Ecotoxicology and Environmental Safety 221 (2021) 112443

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Using quantitative ion character–activity relationship (QICAR) method in evaluation of metal toxicity toward wheat

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ARTICLE INFO

Edited by: Dr. Hao Zhu

Keywords:
Metal
Quantitative ion character–activity relationship (QICAR)
Toxicity
Biotic ligand model (BLM)
Wheat

ABSTRACT

It is important to assess the toxic effects posed by soil pollutants toward plants. However, plant toxicology experiments normally involve a considerable amount of manpower, consumables and time. Therefore, the use of metal toxicity prediction models, independent of toxicity tests, is critical. In this study, we investigated the toxicity of different metal ions to wheat using hydroponic experiments. We employed the methods of soft–hard ion grouping, soft–hard ligand theory and *K* (conditional binding constant based on the biotic ligand model principle) in combination with hydroponic experiments to explore the application of quantitative ion character-activity relationships in predicting phytotoxicity. The results showed that the toxicity of the 19 metal ions tested varied significantly, with EC_{50} ranging from 0.27 μM to 4463.36 μM. The linear regression relationships between the toxicity of these metal ions and their physicochemical properties were poor ($R^2 = 0.237–0.331$, $p < 0.05$). These relationships were improved after grouping the metals according to the soft–hard theory ($R^2 = 0.527–0.744$ and $p < 0.05$ for soft ions; $R^2 = 0.445–0.743$ and $p < 0.05$ for hard ions). The application of soft–hard ligand theory, based on the binding affinity of the metals to the ligands, showed poor prediction of the phytotoxicity of metals, with $R^2 = 0.413$ ($p = 0.024$) for the softness consensus scale (σ_{Con}) and $R^2 = 0.348$ ($p = 0.218$) for the normalized hard ligands scale (HLScale). However, the method of *K* provided the closest fit in predicting toxicity ($R^2 = 0.803$, $p < 0.001$). Our results showed that the application of soft–hard ion grouping and log *K* can improve prediction of the phytotoxicity of metals relatively well, which can potentially be used for deriving the toxicity of elements with limited toxicity data.

1. Introduction

In recent years, soil has been severely affected by inorganic pollutants, such as metals, including Cu, Zn and Ni. Some metals that were uncommon in the past have also entered the soil environment with increased industrialization such as La, Se and V. (Gong et al., 2019; Ji et al., 2020). However, there is limited information on the ecological risks of these elements. It is therefore important to understand the ecotoxicity and risks, and establish assessment models for these emerging elements.

Quantitative ion character–activity relationship (QICAR) models have been used to predict the toxicity of metals on the basis of the relationship between the physicochemical properties of the ions and their toxicity (Bogaerts et al., 2001; Walker et al., 2003). Newman et al. (1998) first proposed the feasibility of applying the QICAR model to toxicity prediction by exploring the effect of metals on the marine bacterium (*V. fischeri*). Subsequently, this model has been increasingly used in the aquatic environment. Li et al. (2012) used the QICAR model to study the metal toxicity of the non-marine ostracod *Cypris subglobosa* in freshwater, correlating the toxicity with physicochemical properties such as atomic number (AN), electronegativity (Xm), and the log of the first hydrolysis constant ($|\log(-K_{OH})|$) and so on. Similarly, Wolterbeek and Verburg (2001) correlated the physicochemical properties of a wide range of species with the toxic effects of cations and found accurate prediction of ion toxicity using these properties.

A recent study found it difficult to establish a relationship between multiple elements and their ecotoxicity, using one or more physicochemical properties (Chen and Wang, 2007). This may be due to the large differences in the properties of the elements, which resulted in completely different dosage-toxicity responses of organisms to

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https://doi.org/10.1016/j.ecoenv.2021.112443
Received 19 April 2021; Received in revised form 31 May 2021; Accepted 18 June 2021
Available online 21 June 2021

individual elements (Newman et al., 1998; Zamil et al., 2009). Thus, QICARs established after hard–soft grouping (ions with higher charge density, smaller radius and lower polarizability are hard ions, those with the opposite characteristics are soft ions) have been used in predicting metal toxicity (Newman et al., 1998; Pearson and Mawby, 1967; Tatara et al., 1998; Zamil et al., 2009). For instance, Meng et al. (2019b) used the QICAR model to investigate the relationships between metal ion characters and the ecological soil screening levels (Eco–SSLs). They found that QICAR models based on classifying metal ions as either soft or hard can accurately predict Eco-SSLs.

Similarly, the QICAR model has also been applied to terrestrial systems. Many studies have attempted to apply QICAR to estimate the toxicity of elements to plants, as plants are among the most commonly used organisms in terrestrial risk assessments. However, there are various ligands of plant roots, such as hard ligands containing oxygen or nitrogen (carboxyl, hydroxyl or amine groups) and soft ligands containing sulfur (sulfhydryl, alkene or aromatic groups), which have different complexing ability with metals (House, 2013; Kopittke et al., 2014). Therefore, it is difficult to quantify the binding ability of these ligands to metals by using single properties of these elements.

In order to better use QICAR in plants, a normalized hard ligands scale (HLScale) was proposed by Kinraide (2009), which was obtained by averaging and normalizing the binding strengths of metal cations to 13 hard ligands (including oxalate, citrate, hydroxide and carbonate, etc.). Kinraide (2009) believed that most metal ions were bound to hard ligands, and that binding affinity to hard ligands could predict the binding ability of metals to biological materials, such as plasma membranes and cell walls. However, the toxicity of some soft ions, such as Ag^{+}, Cu^{2+} and Tl^{+}, was found to be poorly correlated with HLScale, indicating that the toxicity of these ions may not reflect the binding ability with hard ligands, and may exhibit different toxicity mechanisms from other ions.

As a consequence, Kinraide (2009) presented a consensus scale related to the soft ions, termed the softness consensus scale (σ_{Con}), which was obtained by averaging and normalizing ten other scales related to chemical softness. He reported that σ_{Con} was strongly positively correlated with the binding strength of metals to soft ligands, and that its coupling with charge (Z) could closely predict the phytotoxicity of unknown elements ($R^2 = 0.923$, p-value was not reported). Kopittke et al. (2011) also studied the toxicity effects of 26 metals on cowpea roots, using both HLScale and σ_{Con} methods. It was found that HLScale ($R^2 = 0.658$, p was not reported) showed more accurate toxicity prediction than did σ_{Con} ($R^2 = 0.446$, p was not reported). Although the soft–hard ligand theory has achieved good results in predicting the toxicity of individual elements, there are still some elements such as Ag^{+} and Tl^{+} that have poorly predicted effects. In addition, these predictive results need to be further verified using different plants.

