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Envronmental toxicology

Development of QICAR Models to Address the Lack of Toxicological Data for Technology-Critical Elements

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Abstract: recent industrial developments have resulted in an increase in use of so-called technology-critical elements (TCEs), for which the potential impacts on aquatic biota remain to be evaluated. In the present study, quantitative ion character activity relationships (QICARs) have been developed to relate intrinsic metal properties to their toxicity towards freshwater aquatic organisms. In total, 23 metal properties were tested as predictors of acute ec_{50} values for 12 *data-rich* metals, for algae, daphnids and fish (with and without species distinction). Simple and multiple linear regressions were developed using the toxicological data expressed as a function of the total dissolved metal concentrations. The best regressions were then tested by comparing the predicted ec_{50} values for the tces (germanium, indium, gold, rhenium and platinum group elements – pges – which include iridium, platinum, palladium, rhodium, and ruthenium) with the few measured values that are available. The eight 'best' QICAR models (adjusted $r^2 > 0.6$) used $\chi_m^2 r$ as the predictor. For a given metal ion, this composite parameter, also known as the covalent index, is a measure of the importance of covalent interactions relative to ionic interactions. The toxicity of the tces was reasonably well predicted for most of the TCEs, with values falling within the 95 percent prediction intervals for the regressions of the measured versus predicted ec_{50} values. Exceptions included au(i) (all test organisms), au(iii) (algae and fish), pt(ii) (algae, daphnids), ru(iii) (daphnids) and rh(iii) (daphnids, fish). We conclude that qicars show potential as a screening tool to review toxicity data and flag 'outliers', which might need further scrutiny, and as an interpolating or extrapolating tool to predict TCE toxicity.

KEYWORDS:Platinum group elements, precious metals, Ge, In, Re, Quantitative Ion Character Activity Relationships

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INTRODUCTION

Until the beginning of the 21st century, industrial uses of nonferrous metals were mostly focused on 'base metals', *i.e.*, elements such as copper, lead, nickel or zinc, that are found in the first four rows of the Periodic Table of the Elements. However, recent developments in areas such as electrical engineering, computing, renewable energy technologies and medicine have expanded this range of metals. Gold, indium, germanium, rhenium and the platinum group elements (PGEs) belong to this group of newly-used 'Technology-Critical Elements' (TCEs), where the term 'critical' refers to the increasing technological importance of these elements and their limited number of sources (Gulley et al. 2018). Extensive ecotoxicological databases exist for the base metals, for example in the US-ECOTOX database (US EPA 2020) and the European ECHA database (ECHA 2020), and they are used in environmental risk assessments around the world. In the case of most TCEs, however, these data are still very scarce, limiting our ability to properly assess their possible environmental impacts.

When new organic molecules are synthesized and are poised to enter commerce, Quantitative Structure-Activity Relationships (QSARs) can be used to predict their environmental fate and toxicity. These models relate the properties of the new molecule (e.g., water solubility, octanol-water partition coefficient) to its biological reactivity (Boethling and Mackay 2000). For metals, the development of such models has been less comprehensive since far fewer 'new' inorganic compounds have been introduced commercially compared to new organic chemicals. However, there have been a number of successful efforts to relate the toxicity of various base metals to their ionic properties (Walker et al. 2013). These Quantitative Ion Character Activity Relationships (QICARs)

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have used ion properties such as electronegativity, softness, ionization potential and electrochemical potential as predictors (Kinraide 2009; Wang et al. 2016; Wolterbeek and Verburg 2001). To date, these models have been developed for and tested with *data-rich* metals and target species such as algae (Fujiwara et al. 2008; Hickey et al. 1991; Mu et al. 2014), daphnids (Kaiser 1980; Tatara et al. 1997; Wu et al. 2013) and fish (Ownby and Newman 2003; Wu et al. 2013). However, they have not been systematically tested with *data-poor* metals such as the TCEs.

The objective of the present study was to develop robust organism-specific QICAR models for a wide range of *data-rich* metals, and then apply them to *data-poor* TCEs (germanium, indium, gold, rhenium and platinum group elements – PGEs – which include iridium, platinum, palladium, rhodium, and ruthenium) to predict their acute aquatic toxicity (algae, daphnids, fish) on the basis of their ionic properties. Many of the TCEs exist in oxidation states of +III or higher, which is not the case for the majority of the *data-rich* elements. Our study was thus designed to test whether this difference in oxidation state would affect the ability of QICARs developed for *data-rich* elements to predict the toxicity of the TCEs.

