4) and increased with running time and antibiotic concentration (Fig.4bc). Based on existing research, the main mechanisms of antibiotics removal by phytoremediation are adsorption, plant uptake, and microbial degradation. Because adsorption occurs relatively quickly, it is generally the key mechanism of antibiotics removal by phytoremediation in the initial stage. However, with increasing contact time, the availability of active sites and the forces driving antibiotics to these sites decrease, so the adsorption rate slows down. As a result, plant uptake and microbial

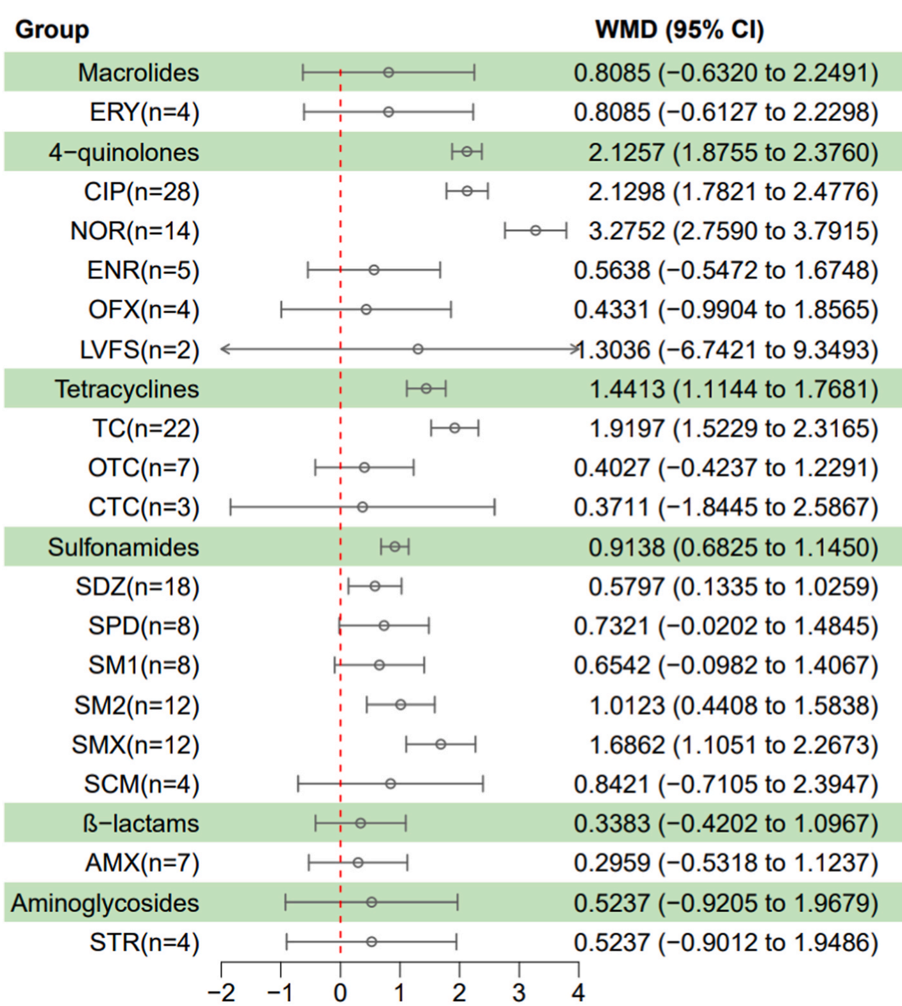

Fig. 3. The removal rate of antibiotics varies with antibiotic type, as indicated by the mean effect size and 95% confidence interval. The numbers following the name of each variable indicate the number of observations conducted. ERY: erythromycin; CIP: ciprofloxacin; NOR: norfloxacin; ENR: enrofloxacin; OFX: ofloxacin; LVFS: levofloxacin; TC: tetracycline; OTC: oxytetracycline; CTC: chlortetracycline; SDZ: sulfadiazine; SPD: sulfapyridine; SM1: sulfamerazine; SM2: sulfamethazine; SMX: sulfamethoxazole; SCM: sulfacetamide; AMX: amoxicillin; STR: streptomycin.