Recently, a biotic ligand model (BLM) theory has been proposed to predict element toxicity (Niyogi and Wood, 2004; De Schamphelaere and Janssen, 2002). The BLM assumes bioactive receptor sites to be biotic ligands (BLs). The BL site may be a physiologically active site, leading to a direct biological response, or it may be a transport site, leading to an indirect biological response. According to BLM theory, toxicity will occur when a certain number of ions bind to BLs. The toxicity of ions depends on the binding affinity (conditional binding constant, K) of metal ions to the BL sites. For the last few years, the BLM theory has been used to determine the binding constants of some common elements, such as Cu, Zn and Ni, to specific organisms (e.g., barley and wheat) (Lock et al., 2007; De Schamphelaere and Janssen, 2002; Wang et al., 2010).

In the present study, we compared the methods of soft–hard ion grouping, soft–hard ligand theory (HLScale and σ_{Con}) and K based on the BLM principle to explore the application of the QICAR model in predicting the phytotoxicity of multiple metal elements. The results of this study can potentially be used for deriving guideline values for ecological toxicity and can provide fundamentals for the toxicology database.

2. Materials and methods

2.1. Experimental design

The acute toxicity of metal ions on wheat root elongation after short-term hydroponic culture was conducted using a simplified nutrient solution. The test solutions consisted of 19 element treatment groups, including 13 metals (Ag (I), Cu (II), Zn (II), Ni (II), Co (II), Cd (II), Mg (II), La (III), Sc (III), Cr (III), Cr (VI), V (V), Mo (VI)) and six metalloids (As (III), As (V), Se (IV), Se (VI), Sb (III), Sb (V)). Here, we refer to the metalloids collectively as metals for convenience. Each treatment group consisted of seven different concentration levels and a control (no-metal) sample. The concentration range of metal ions used ensured non-toxic to lethal concentrations, which were determined in preliminary tests (Table S1, support information), yielding a total of 456 treatments, each treatment consisting of three biological replicates.

2.2. Preparation of test solutions

The basic culture solution for the 19 metals was 0.2 mM $CaCl_2$ prepared in deionized water. The pH values of all test solutions were adjusted to 6.0 ± 0.1 with the buffer, 2-(*N*-morpholino) ethanesulfonic acid (MES), which is recommended by the United States Environmental Protection Agency (USEPA) due to its lack of complexation with metals and its lack of effect on the biological toxicity of metals. All reagents used were of analytical grade or higher. The solutions were equilibrated for 24 h prior to use.

2.3. Toxicity tests

Root elongation inhibition tests were performed with wheat (*Triticum aestivum* L., cv. Changfeng 2112) following the methods described in ISO 11269–1. Seeds of wheat were surface sterilized in 0.5% NaClO for 15 min, then thoroughly rinsed with deionized water, before being placed in a glass petri dish lined with filter paper moistened with sterile deionized water. Subsequently, the seeds were transferred to an incubator (25 ± 1 ℃, 80% relative humidity, in the dark) for 24 h. Seeds with white radicles protruding were transferred to a nylon net immobilized on the surface of a plastic culture tank filled with 250 mL of the test solution. Six seeds were placed on each tank, and all the culture tanks were randomized within the incubator chamber. The temperature was maintained at a 20/18 ℃ day/night temperature regime (under a 16-/8-h day/night photoperiod), under a light intensity of approximately 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The test solutions were changed every two days. Root and shoot elongation (reflecting the close dose-response relationship between toxicity exhibited by some elements and shoot elongation inhibition, according to Li et al., 2016) were measured after four days.

2.4. Scanning electron microscopic observations of root tips

Root samples were prepared for scanning electron microscopy (SEM) according to Xu et al. (2017). Wheat root tips (ca. 10 mm long) were cut off and placed in a cell culture plate with a pore size of 8 mm and a depth of 17 mm, and soaked in *tert*-butanol. After soaking, the cell culture plate, containing root tips and *tert*-butanol, was wrapped in parafilm and aluminum foil, prior to being moved to a freezer at −20 ℃ for 12 h. Lyophilization of root tips was conducted (−20 ℃, 14 Pa) using a vacuum freeze dryer (LGJ-18S; Beijing, China). Subsequently, the root tips were coated with Au (ca. 15 nm particle diameter) using an JFC-1100 (JEOL; Tokyo, Japan) ion sputterer. The root tips were then observed using a field emission SEM (ZEISS Gemini 300; Oberkochen, Germany) at 5 kV and a 9.3-mm working distance.

2.5. Chemical measurements

The pH values of all test solutions were measured with a pHS-3 C

(INESA; Shanghai, China) precision acidity meter. The free ion activity of the metals in the test solutions was calculated using Visual MINTEQ 3.1 (available at http://hem.bredband.net/b108693/). The input parameters included temperature, solution pH, and the concentrations of metal ions and other coexisting ions.

2.6. Data analysis and modeling

Wheat root elongation and shoot elongation were measured after four days of culture. Based on these parameters, the relative elongation (RE %) was calculated as follows:

$$\mathrm{RE}\ (\%) = \frac{E_t}{E_c} \times 100 \quad (1)$$

where E_t (cm) is the elongation of root or shoot in the metal ions treatment; E_c (cm) is the elongation of root or shoot in the control group.

The dose-effect relationship between metals toxicity concentration (shown as free metal ions activity) and RE was fitted by the log-logistic model (Eq. (2)), allowing calculation of the EC_{50} values (the median half-maximal effective concentration, representing the concentration corresponding to that which inhibited the wheat root or shoot elongation by 50%).

$$\mathrm{RE}\ (\%) = \frac{Y_0}{1 + e^{b(X-M)}} \times 100 \quad (2)$$

where X is the concentration of metal ions (free metal ion activity, μM) and Y_0, M (lg {EC_{50}}) and b are the fitting constants.

The relationships between toxicity and physicochemical properties were analyzed using the QICAR method. In the present study, 23 physicochemical properties of metals and metalloids were selected, namely atomic number (AN), atomic mass (AW), atomic radius (AR) (Wolterbeek and Verburg, 2001), covalent radius (CR), Pauling ionic radius (r) (Shannon and Prewitt, 1970), electron density (AR/AW) (Base and Mesmer, 1976), density (D), boiling point (BP), melting point (MP), ionization potential (IP) (Lide and Haynes, 2013), difference in ionization potentials between the ion oxidation numbers OX and OX^{-1} (ΔIP) (Lide and Haynes, 2013), ionic charge (Z), electronegativity (Xm) (Newman and McCloskey, 1996; Wolterbeek and Verburg, 2001), electrochemical potential (ΔE_0) (Kaiser, 1980; Lide and Haynes, 2013; Newman and McCloskey, 1996), first hydrolysis constant ($|\log(-K_{OH})|$) (Base and Mesmer, 1976), softness index(σp) (Pearson and Mawby, 1967), atomic ionization potential(AN/ΔIP), covalent index (Xm^2r), polarization force parameters (Z^2/r, Z/r^2, Z/r) and similar polarization force parameters (Z/AR, Z/AR^2).