The toxicological data used to construct the models and the values predicted with the models were expressed as dissolved EC_{50} values (total dissolved metal concentration that elicits a 50% response in the studied population). Given the important influence of metal speciation on metal bioavailability and toxicity, a second study was also conducted to predict toxicity from free metal ion concentrations and ionic properties. These results will be published in a follow-up paper.

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MATERIALS AND METHODS

QICAR modeling involves establishing a mathematical relationship between two separate databases. Here, these databases are a compilation of the ion characteristics of the metals in the two groups (the training set of *data-rich* metals and the prediction set of *data-poor* TCEs), and a compilation of the toxicological data that are available for each group.

Building of the metal characteristics database

To construct the metal characteristics database, the most significant metal characteristics that had been previously reported in QICAR studies were compiled (Walker et al. 2013). These characteristics can be grouped into five categories, i.e. *physical properties* (atomic weight – AW; atomic volume – V; density - ρ; melting point – MP; polarizability - α; molar refractivity – MR); *electronic structure* (atomic number – AN; ionization energy and potential – IP and ΔIP, respectively; electron affinity – E*); *redox properties* (oxidation number – ox; standard electrode potential -E^0 and electrochemical potential, ΔE^0); *binding properties* (ionic radius – r; covalent radius – CR; Van der Waals radius – Vdw; electronegativity – χ_m) and various composite *indices* (ionic potential – z/r, where z = the charge on the ion; ionic index – z^2/r; covalent index $\chi_m{}^2 r$; covalent binding stability – $\Delta\beta$; Pearson softness parameter – σ_p; absolute value of the first hydrolysis constant – log K_{OH}; AN/ΔIP). These ion characteristics were mostly found using the *Handbook of physics and chemistry* (Rumble 2018). Exceptions included σ_p (Ahrland 1968; Williams et al. 1982), log K_{OH} (Academic Software 2001; Smith et al. 2004) and $\Delta\beta$, which was either retrieved from Newman and McCloskey (1996) or

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calculated from stability constants found in the IUPAC (Academic Software 2001) or NIST databases (Smith et al. 2004).

The first step of the database building was thus to compile the data and verify whether the metal characteristics were available for the metal training dataset, i.e., for Ag, Ca, Cd, Co, Cu, K, Mg, Mn, Na, Ni, Pb, Zn and for the studied TCEs, i.e. Au(I), Au(III), Ge (IV), Ir(III), In(III), Pd(II), Pd(IV), Pt(II), Pt(IV), Re(V), Re(VII), Rh(III), Ru(III), Ru(IV). When one of the ion characteristics was missing for a particular metal, the characteristic was eliminated from the modeling process. The second step was to perform a Principal Component Analysis (PCA) to determine correlations among metal characteristics, in order to reduce the number of characteristics to be included in the models. For the construction of the QICARs, we prioritized non-redundant metal characteristics that were available for the training metals and that best bracketed the characteristics for the *data-poor* TCEs.

Preparation of the toxicity database

Freshwater toxicological data were obtained from different sources, such as national curated databases and from specific searches of the recent literature for updated or missing data. Data were compiled for short-term exposures conducted with algae (48 h, 72 h or 96 h, depending on the species), daphnids (48 h) and fish (96 h). Their measured responses (growth inhibition; immobilization; mortality) were noted, along with the species that had been tested, the experimental conditions (exposure duration, temperature) and details regarding the composition of the exposure media. For model construction, we only retained EC_{50} values that had been derived from toxicity tests where the metal concentrations had been measured (i.e., not just nominal metal

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concentrations). The compiled EC_{50} values were analyzed for outliers using boxplot representations (data not shown). Values were considered as outliers when they fell outside the interval of three times the interquartile range (Addinsoft 2018).

Growth inhibition was the only endpoint used for algae, whereas both immobility and survival were used as toxicity endpoints for daphnid tests. All the daphnid EC_{50} values were pooled for a given metal, regardless of the endpoints, since no outliers were revealed with this approach. In the Cu and Cd fish databases, the toxicity tests were performed with different life stages (from larvae to adults). Since the study of outliers did not reveal any trends that would have justified the exclusion of certain life stages for any metals, results for these life stages were pooled.

Model building

Simple linear regressions (SLR) were constructed between each selected training metal characteristic (as well as its logarithm) and the logarithms of the mean measured EC_{50} values, without species distinction. The log-transformation of the metal characteristics was additionally performed to reduce their variances as compared to that of the series of -log EC_{50} values. A second set of SLRs was constructed with species-specific EC_{50}s for the species for which the number of independent tests was the largest, *i.e.*, *Pseudokirchneriella subcapitata* for algae, *Daphnia magna* and *Ceriodaphnia dubia* for daphnids, and *Oncorhynchus mykiss* and *Pimephales promelas* for fish.