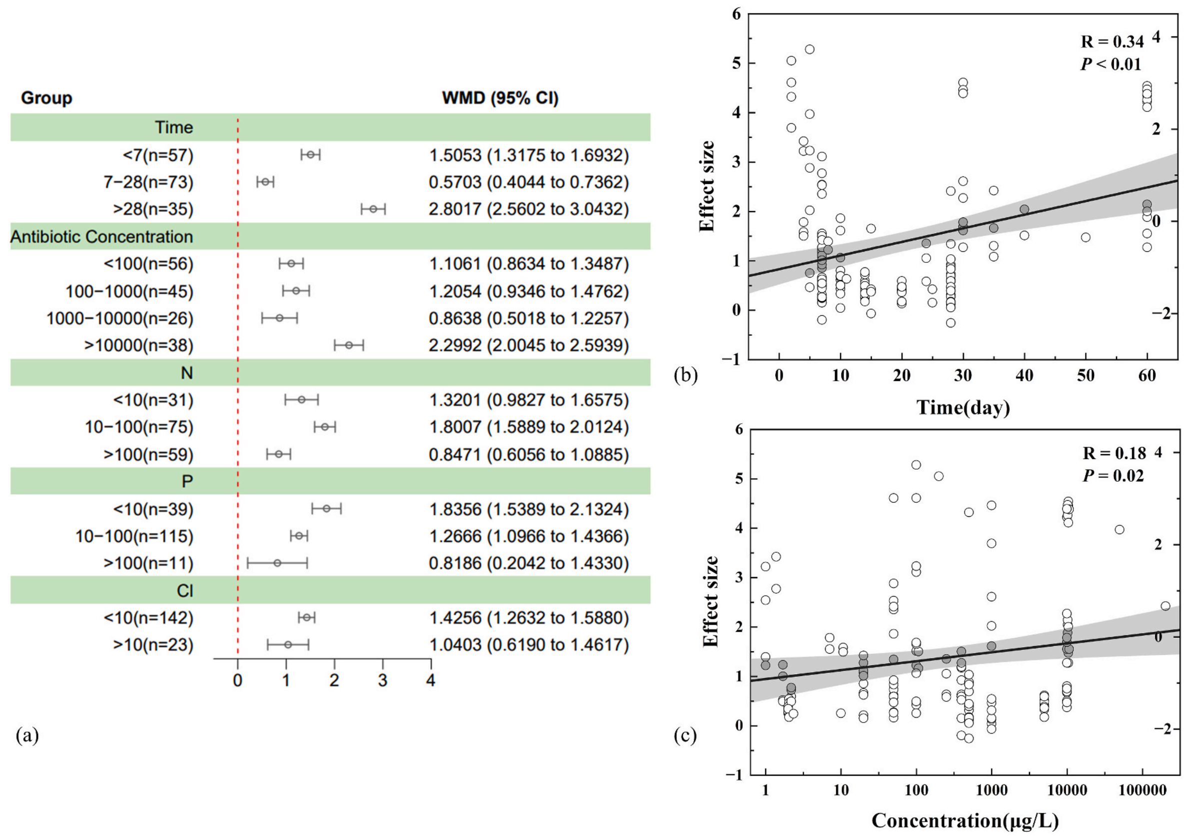

Fig. 4. (a) The removal rate of antibiotics varies with Time, Antibiotic concentration, N concentration, P concentration and Cl concentration, as indicated by the mean effect size and 95% confidence interval. The numbers following the name of each variable indicate the number of observations conducted. (b) The relationship between effect size of antibiotics removal (lnRR) and time. (c) The relationship between effect size of antibiotics removal (lnRR) and concentration.

degradation are mostly joint action in the middle and late stages of phytoremediation (Guo et al., 2019), and the removal process frequently following first- or zero-order removal kinetics (Chen et al., 2021). Moreover, with the increase of antibiotic concentration, the total amount of antibiotics adsorbed by plants increases, and the time required to reach adsorption equilibrium is longer. Furthermore, in view of the fact that antibiotics are mostly absorbed by plants through passive transport (Hu et al., 2021), and an increase in antibiotic concentration enlarges the concentration gradient between the interior and exterior of plant cells, accelerating antibiotic diffusion. Therefore, plants appear to