In addition to the above physicochemical properties, three further properties representing the binding strength of metal ions to ligands, were selected. A total of 13 log K values, corresponding to 13 metals associated with ligands in wheat or barley roots, were selected from the literature. These log K values were obtained from published studies using similar experimental conditions and test species to the present study (Table 1). The other two parameters were the consensus scale for softness (σ_{Con}) and the normalized hard ligand scale (HLScale), which were selected from Kinraide (2009) (Table 1).

The Pearson correlation coefficient (r, shown in italics) and significance level (p) were used to evaluate the correlations between metal toxicity and physicochemical properties. The single-variable linear regression was used to quantify the relationship between toxicity and physicochemical properties, including log K, σ_{Con} and HLScale. To reduce the autocorrelation between physicochemical properties of the metal ions, principal component analysis (PCA) was used to generate a set of new variables, namely principal components (PCs). A relatively small number of PCs can express much of the total variability in the datasets. Thus, multi-variable linear relationships were established on the basis of PCA.

The F-statistic and significance level ($p < 0.05$) from analysis of variance (ANOVA) were to test the significance of regression equations between physicochemical properties and toxicity data; the coefficient of determination (R^2) and root-mean-square error (*RMSE*) were used for testing regression effects. The equation with maximum R^2 and F-statistic, and minimum *RMSE* was regarded as the optimal QICAR prediction equation. All correlations and regression relationships were calculated using SPSS version 24.0 (IBM, NY, USA).

Table 1
Logarithm of the conditional binding constant between metals and biotic ligands in the biotic ligand model (BLM) theory (log K), the softness consensus scale (σ_{Con}) and the normalized hard ligands scale (HLScale) for different elements, and half-maximal effective concentration {EC_{50}} values fitted using the dose-response relationship between relative root elongation and free ion activity of elements. {} refers to the free ion activity.

Ion	log K[a]	σ_{Con}[b]	HLScale[c]	{EC_{50}} μM
Ag^+	–	0.84	−0.28	0.27 {0.18–0.42}
Cu^{2+}	6.13	0.57	−0.09	0.36 {0.27–0.49}
Ni^{2+}	4.83	0.29	−0.41	1.31 {0.90–1.90}
*Y^{3+}	–	–	–	1.73 {1.49–1.97}
La^{3+}	6.07	−0.53	0.18	2.05 {1.70–2.48}
*Ce^{3+}	6.48	–	–	1.5 {1.28–1.72}
Sc^{3+}	–	–	–	3.61 {1.95–6.68}
Cd^{2+}	5.19	0.17	−0.48	4.47 {3.03–6.59}
Cr^{3+}	7.43	0.02	0.78	5.04 {3.33–7.63}
As^{5+}	4.91	*−0.16*	–	6.60 {4.12–10.57}
Co^{2+}	4.65	4.65	0.27	8.16 {5.76–11.56}
*Ga^{3+}	–	–	–	8.6
Zn^{2+}	4.06	−0.09	−0.41	16.93 {12.56–22.83}
As^{3+}	–	–	–	58.54 {31.54–108.65}
Se^{4+}	4.48	*0.00*	–	69.54 {48.30–100.05}
V^{5+}	4.06	*−0.13*	–	154.22 {82.30–288.98}
Cr^{6+}	3.89	*0.27*	–	177.63 {113.60–277.75}
Se^{6+}	3.45	*0.19*	–	346.54 {14.89–8065.93}
Mo^{6+}	–	–	–	1219.65 {663.27–2242.75}
Sb^{3+}	–	–	–	1741.20 {851.43–3560.81}
*Mg^{2+}	3.59	-1.02	−0.88	3363.30 {2955.17–3827.79}
*Sb^{5+}	3.08	–	–	4463.36 {1914.47–10,405.84}

*These ions were used in external verification, where the toxicity values of $Y^{3+}$and Ce^{3+} were from Gong et al. (2019) and that of Ga^{3+} were from Wheeler et al. (1993).

[a] The log K was obtained from previously published studies (Gong et al., 2020; Ji et al., 2020; Meng et al., 2019a; Song and Ma, 2017; Wang and Song, 2019; Wang et al., 2020, 2021).

[b] The σ_{Con} values were obtained from Kinraide (2009), where available. Otherwise, σ_{Con} values were computed from $\sigma_{Con} = 0.067I_P * E_0 + 0.0454D$ and are printed in italics, where D is density, E_0 is standard electrode potential and I_p is the first ionization potential (Barbalace (2008) Environmental Chemistry http://environmentalchemistry.com).

[c] The HLScale values were obtained from Kinraide (2009).

2.7. Model validation

Model verification was conducted using internal and external verification methods. Data for internal verification were modeling data. Data for external verification were obtained from the published literature and toxicology database; the test species and test conditions of these data were consistent with those of the present study. Four elements, Ce^{3+}, Ga^{3+}, Sb^{5+} and Y^{3+}, were screened for soft-hard ions and three elements, Ce^{3+}, Mg^{2+} and Sb^{5+} were screened for log K (Table 1). Verification was performed by substituting metal ion characteristics into the prediction equations; predicted toxicity values were compared with observed values and the differences between them were used to evaluate the predictive potentials of the selected models.

3. Results

3.1. Dose-response relationships

The dose-response curves between free ion activity of the 19 metal

ions and the corresponding relative elongation (RE) for wheat root and shoot are shown in Fig. S1. The R^2 and *RMSE* values were slightly greater for roots than for shoots ($p < 0.05$, *RMSE* shown in Table S2), except for V^{5+} and Sb^{5+}. Thus, the root RE served as the toxicity indicator to calculate the {EC_{50}} value.

The {EC_{50}} value ({} referring to the free metal ion activity) (Table 1), reflecting the toxicity power of metal ions to wheat, was fitted by the dose-response relationship between relative root elongation and free ion activity. The lower the {EC_{50}} value, the greater the toxicity. Accordingly, the following toxicity rankings toward wheat of 19 metal ions were obtained:

$$Ag^{+} > Cu^{2+} > Ni^{2+} > La^{3+} > Sc^{3+} > Cd^{2+} > Cr^{3+} > As^{5+} > Co^{2+} > Zn^{2+} > As^{3+} > Se^{4+} > V^{5+} > Cr^{6+} > Se^{6+} > Mo^{6+} > Sb^{3+} > Mg^{2+} > Sb^{5+}$$

The obvious difference in {EC_{50}} values (from 0.27 μM for Ag^{+} to 4463.36 μM for Sb^{5+}) revealed that different ions may exhibit different toxicity mechanisms toward wheat.