In a second step, multiple linear regressions (MLR) were performed using a stepwise approach, with all the selected metal characteristics and their logarithms as input variables. To reduce the number of tested variables, we compared the variances of the -log EC_{50} values with the variances of the metal characteristics and their log-transformed

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values, using the Fisher test ($\alpha = 0.05$). When the original values of a given metal characteristic had a variance similar to that of the -log EC_{50} values, the log transformed metal characteristics were not considered. The 'entry' parameter significance of the MLR was tested using a Student t test at $\alpha = 0.05$ whereas the removal of a variable was tested at $\alpha = 0.1$. Metal parameter multicollinearity was assessed using the Variance Inflation Factor (VIF; < 10 as an indication of negligible collinearity). The 'best' metal characteristics were then selected based on several goodness of fit statistics for the developed model: the highest adjusted r^2 (r^2_{adj}); the lowest root mean square error (RMSE);

the highest F value with a p value lower than 0.05 (the F and p values being obtained with an Analysis of Variance (ANOVA) to test the significance of the model to predict the -log EC_{50} values based on the selected metal characteristics); the closeness of the Mallows Cp coefficient to the target value of Cp (Cp= k+1, k being the number of variables in the model), and the lowest AIC (Akaike's Information Criterion). As was the case for the SLRs, MLRs were constructed with and without species distinction.

The chosen models were then analyzed to determine if the assumptions for linear regression were met. The normality of the residuals was tested using the Shapiro-Wilk test ($\alpha = 0.05$) whereas their lack of autocorrelation was assessed using the Durbin-Watson (DW) test (*Supporting information* and Table SI-1). Results of both tests are provided directly in the text only when the assumptions were not verified. Otherwise, statistical results are given in Table SI-2. Once developed, the models were tested using the log-transformed average EC_{50} values for the TCEs. Model building and correlation statistics were performed using XLSTAT 2018.2 and higher (Addinsoft 2018).

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RESULTS

Metal characteristics database

Almost all the metal characteristics were found for the training metals, with the exception of electron affinity (E*) values for Cd, Mg, Mn and Zn, which were indicated to be 'not stable' (Handbook of Chemistry and Physics – Rumble (2018)) (Table SI-3). For the studied TCEs, no IP or ΔIP values were found for Au(III), Ir(III), Pd(IV), Pt(IV), Re(V), Re(VII) and Ru(IV), whereas E^0 and ΔE^0 were missing for Re(V) (Table SI-4). However, the lack of data was the most acute for Δβ, σ_p and log K_{OH}, with data available for only 5 out of 17 for Δβ, 8 out of 17 for σp and 11 out of 17 for log K_{OH}, which thus precluded the use of Δβ, σ_p and log K_{OH} in the subsequent model development. This situation reflects the lack of thermodynamic data describing the binding of these TCEs to inorganic ligands.

The metal characteristics that remained at this stage for building the QICAR models were AW, V, ρ, MP, α, AN, r, CR, Vdw, $\square_m$, z, z/r, z^2/r and $\square_m^2 r$. Among these characteristics, only the values for V, AN, CR and Vdw bracketed those of the TCEs (Table SI-5), and they were prioritized over the other characteristics to construct the models in the subsequent steps. At this point, a PCA was performed with the 14 remaining metal characteristics to identify possible redundancies among them (Figure 1). A first group of metal characteristics was composed of V, which correlated with α (r = 0.889), CR (r = 0.931) and Vdw (r = 0.720). V was chosen because the range of its values for the training metals bracketed the V values for the studied TCEs and it represents two variables (V = AW/ρ). It was also chosen in preference to CR because of the potential difficulty in differentiating between CR and r. A second group of characteristics was

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constituted of AN, which correlated with AW (r = 0.999) and ρ (r = 0.908). AN was chosen because, as was the case for V, its range of values for the training metals included the AN values for the studied TCEs. In the third PCA group, z^2/r strongly correlated with z (r = 0.984) and z/r (r = 0.937). The ratio z^2/r was chosen due to its prominence in earlier QICAR models (Newman and McCloskey 1996; Van Kolck et al. 2008). The electronegativity, □$_m$, correlates with ρ (r = 0.797), which had already been eliminated, and with □$_m^2$r (r = 0.777); both parameters were kept but were not used concurrently in the models. MP and r did not correlate with any other characteristics and were thus also kept. To summarize, V, AN, z^2/r, χ_m or $\chi_m^2 r$, MP and r were retained to construct the QICAR models.