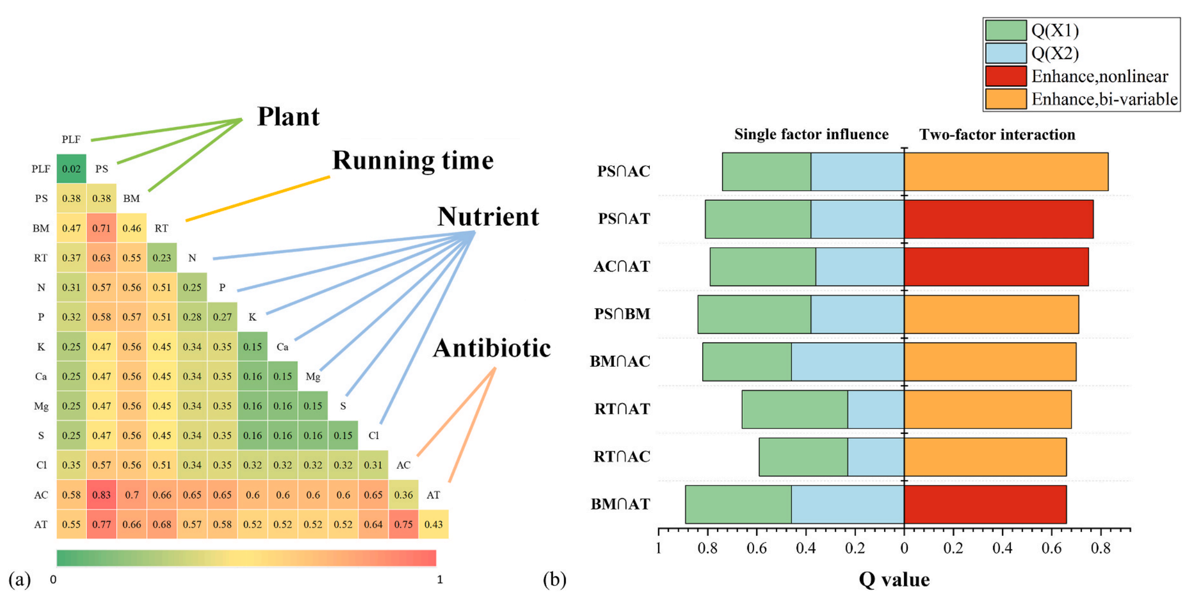

Fig. 5. (a) The Q value interaction matrix of the interaction detector. PLF: plant life form; PS: plant species; BM: biomass; RT: running time; N: nitrogen; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; S: sulfur; Cl: chlorine; AC: Antibiotic concentration; AT: Antibiotic type. (b) The 8 largest combinations of interactions of Q (X1∩X2) values for all interactions. "∩"indicated the presence of an interaction between two factors.

have a greater removal efficiency at high antibiotic concentrations.

In addition, antibiotics were also significantly removed by plants at different nutrient concentrations (Fig. 4a). To date, the test plants selected in this research field were mainly wetland plants, which have strong adaptability to the environment. Additionally, most of the studies were conducted in a laboratory setting in which sufficient nutrients were provided for plant growth. These might be the reasons for this result.

3.2.5. Interactions between influencing factors

The maximum Q value of the influencing factors was only 0.46 (Fig. S4). This indicated that the 13 selected influencing factors may not adequately represent changes in antibiotics removal efficiency and may be influenced by each other. Therefore, interactions between factors should be further explored.

The Q value matrix of the interaction detector was shown in Fig. 5a, which revealed the effect of different factor interactions on antibiotics removal. The number of interaction types in the matrix showed that univariate weakening, nonlinear-weakening, bivariate enhancement and nonlinear-enhancement accounted for 7.79%, 1.28%, 56.41% and 34.62%, respectively (Tables S2 and S3). The enhancing effect predominated among the types of interaction, indicating that the combination of two factors had a larger effect on antibiotics removal than each single acting. Plants removed antibiotics directly or indirectly by adsorption, plant uptake, and microbial degradation, which are influenced by multiple environmental factors (Zhang et al., 2014; Hu et al., 2021). Among the eight maximum interaction Q values, plant species, antibiotic concentration, antibiotic types, biomass, and running time were the factors that contributed the most to the interactions (Fig. 5b), which was consistent with the results of subgroup analysis (Fig. S4). It can be seen that plant species, running time and biomass have obvious interactions with antibiotic concentration and antibiotic types (Fig. 5b). This suggested that in future practical applications, specific plants with high tolerance and removal efficiency should be screened for different kinds of antibiotics. Depending on the actual situation of antibiotic pollution (type, concentration), biomass, running time, or a combination of many special plants should be selected in order to achieve phytoremediation effects while decreasing the cost in both time and money.