3.2. The relationship between toxicity and physicochemical properties of 19 metal ions

Correlations between the toxicity (expressed as log {EC_{50}}) and 23 physicochemical properties for 19 metal ions are shown in Fig. 1. log {EC_{50}} was significantly correlated with six physicochemical properties ($p < 0.05$), which were ranked in descending order of Pearson correlation coefficient ($r = 0.575$–0.487, $p = 0.010$–0.035) as Z, r, Z/AR, IP, Z/r, Z^2/r. The physicochemical properties were positively correlated with log {EC_{50}}, except for the ionic radius (r).

Single-variable linear regression equations between log {EC_{50}} and these physicochemical properties with $p < 0.05$ (Fig. 2 and Table 2) indicated that the best single-variable linear relationship was between Z and log {EC_{50}}. Furthermore, other properties such as r and IP also contributed to toxicity prediction to some extent. Overall, although these six properties were statistically significant ($p < 0.05$), they could explain only 23.7–33.1% of the toxicity variation ($R^2 = 0.237 - 0.331$).

Because of some autocorrelation between physicochemical properties of the metal ions, we also studied the multi-variable linear relationships between log {EC_{50}} and physicochemical properties, based on PCA. The newly independent variables (PC_x), created by PCA, were combinations of different properties, with a cumulative contribution rate of > 85%. The results indicated that PC_{x1}, including Z and r, achieved the best multi-variate linear relationship ($R^2 = 0.341$, $p = 0.009$). In fact, the multi-variable linear relationships, based on PCA, were not much different from the single-variable linear relationships by comparing R^2, *RMSE*, *F*-statistics and *p* (Table S3). Hence, investigating the relationships between wheat toxicity and the physicochemical properties of 19 metal ions cannot achieve the ideal effect for toxicity prediction.

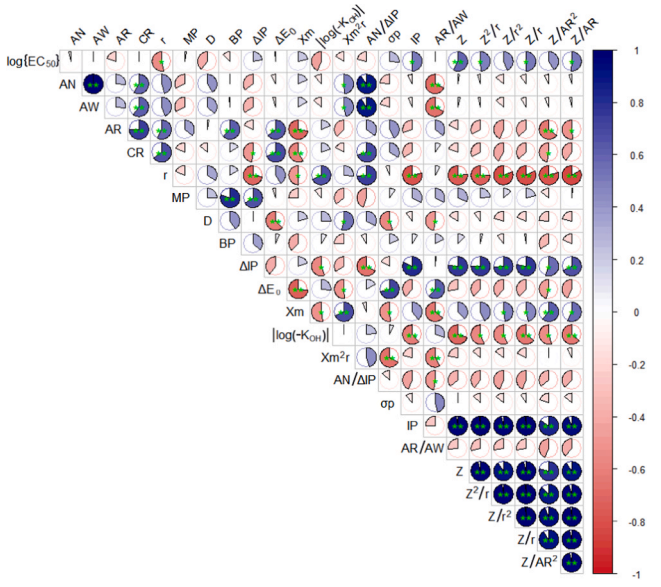

Fig. 1. Correlation coefficients between the logarithm of the half-maximal effective concentration {EC_{50}} value (i.e., log {EC_{50}}, with {} referring to free ion activity) and each of 23 physicochemical properties of 19 metal ions, where blue refers to a positive correlation and red refers to a negative correlation. The correlation varies with the color and the size of the pie; the darker the color and the larger the pie, the greater the correlation. * * $p < 0.01$, * $p < 0.05$; Pearson correlation.

3.3. The relationship between toxicity and physicochemical properties of soft and hard ions

The ions were divided into soft ions and hard ions according to Pearson's hard-soft-acid-base (HSAB) theory (Pearson and Mawby, 1967), and the correlation between toxicity and physicochemical properties was investigated separately for each ion grouping (Fig. S2 and Fig. S3). For the soft ions, toxicity was significantly correlated with eight properties: Z, D, |log (-K_{OH})|, Z^2/r, AR/AW, Z/AR, IP and Z/r ($p < 0.05$). Among them, D ($r = -0.839$, $p = 0.009$), |log ($-K_{OH}$)| ($r = -0.815$, $p = 0.014$) and AR/AW ($r = -0.757$, $p = 0.030$) were negatively correlated with log{EC_{50}} for soft ions, whereas the other properties were positively correlated. For the hard ions, toxicity was significantly correlated with seven properties: Z, IP, Z^2/r, Z/r, ΔE_0, Z/r^2 and Z/AR ($p < 0.05$). With the exception of ΔE_0 ($r = -0.743$, $p = 0.022$), the others were significantly positively correlated with log {EC_{50}} for the hard ions. Nevertheless, regardless of whether they were soft ions or hard ions, the *r* and *p* between toxicity and physicochemical properties were more significant than that for all 19 metals, as described in subsection 3.2.

The single-variable and multi-variable linear relationships for soft ions and hard ions were established separately. For the single-variable linear relationships (Fig. 3), Z showed the closest linear relationship with log {EC_{50}} for either soft or hard ions, and could explain 74.4% of the toxicity variation for soft ions ($R^2 = 0.744$, $p = 0.006$) and 74.3% for hard ions ($R^2 = 0.743$, $p = 0.003$). The R^2 values were significantly higher than the corresponding values obtained when soft and hard ions were considered together, as described in subsection 3.2 ($R^2 = 0.331$, $p < 0.05$). This result indicates that the toxicity prediction is significantly improved when ions are classified according to the HSAB theory.

The multi-variable linear relationships for soft and hard ions showed similar results. A comparison of the R^2, *RMSE*, *F*-statistic and *p* showed that there was little difference between the single-variable and multi-variable linear relationships. Given that the linear relationship involving multiple variables may result in greater error and autocorrelation, we chose the single-variable linear relationship with Z to be the final linear relationship, with Eq. (7) and Eq. (15) (Shown in bold in Table 2) recommended to be the best QICAR predictive equations for soft and hard ions, respectively.