Toxicity database

Algae. Table SI-6 presents a summary of the algal toxicity data for the training metals. The lowest EC_{50} values are those obtained for Ag, with data ranging between 4.6×10^{-9} and 1.7×10^{-7} M, and for exposure experiments conducted with various algal species. The cations Ca, K, Mg and Na have the highest EC_{50} values, within the mM range, which came predominantly from tests performed with *P. subcapitata* (except *Chlorella* sp. for the Mg data). Cadmium, Co, Cu, Ni and Zn have comparable EC_{50} values (low μM) whereas Mn (5.6×10^{-5} M – 1.1×10^{-3} M) has higher EC_{50} values. These toxicological data were obtained from experiments performed on several algal species, except in the case of Co and Zn, the datasets for which were composed only of *P. subcapitata* values. EC_{50} values considering all algal species at the same time and *P. subcapitata* values only were analyzed for outliers, the numbers of which varied as a function of the metal (Table SI-6). The toxicological data for the studied TCEs were

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much less extensive than those for the training metals (Table SI-7). Indeed, the largest dataset was the one for Pd(II) with only five EC_{50} values and was composed of tests performed on three different species, including *P. subcapitata*. Both Pd species, Pd(IV) and Pd(II), were found to be the most toxic of the TCEs, with EC_{50} values of 2.8×10^{-8} M for Pd(IV) and $(1.7 \pm 1.2) \times 10^{-7}$ M for Pd(II) (mean ± standard deviation when n > 2). Six TCEs [Au(III), In(III), Rh(III), Ru(III), Ru(IV), Pt(IV)] have EC_{50} values ranging between 1.1×10^{-6} M [Ru(IV)] and 1.4×10^{-5} M ([In(III)]. The least toxic elements among the studied TCEs are Au(I), Ge(IV), Pt(II) and Re(VII), with EC_{50} values between 1.0×10^{-4} M [Au(I)] and 1.2×10^{-3} M [Ge(IV)].

Daphnids. The EC_{50} values for the training metals used to construct the daphnia models are presented in Table SI-8; comparable data for the TCEs are presented in Table SI-9. Among the studied metals, the cations Ca, Mg, Mn and Na are the least toxic with average EC_{50} values of about 1×10^{-2} M. Zinc and Co have comparable toxicity, with average EC_{50} values of $(1.4 \pm 1.1) \times 10^{-5}$ M for Zn and $(4.6 \pm 2.7) \times 10^{-5}$ M for Co. Cadmium, Cu and Pb toxicity values are in the µM range whereas Ag is the most toxic of the studied metals with EC_{50} values ranging between 1.0×10^{-9} M and 1.5×10^{-6} M. Several daphnia species were examined for each metal; for example, Ni toxicity was assessed using 13 different species. Nevertheless, *D. magna* and *C. dubia* were the most studied species. Each metal data set was examined for outliers, first by taking into account all species, then only *D. magna* and finally *C. dubia* (Table SI-8). Among the studied TCEs, Au(I) has the lowest EC_{50} value (6.9×10^{-7} M), whereas the toxicity of Au(III), Pd(II) and Pt(IV) are in the µM range. The least toxic TCEs are Ir(III), Pt(II),

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Rh(III) and Ru(III), with EC_{50} values ranging between 1.6 x 10^{-5} M [Ir(III)] and 1.9 x 10^{-4} M [Ru(III)] (Table SI-9).

Fish. The compiled toxicity test data are presented in Table SI-10 (training metals) and in Table SI-11 (TCEs). Among the training elements and taking into account all the fish species, Ag is the most toxic metal with an average EC_{50} value of (2.0 ± 3.4) × 10^{-7} M. The least toxic cation is Na with EC_{50} values ranging from 9.6 to 11 x 10^{-2}, followed by Mg, Ca and K, the EC_{50} values of which are in the 1 x 10^{-2} M range. Lead and Cu were found to be more toxic (µM range) than Mn, Cd, Ni and Zn, based on their average EC_{50} values. However, a wide dispersion around the average EC_{50} values was observed for Cd (five orders of magnitude) and Ni (four orders of magnitude), which suggests that such comparisons of metal toxicity should be undertaken with caution. Although many fish species (n=24) were present in the database, the most studied species were *O. mykiss* and *P. promelas* for each of the studied metals. Pd(IV) is the most toxic TCE for fish with an EC_{50} value of 1.4 x 10^{-6} M, followed by both Au species with EC_{50} values of 2.0 x 10^{-5} M for Au(I) and 4.6 x 10^{-5} M for Au(III). Ge(IV), Mn(II), Pd(II), Rh(III) and Pt(IV) have EC_{50} values within the concentration range between 1.3 x 10^{-4} M [Pt(IV)] and 9.9 x 10^{-4} M [Ge(IV)].