3.3. Effect of antibiotics on plant physiological indexes

The results in Section 3.2 demonstrated that phytoremediation was effective at removing antibiotics from the water phase and had certain application potential. However, some research discovered that antibiotics can affect growth, photosynthesis, and enzyme systems in aquatic plants by inducing oxidative stress or inhibiting eukaryotic protein synthesis (Caverzan et al., 2012; Deng et al., 2022). Therefore, it is necessary to investigate the physiological effects of antibiotics on plants, which is critical to the long-term operation of phytoremediation. The results showed that plant physiological indexes changed significantly under antibiotic stress, and these changes were greatly impacted by antibiotic concentration, running time, and nutrient concentration (Fig.6ab).

Antibiotics did not have a negative effect on plants at all concentrations; for example, when the antibiotic concentration was below 100 μgL^{-1}, there was no significant change in plant growth. However, plant growth was inhibited with increasing antibiotic concentration (Fig. 6b). Plant growth decreased when the antibiotic concentration was above 100 μgL^{-1} (Fig. 6a). Gomes et al. (2017) also found the similar phenomenon in which antibiotics were harmful to *Lemna minor* at concentration of 1.05 mgL^{-1}, whereas low concentrations promoted its growth. The submerged plant *Vallisneria natans* also showed significantly lower growth rates under 30 and 50 mgL^{-1} concentrations of sulfonamides antibiotics exposure than the antibiotic-free control (Zhu et al., 2020). Moreover, high concentrations of N, P, and Cl inhibited plant growth (Fig. 6b), which may be due to the fact that high concentrations of nutrient salts were similar to other organic pollutants, resulting in stress on plants (Barker et al., 2008; Zhou et al., 2017; Rao et al., 2018).

Based on the overall analysis, plant chlorophyll *a* and chlorophyll *b* decreased by 12% and 21% under antibiotic stress (Fig. 6a). These effects were not surprising, as it was found in several previous studies that plants had significantly lower chlorophyll content when exposed to antibiotics (Aristilde et al., 2010; Gomes et al., 2020). Antibiotics have toxic inhibitory effects on the donor side, electron transfer, and acceptor side of photosystem II (Geoffroy et al., 2003; Pan et al., 2008; Rocha et al., 2021). Moreover, antibiotics may inhibit the synthesis of the light-trapping chlorophyll *a/b* protein complex, leading to a decrease in energy conversion efficiency (Alberte et al., 1981). Consequently, there was a negative correlation between antibiotic concentration and chlorophyll content (Fig. 6b).

The antioxidant enzyme system of plants is an important mechanism for maintaining redox homeostasis in plant cells, enhancing plant resilience and adaptability under environmental stress (Caputo et al., 2012). The major defense response processes of plants under antibiotic stress conditions were quantified through meta-analysis. The results showed that APX and CAT increased by 65% and 33%, respectively (Fig. 6a). Antioxidant enzymes (e.g., SOD, CAT, POD) are considered to be the first line of defense in protecting plants against stress (Spengler et al., 2017). CAT activity in plants increased significantly under