3.4. The relationships between the toxicity of metals and their binding constants to ligands

The ligands in roots are an important avenue by which ions can exert their toxic effects. Since root tissues contain a large number of ligands, it is important to explore the relationships between toxicity and the binding of ions to ligands. Three properties representing the binding strength of ions to ligands were selected, including the parameter of the BLM (i.e., log *K*), the consensus scale of softness (σ_{Con}) and the normalized hard ligand scale (HLScale). Basically, the greater the log *K*, the closer the ions bound to the ligands, and the greater the toxicity. Similarly, the larger the σ_{Con} values, the softer the ions and the stronger the corresponding soft–soft interactions. For HLScale, the value 0.0

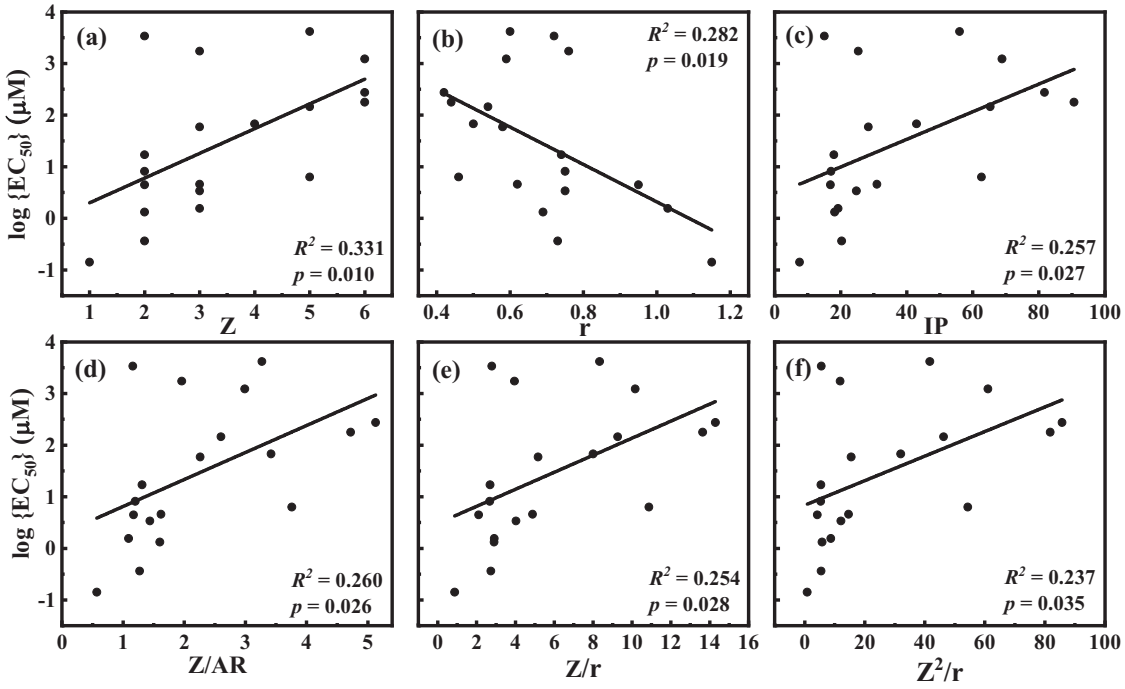

Fig. 2. Single-variable linear regression relationships between the logarithm of the half-maximal effective concentration {EC_{50}} value (i.e., log {EC_{50}}, with {} referring to free ion activity) of 19 metal ions and six physicochemical properties with $p < 0.05$ (Z, r, Z/AR, IP, Z/r, Z^2/r). IP represents the orbital energy of metal atoms required to form ions; Z/r and Z^2/r are polarization force parameters, which are a combination of the ionic charge and Pauling ionic radius; Z/AR is a measure of the polarization power of metal ions.

represents the average binding strength, while the values of –1.0 and 1.0 represent one standard deviation below or above the average value, respectively. The correlations and linear relationships between each of the three properties and log {EC_{50}} were established (Fig. S4 and Fig. 4). Some outliers such as Cr^{3+}, La^{3+} and Ag^{+}, were excluded from the relationships for the following reasons. The higher values of log *K* and HLScale for Cr^{3+} overestimated toxicity; the σ_{Con} value of La^{3+} underestimated toxicity due to its weak-binding to soft ligands; and the deviation of Ag^{+} in HLScale (the HLScale value of Ag^{+} was < –1.0, which is below one standard deviation) was due to its strong binding to soft ligands.

Log *K* and σ_{Con} were significantly correlated with log {EC_{50}} ($p < 0.05$), whereas the correlation between HLScale and log {EC_{50}} was not significant ($p = 0.218$) (Fig. S4). Log *K* exhibited a strong negative correlation with log {EC_{50}}, which indicated that the closer the binding of ions to ligands in the roots, the greater the toxicity. Meanwhile, the ranking of metals with respect to toxicity based on log *K* was almost identical to the toxicity rankings described in subsection 3.1, which further illustrated the close relationship between log *K* and toxicity (Fig. 4a). Although the rankings of metal toxicity based on σ_{Con} were not much different, some ions, such as Se^{6+} and Cr^{6+}, were not in the 95% confidence interval, resulting in a weaker negative correlation with log {EC_{50}} (Fig. 4b). In terms of toxicity prediction, compared with σ_{Con} ($R^2 = 0.413$, $p = 0.024$) and HLScale ($R^2 = 0.348$, $p = 0.218$), log *K* showed the closest fit ($R^2 = 0.803$, $p < 0.001$), with the best predictive equation shown below:

$$\log \{EC_{50}\} = -1.009 \log K + 5.835 \quad (3)$$

3.5. Validation of predictive abilities of QICAR models developed by different methods

We conducted internal and external verification to evaluate the accuracy of predictive models developed on the basis of log *K* and the soft–hard ion grouping methods (Fig. 5). The errors between the measured and predicted log {EC_{50}} of the log *K*-based model ($n = 14$) were within 1.5 orders of magnitude. Notably, the toxicity values of Cu^{2+}, Cd^{2+}, As^{5+} and Se^{6+} used for internal verification were well reproduced by this model with differences of less than 0.10-fold, and those of Sb^{5+} and Ce^{3+} used for external verification were within an order of magnitude. Analogously, for the model based on the soft–hard ion grouping method ($n = 13$ for hard and $n = 8$ for soft ions), the differences between measured and predicted log {EC_{50}} were within an order of magnitude except for Sb^{5+} and As^{5+}. The above results showed that the models based on both the log *K* and soft–hard ion grouping methods can provide reasonable predictions for the selected metals.

4. Discussion

The present study showed that root and shoot length were inhibited to varying degrees when wheat was exposed to 19 metal ions separately. We tested the inhibitory effects of the 19 metal ions on wheat roots and shoots. The dose-response relationships of root elongation with ions were superior as toxicity indicators to those of shoots, except for V^{5+} and Sb^{5+}. Generally, the roots, as the initial site of contact for metal ions, were damaged more than shoots and leaves of the same plant. In previous studies, acute toxicity tests on plants (such as barley and wheat) usually employed roots as toxicity indicators. However, Yeasmin et al. (2019), investigating the toxicity effects of As, Cr, Se and Mo on the roots and shoots of cucumber (*Cucumis sativus* L.), found that high-valence ions (As^{5+}, Cr^{6+}, Se^{6+} and Mo^{6+}) showed closer dose-response relationships with shoots, a finding which was different from the results reported from the current study. This discrepancy between the two studies may be related to differences in the plant species tested, the age of the plants tested and the duration of the testing period.

The 19 metal ions we tested showed different toxicity effects on wheat, with Ag^{+} exhibiting the most toxic effect, reflected by an {EC_{50}} = 0.27 μM. The high toxicity of Ag^{+} has been reported earlier. For example, Blamey et al. (2010) determined the median effective concentration for cowpea root elongation in Ag^{+} solutions, ranging from

Table 2
The single-variable linear regression equations between the physicochemical properties and the log {EC_{50}} of all metal ions, soft ions and hard ions. log {EC_{50}} is the concentration reducing root elongation by 50%, i.e., the half-maximal effective concentration {EC_{50}}, with {} referring to free ion activity. R^2 is the coefficient of determination, *RMSE* is the root-mean-square error and *p* is the statistical level of significance.

Type	Regression equation	R^2	*RMSE*	*F*	*p*	
All 19 ions	log {EC_{50}} = 0.479 Z − 1.181	0.331	1.13	8.405	0.010	(1)
	log {EC_{50}} = −3.628 r + 3.945	0.282	1.17	6.669	0.019	(2)
	log {EC_{50}} = 0.523 Z/AR + 0.288	0.260	1.19	5.980	0.026	(3)
	log {EC_{50}} = 0.027 IP + 0.459	0.257	1.19	5.875	0.027	(4)
	log {EC_{50}} = 0.165 Z/r + 0.484	0.254	1.19	5.787	0.028	(5)
	log {EC_{50}} = 0.024 Z^2/r + 0.835	0.237	1.21	5.278	0.035	(6)
Soft ions	log {EC_{50}} = 1.75 Z - 2.89	0.744	0.71	17.463	0.006	**(7)**
	log {EC_{50}} = −0.707 D + 6.612	0.704	0.76	14.248	0.009	(8)
	log {EC_{50}} = −0.348 | log($-K_{OH}$)| + 3.596	0.664	0.81	11.881	0.014	(9)
	log {EC_{50}} = 0.217 Z^2/r - 0.646	0.595	0.89	8.803	0.025	(10)
	log {EC_{50}} = −0.184 AR/AW + 4.509	0.572	0.92	8.031	0.030	(11)
	log {EC_{50}} = 1.879 Z/AR - 1.834	0.566	0.92	7.831	0.031	(12)
	log {EC_{50}} = 0.156 IP – 2.125	0.552	0.94	7.395	0.035	(13)
	log {EC_{50}} = 0.749 Z/r - 1.335	0.527	0.97	6.68	0.042	(14)
Hard ions	log {EC_{50}} = 0.66 Z – 1.458	0.743	0.55	20.102	0.003	**(15)**
	log {EC_{50}} = 0.032 IP – 0.182	0.645	0.65	12.708	0.009	(16)
	log {EC_{50}} = 0.027 Z^2/r + 0.347	0.611	0.68	10.977	0.013	(17)
	log {EC_{50}} = 0.19 Z/r – 0.094	0.573	0.71	9.403	0.018	(18)
	log {EC_{50}} = −0.873 ΔE_0 + 2.25	0.553	0.73	8.649	0.022	(19)
	log {EC_{50}} = 0.063 Z/r^2 + 0.47	0.445	0.81	5.611	0.050	(20)
	log {EC_{50}} = 0.476 Z/AR + 0.134	0.445	0.81	5.617	0.050	(21)

0.01 μM to 0.021 μM over the first 4–8 h of exposure. The results indicated that Ag^+ has rapid and highly toxic effects on cowpea roots at concentrations similar to those which are toxic to freshwater biota. Similarly, high phytotoxicity effects of Ni^{2+} ({EC_{50}} = 0.85 μM) and Cd^{2+} ({EC_{50}} = 7.5 μM) have also been reported by Kopittke et al. (2011) and Li et al. (2011), respectively. The highly toxic effects of these ions may be due to their accumulation in the outer cortex and the meristem of roots (Kopittke et al., 2017). Our SEM findings confirmed this inference (Fig. S5), where we found that the toxicity symptoms of wheat roots exposed to Cd^{2+} (Fig. S5(a)) or Ni^{2+} (Fig. S5(b)) included a large number of ruptures.

There are often differences in toxicities between different valence states of the same element (such as $\{EC_{50}\}^{3+}_{Cr}$ = 5.04 μM, $\{EC_{50}\}^{6+}_{Cr}$ = 177.63 μM (Table 1) in the current study). Song et al., (2014, 2017) obtained similar results by examining the toxic effects of Cr^{3+} and Cr^{6+} on barley, where $\{EC_{50}\}^{3+}_{Cr}$ was 7.94 μM and $\{EC_{50}\}^{6+}_{Cr}$ was 128 μM. Kopittke et al. (2012) suggested that ions exhibiting high toxicity accumulated in the root apex, resulting in tissue shedding, whereas ions with low toxicity continued to move, entering the stele. Compared with highly toxic Cd^{2+} and Ni^{2+}, the symptoms of roots exposed to Cr^{6+} showed smooth surfaces, with few ruptures (Fig. S5(c)), indicating that Cr^{6+} exhibited low toxicity. On the other hand, ions that are necessary for plant growth, such as Mg^{2+} and Ca^{2+}, are generally not toxic. They usually compete for biologically active sites with toxic ions, thereby reducing the toxicity of the latter (Clifford and Mcgeer, 2010). However, they can also inhibit plant growth at high concentrations. Kopittke et al. (2011) studied the toxic effects of 26 metals on cowpea root and reported a 50% inhibition in root elongation at approximately 14,000 μM for Mg^{2+}, indicating that the toxicity of Mg was far lower than that of toxic ions.

Based on the QICAR method, separation of ions on the basis of their classification according to HSAB theory improved the linear regression predictive effect for root toxicity. log {EC_{50}} of soft ions was significantly correlated with eight physicochemical properties. Z, which had the greatest correlation coefficient with log {EC_{50}} for soft ions, was also the most effective variable for establishing predictive equation. Z is one of the auxiliary criteria by which soft and hard ions were distinguished. Generally, the toxicity of soft ions is attributed to soft–soft interactions (Pearson, 1963, 1968), and soft receptors are usually characterized by low charge or large radius (Wolterbeek and Verburg, 2001). Thus, the ion charge is of overriding importance for the toxicity of soft ions. Hard ions exhibited significant correlations between log {EC_{50}} and seven physicochemical properties. Similar to the results from soft ions, the single-variable linear relationship based on Z of hard ions showed the beat prediction of toxicity effects.

In general, the establishment of a good toxicity predictive model requires a large volume of data, which are lacking for many metals. Modeling based on the soft–hard ions classification required more toxicity data for metal elements, which caused difficulties in achieving modeling. In addition, the types and number of metal elements decreased after soft–hard ion grouping. Although R^2 was improved, this effect may be the result of spurious correlation due to a reduction in sample number (i.e., the types of metal elements). As a consequence, the classification of soft–hard ions, using QICAR, needs to be verified over a larger number of elements.