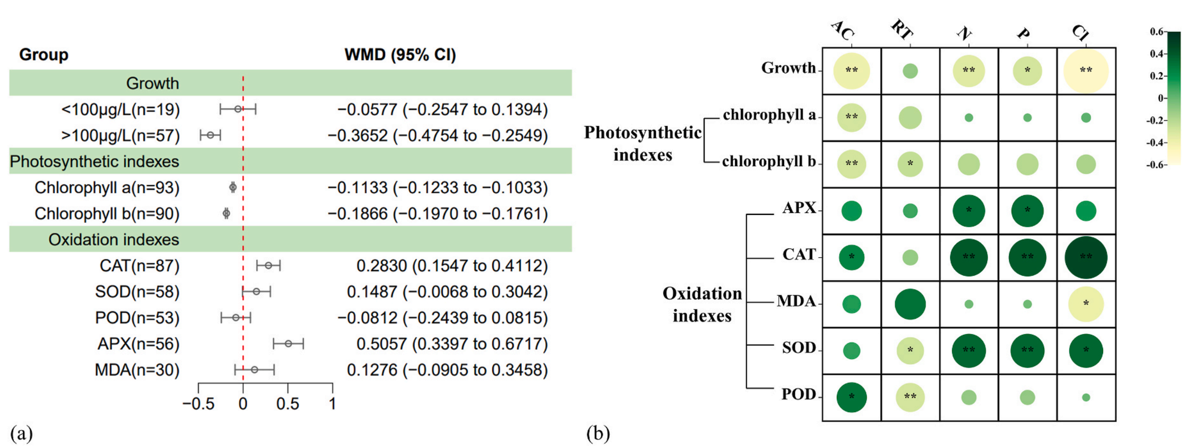

Fig. 6. (a) Changes in toxicity of antibiotic on plants for different functional indexes, as indicated by the mean effect size and 95% confidence interval. The numbers following the name of each variable indicate the number of observations conducted. APX: ascorbate peroxidase; CAT: catalase; MDA: malondialdehyde; SOD: superoxide dismutase; POD: peroxidase; AC: Antibiotic concentration; RT: running time; N: nitrogen concentration; P: phosphorus concentration; Cl: chlorine concentration. (b) The relationship between plant physiological indexes and environmental factors.

antibiotic stress, accelerating the reduction of hydrogen peroxide (H_2O_2), thereby eliminating harmful substances more completely from cells (Gomes et al., 2017), such as peroxides and hydroxyl radicals. However, SOD and POD activity did not significantly change (Fig. 6a), indicating that the protective effect of the two enzymes under antibiotic stress was limited. This may be due to the fact that only CAT was capable of efficiently degrading H_2O_2 without the energy provided by cells, and its activity increased linearly with the concentration of H_2O_2 in a considerable range (Dat et al., 2001; Arora et al., 2002; Chelikani et al., 2004), but further investigation of the underlying mechanism is necessary in the future. The APX activity involved in the second stage of plant detoxification was also significantly increased, allowing plants to convert ascorbic acid ions to dehydroascorbic acid and release a large number of electrons to decrease H_2O_2 in chloroplasts (Asada, 1992; Caverzan et al., 2012). MDA was the product of plant peroxidation under environmental stress, and its accumulation reflected the degree of damage to cell membranes induced by oxygen free radicals (Yajima et al., 2009). In this research, MDA did not increase significantly when compared to plants treated without antibiotics (Fig. 6a). These results indicated that plants had a certain resistance to antibiotic pollution, and CAT and APX may be sensitive to antibiotic stress for aquatic plants. Meanwhile, the oxidative stress indexes for APX, CAT, and SOD enzyme activity increased in eutrophic water bodies (Fig. 6b), containing high concentrations of N, P, and Cl, to protect cells from oxidative damage (Zhao et al., 2019).

In conclusion, there was an elimination concentration threshold for antibiotics removal by plants that varied based on plant species and antibiotic types. When the antibiotic concentration exceeded this threshold, plants were negatively affected or even died (Maldonado et al., 2022). Phytoremediation was shown to be more suitable for wastewater containing antibiotics at concentrations below 100 μgL^{-1}, which was much higher than the concentration of antibiotics in common wastewater, and had the potential for long-term sustainable applications. Moreover, phytoremediation could be used in combination with other technologies, such as constructed wetlands and plant ponds, to treat wastewater containing higher concentrations of antibiotics.

3.4. Antibiotic accumulation and translocation in plants

Plants absorb antibiotics by diffusion, and then they can be removed continuously by plants through metabolism, translocation, and accumulation (Hu et al., 2021). According to the results of the regression analysis, BCF was negatively correlated with antibiotic concentration (Fig. 7a). Possible explanation for this trend was that plants had limited uptake rate to antibiotics. Despite the high concentration of antibiotics in the water, the rate of plant uptake had reached saturation (Azanu et al., 2016; Yan et al., 2020). Simultaneously, high antibiotic concentrations may stress plants and affect root uptake (Gomes et al., 2017; Yan et al., 2021). Because antibiotic metabolism occurred inside the plants, BCF also decreased over time (Fig. 7b). The majority of antibiotics were gradually metabolized in plants, with just a tiny fraction remaining in the plant as parent compounds, and the amount of accumulation in the plant would become lower and lower over time (Table S4). For example, after 10 d of phytoremediation by *Arabidopsis thaliana*, only 1.10% SMX remained in its original form in plant tissues, and the rest was metabolized through acetylation, oxidation and methylation (Huynh and Reinhold, 2019; Tai et al., 2019).