Alternatively, we explored the relationship between metal toxicity and the physicochemical parameters σ_{Con}, HLScale and log *K*, based on the QICAR method. The parameters showed different predictive effects for metal phytotoxicity. The correlation between toxicity and σ_{Con} values was not significant when considering all metal ions ($p = 0.185$), but was improved after excluding La^{3+} and Mg^{2+} ($R^2 = 0.413$, $p = 0.024$). This indicated that σ_{Con} may not explain the toxicity of all ions. Kinraide (2009) investigated the relationship between the σ_{Con} values and toxicity of low-valence ions ($Z < 3$) and found that the combination of charge and σ_{Con} could predict toxicity well ($R^2 = 0.923$; *p*-value was not reported). However, this relationship was limited to only low-valence metal elements.

In the current study, we found that the toxicity predictive effect based on the HLScale method was even worse ($R^2 = 0.348$, $p = 0.218$) than that based on σ_{Con}. On the other hand, Kopittke et al. (2011) investigated the relationship between HLScale and rhizotoxicity of 26 metal ions to cowpea, and found that the closeness of fit was good ($R^2 = 0.658$, *p*-value was not reported). There are several possible reasons for these disparate results between the two studies. Firstly, roots from different plant species may contain different ligands. The cowpea (dicotyledon) used by Kopittke et al. (2011) may contain more of the same ligands that Kinraide (2009) used to calculate HLScale. Thus, the HLScale can predict the toxicity of metals to cowpea roots better than to the roots of the wheat (monocotyledon) we used. Secondly, there are limited data on metal species and toxicity involved in the HLScale method. The HLScale method we used only involves eight metal elements, including two subsequently excluded outliers (Ag^+ and Cr^{3+}). This small number of elements has a strong influence on the regression relationship between HLScale and metal toxicity. Thirdly, there are different phytotoxicity mechanisms for different metal elements. Previous studies have pointed out that binding of metal ions to hard ligands is an important, non-specific mechanism that causes toxicity directly by

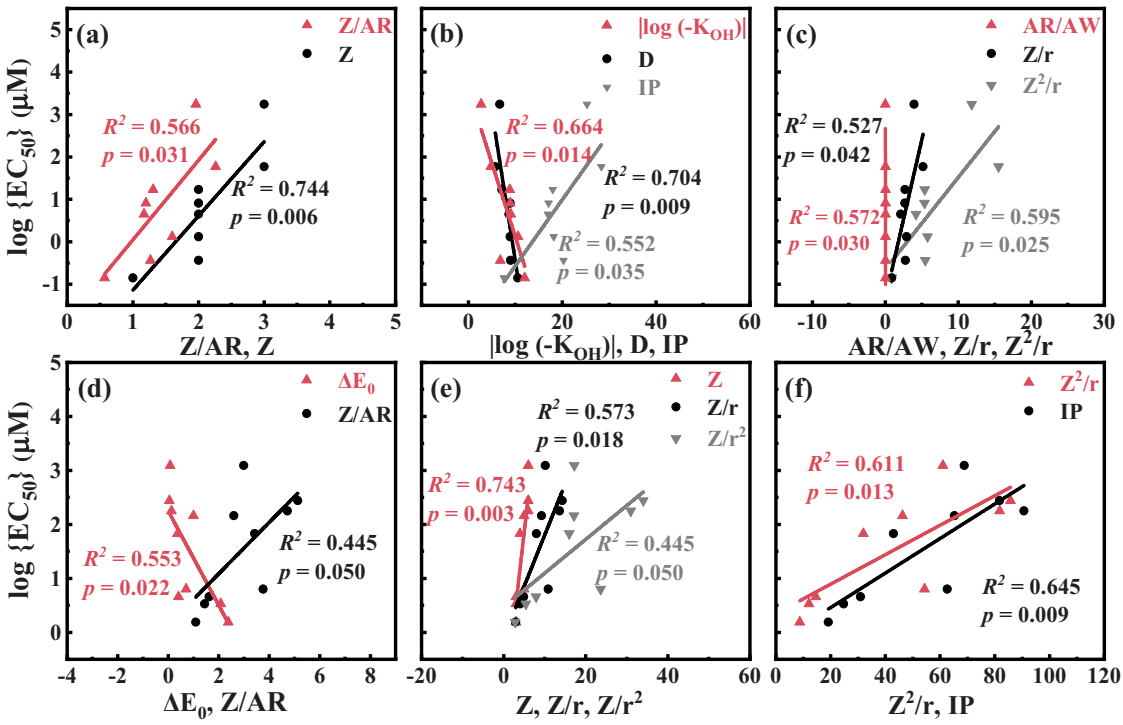

Fig. 3. Single-variable linear regression relationships between toxicity values (expressed as logarithm of the half-maximal effective concentration {EC_{50}} value, with {} referring to free ion activity) of soft–hard ions and their correlated physicochemical properties. (a-c) eight properties with $p < 0.05$ (Z, D, |log ($-K_{OH}$)|, Z^2/r, AR/AW, Z/AR, IP and Z/r) for soft ions. (d-f) seven properties with $p < 0.05$ (Z, IP, Z^2/r, Z/r, ΔE_0, Z/r^2 and Z/AR) for hard ions. D represents the density of metal ions; |log($-K_{OH}$)| is a measure of the affinity of metal ions in forming metal hydroxides; AR/AW is a measure of the electron density of ions, where AR is atomic radius and AW is atomic weight; ΔE_0 is the absolute value of the electrochemical potential in volts between the ion with a certain oxidation number OX and its first stable, reduced form, the elemental state OX^{-1} of the metal.

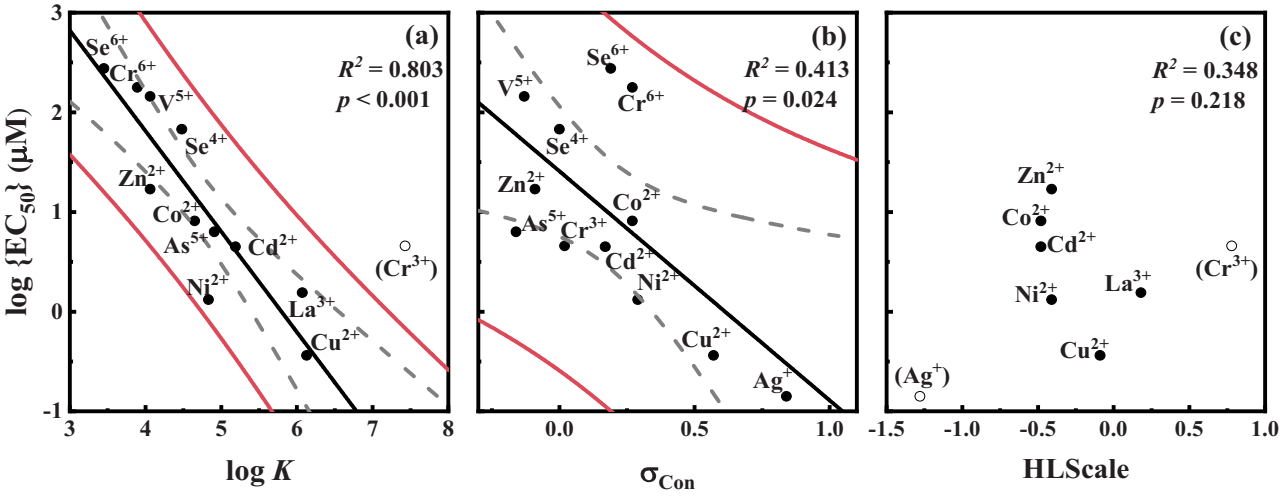

Fig. 4. (a) Linear regression relationships between the logarithm of the half-maximal effective concentration {EC_{50}} values (i.e., log {EC_{50}}, with {} referring to free ion activity) and the logarithm of the conditional binding constant (log *K*) based on the biotic ligand model (BLM) theory; (b) linear relationships between log {EC_{50}} and the softness consensus scale (σ_{Con}); (c) linear relationships between log {EC_{50}} and the normalized hard ligands scale (HLScale). The dotted and red lines represent the 95% confidence interval and prediction interval bands, respectively.

inhibiting the controlled relaxation of the cell wall required for cell elongation (Kopittke et al., 2011). However, this may not apply to all ions. In other words, toxicity may not be associated with the binding strength to the hard ligands for all ions. For example, some soft ions, such as Ag^+, may strongly bind to the R-S-functional group (soft ligand) in metallothionein, and in this process, the metal complex is bound to glutathione and sent to the vacuole, thereby exerting toxicity effect (Bell et al., 2002). Analogously, Kopittke et al. (2012) investigated the toxicity mechanism of As^{3+} in cowpea roots and found that the phytotoxicity of As^{3+} is due to its reaction with dithiol groups on proteins and the inhibition of enzyme reactions that require free sulfhydryl groups (soft ligand) (Horswell and Speir, 2006). The theory of different phytotoxicity mechanisms for different metal elements is also supported in the current study by the SEM images (Fig. S5), whereby the soft metal ions, Cd^{2+} (Fig. S5(a)) and Ni^{2+} (Fig. S5(b)), seriously damaged the roots and were more toxic than Cr^{6+} or Mg^{2+}, indicating that they bound

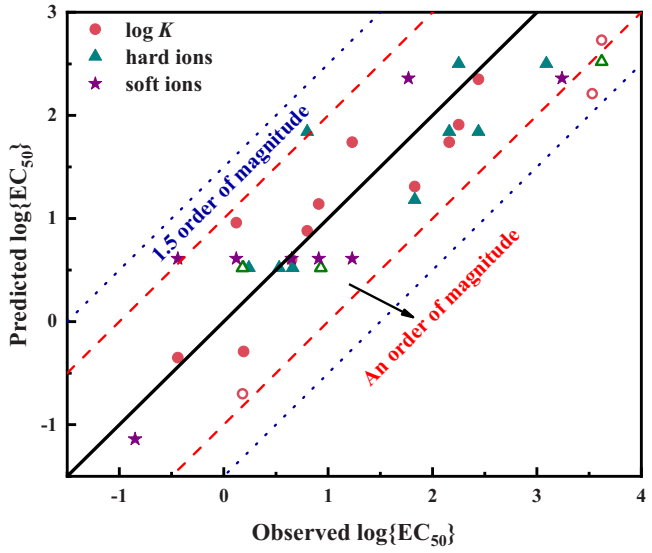

Fig. 5. Relationships between observed and predicted toxicity values obtained by the QICAR established on the basis of soft–hard ion grouping and log *K* methods. *K* is the conditional binding constant in the biotic ligand model. Toxicity is expressed as the logarithm of the half-maximal effective concentration {EC_{50}} values, with {} referring to free ion activity. The black solid line represents the 1:1 line, and the red and blue dashed lines indicate 1 and 1.5 order of magnitude differences between predicted and observed toxicity values, respectively. The solid and hollow shapes indicate internal and external verifications, respectively ($n = 14$ for log *K*, $n = 13$ for hard ions and $n = 8$ for soft ions).

strongly to the soft ligands. On the contrary, Cr^{6+} (Fig. S5(c)) and Mg^{2+} (Fig. S5(d)) were less toxic and bound only weakly to the hard ligands, indicating that the binding strength of these metal ions to different ligands varied.

Our results confirmed that the *K* value, based on BLM theory, can predict the phytotoxicity caused by metal elements well ($R^2 = 0.803$, $p < 0.001$). The BLM considers all ligands together, calculating parameters based on toxicity. This parameter (*K*) is obtained for a specific plant species tested, so it has more advantages in predicting phytotoxicity. However, the available *K* values are limited as the BLM involves only a few metal elements. In addition, although some elements, especially those which are essential for plant growth, such as Mg and Ca, have *K* values, these need to be excluded in the establishment of QICAR due to their low toxicity or non-toxic nature. Further study on the toxicity of different elements toward different organisms is required. A common ligand parameter suitable for all organisms by averaging and normalizing their corresponding *K* values may further improve the application of this parameter in QICAR.

5. Conclusion

In this study, we investigated the phytotoxicity of different metal ions to wheat in a hydroponic experimental system. The soft–hard ion grouping, log *K*, σ_{Con} and HLScale were employed to explore the application of the QICAR model in predicting phytotoxicity. The results indicated that the method distinguishing between soft and hard ions achieved more accurate prediction for metal phytotoxicity than that based on all 19 metal ions. Compared with σ_{Con} and HLScale, the log *K*, taking into account specific plant species and comprehensive root ligands, could provide close prediction of the phytotoxicity of metals to wheat. Nevertheless, further research efforts are required to verify the conclusion as limited data are available for the BLM and the impact factors were not considered. In addition, the measures of phytotoxicity of metal ions obtained in the present study were from a hydroponic system. Further work needs to be carried out using soil, which will help to clarify the toxicity mechanisms and provide an important reference for deriving guideline values for ecological toxicity.

CRediT authorship contribution statement

Luo Xiaorong: Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft. **Wang Xuedong:** Conceptualization, Methodology, Formal analysis, Investigation, Project administration, Writing - original draft, Writing - review & editing, Funding acquisition **Tang Yujie:** Investigation, Validation **Yanju Liu:** Software, Writing - review & editing. **Wang Ying:** Writing - review & editing.

Funding

The work was financially supported by the National Natural Science Foundation of China (grant number 41877496).

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112443.

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