The results indicated that most plants exhibited low TF values.

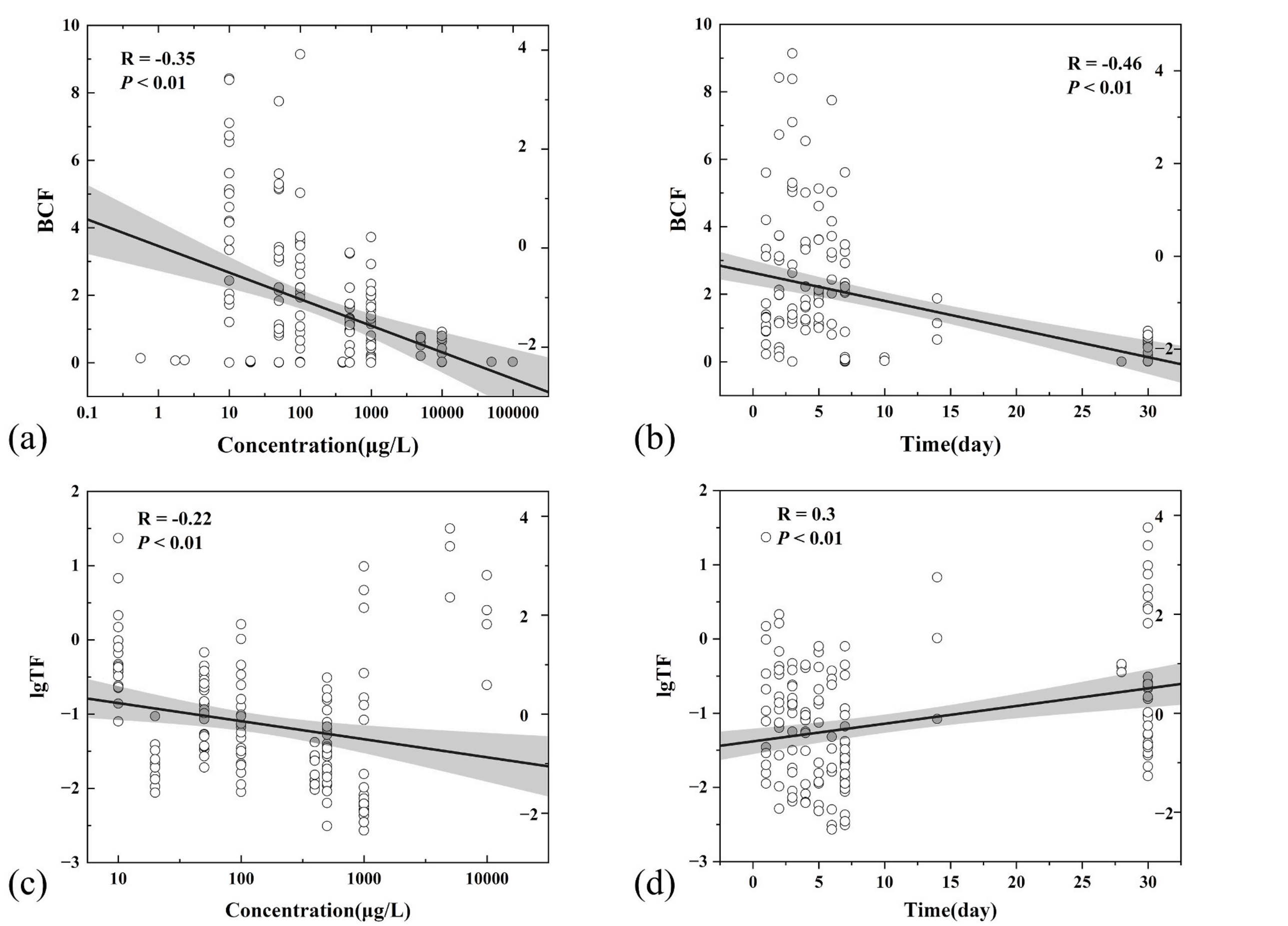

Fig. 7. (a) The relationship between bioconcentration factors (BCF) and concentration. (b) The relationship between BCF and time. (c) The relationship between the denary logarithm of translocation factor (lgTF) and concentration. (d) The relationship between lgTF and time.

Additionally, it was observed that TF decreased further as the concentration of antibiotics increased (Fig. 7c). Due to the increasing of antibiotic concentration in the solution, there was a corresponding reduction in the disparity of the ion concentration between the plant and the surrounding solution. This led to a drop in the osmotic pressure within the plant and a subsequent deceleration in the rate of water transport (Yan et al., 2020). Moreover, when exposed to high concentrations of antibiotics, plants may experience physiological stress and an inhibition in biological activity, resulting in a reduction in the rate at which antibiotics migrate upwards (Yan et al., 2019b). The results also found that TF increased slightly with the increase of time (Fig. 7d), owing to the fact that plant roots are the main accumulation and metabolism site for most antibiotics, and metabolism in leaves is relatively slow (Herklotz et al., 2010).

In general, antibiotics were mainly removed through plant metabolism and microbial degradation, and the amount of antibiotics accumulated in plants was almost negligible (Huynh and Reinhold, 2019; Tai et al., 2019), which was consistent with most studies (Table S4). Therefore, almost no antibiotics can return to the environment through dead plant materials, and the risk of secondary pollution is minimal in phytoremediation.

3.5. Deficiencies and uncertainties

It is worth noting that the following deficiencies and uncertainties remained in this study. (1) The results showed that biomass had significant effects on antibiotics removal efficiency, but finding a uniform unit was difficult due to differences in biomass between plant species. Therefore, the subgroup analysis was not performed for this factor. (2) Due to the different objectives of the articles, the distribution of some variables in different subgroups was imbalanced, for example, there were relatively few studies on macrolides, aminoglycosides, and β-lactams antibiotics. Because of the limited number of researches, it is presently impossible to adequately analyse the removal efficiency of different plant species for a particular antibiotic; (3) The efficacy of phytoremediation is influenced by temperature, which impacts the adsorption of antibiotics, microbial degradation, and plant uptake (Gomes et al., 2018a; Lu et al., 2021). Unfortunately, the statistical analysis did not incorporate temperature as a variable due to the consistent range of temperatures observed in the experiments, which were concentrated at 20–30 °C (Table 1). However, it has been documented that a suitable temperature could considerably increase the growth of microorganisms and plants, so the removal efficiency is higher in the summer than in the winter (Liu et al., 2014). In the future, more long-term experiments across seasons are needed to further explore the effects of temperature on antibiotics removal in plants.

4. Conclusions and perspectives

The meta-analysis revealed that phytoremediation had a significant effect on antibiotics removal, which was influenced primarily by plant species, biomass, running time, antibiotic type and antibiotic concentration, and there were also significant interactions among these influencing factors. Most plant species demonstrated resistance to antibiotic wastewater at concentrations below 100 μgL^{-1}. Antibiotic phytoremediation can be considered a low-risk green remediation technology, because our finding revealed that the amount of antibiotics accumulated in plants was extremely little, the majority of antibiotics were removed by biodegradation. These results can help provide a comprehensive understanding of the influence of various factors on antibiotics removal by phytoremediation and provide a theoretical basis for its practical application in the future. In addition, the results have reference value for the exploration of influencing factors in more complex environments (constructed wetlands) and provide concepts and directions for improving the efficiency of sustainable and environmentally friendly remediation methods for antibiotic pollution.

During the meta-analysis, we realize that the present understanding of antibiotics removal by phytoremediation is far from satisfactory and some future research directions need to be further explored: (1) It is necessary to establish a data sharing mechanism and platform for systematic integration research. Due to the lack of original data, the existing analysis results have certain limitations, hence data sharing and improving the accessibility and reusability of original data are essential. (2) Microorganisms play a crucial role in the phytoremediation of antibiotics, but there are few related research articles, which makes it hard to deeply explore the mechanism of phytoremediation and quantify the impact of various action pathways. Therefore, the exploration of related directions should be strengthened. (3) In the future, more field studies are needed to verify the feasibility of phytoremediation under complex and realistic conditions, and long-term testing data are also needed. (4) Simultaneously, it is essential to proactively advance the development of multi-technology combined treatment systems to further reduce the temporal and financial costs associated with antibiotics removal.

CRediT authorship contribution statement

Tong Zhou: Data curation, Formal analysis, Visualization, Writing – original draft. **Qiuying An:** Data curation, Writing – review & editing. **Ling Zhang:** Writing – review & editing. **Ce Wen:** Visualization. **Changzhou Yan:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117516.

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