Modeling

All-species Algae and P. subcapitata models (designated model A- and model PS) Fourteen SLRs and four MLRs were constructed with the metal characteristics and the -log EC_{50} values compiled for algae (without species distinction) exposed to the studied training metals. The best model (Model A12) was the SLR built with log $\square_n{}^2r$ as the predictor, yielding a r^2_{adj} of 0.89 and a RSME of 0.722 (Table SI-12;

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Figure 2A; Figure SI-1). Among the constructed MLRs, Model A16 had a very high r^2_{adj} (0.93), a lower RMSE than Model A12 and a VIF under 10 (Table SI-13). However, testing model A16 with the TCEs demonstrated that their measured toxicities were poorly predicted, with Au(I), Ge(IV), Pd(IV), Pt(II), Re(VII) and Ru(IV) all falling outside the prediction interval bands (Figure SI-2). In contrast, the toxicity of the studied TCEs was reasonably well described with Model A12, except for Au(I), Au(III) and Pt(II), which lie outside the predicted error ranges (Figure 2B).

SLRs and MLRs were also constructed with -log EC_{50} values restricted to those for *P. subcapitata* (data not shown). Log $\square_m^2$r was again the best predictor of metal toxicity towards *P. subcapitata* (Model PS) with a higher r^2_{adj} of 0.94 and a lower RSME of 0.500 than the *All-species* model A12 (Figure SI-3A). The residuals were not normally distributed ($p = 0.029$) but they were not autocorrelated (DW = 1.864). Six of the TCEs fell reasonably close to the 1:1 predicted versus measured values line (Figure SI-3B).

All-species daphnids, D. magna and C. dubia models (designated models D-, DM and CD) Among the tested metal characteristics, log $\square_m^2$r was again the best predictor of the -log EC_{50} values (without species distinction) for daphnids exposed to the training metals, as indicated by model D12 (Table SI-14; Figure 3A; Figure SI-4). Among all the D models, its adjusted r^2 value was the highest (0.86) and the RMSE was the lowest with a value of 0.77. The measured and predicted -log EC_{50} values for the training metals fell close to the 1:1 line (Figure 3B). This was also the case for the -log EC_{50} values of Pd(II) and Pt(IV). However, the predicted toxicities of the other TCEs were greater than what has been determined in laboratory toxicity tests, especially in the case of Au(I) for which there is a factor of about 10^3 between measured and predicted values. The best MLR

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model was model D16, which used χ_m and MP × z^2/r to predict the daphnia EC_{50} values and yielded an adjusted r^2 of 0.89 (Table SI-15). When measured TCE EC_{50} values were compared with those predicted using model D16, satisfactory results were found with three TCEs [Au(III), Pd(II) and Pt(II)] but unrealistic predicted EC_{50} values were calculated for Ir(III), Ge(IV), Pt(IV) and Ru(III) (Figure SI-5).

Species-specific models were also constructed based on *D. magna* and *C. dubia* toxicity values (both SLRs and MLRs – data not shown). For both species, the best models were SLRs built with log $\chi_m^2 r$ as the predictor. Both models, *i.e.* Model DM for *D. magna* (Figure SI-6A) and Model CD for *C. dubia* (Figure SI7-A), had a high adjusted r^2 of 0.86. The -log of the measured EC_{50} values of the TCEs fell within the prediction interval bands of model DM, but again with the exception of Au(I) (Figure SI6-B). No TCE toxicity data were available to test our model CD against measured values (Figure SI-7B, Table SI-9).

All-species fish, O. mykiss and P. promelas models *(designated models F-, OM and PP)* The best SLR model to describe the toxicity of the training metals to fish, without species distinction, was model F12 (r^2_{adj} of 0.89) with log $\chi_m^2 r$ as the input variable (Table SI-16; Figure 4A; Figure SI-8). However, plotting the measured metal toxicity values as a function of the model F12-predicted toxicity values showed that the predicted -log EC_{50} values of the TCEs matched poorly with the measured values (Figure 4B). The MLR that best described the fish response to exposure to the training metals included χ_m and the interaction term MP × z^2/r (Model F16, Table SI-17). Its adjusted r^2 was 0.89 with a RMSE of 0.664. However, comparison of measured and predicted -log EC_{50} values for the TCEs shows that the toxicity of the TCEs was not well predicted,

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with unreasonably high predicted EC_{50} values (> 10 M) for Ge(IV), Pd(IV) and Pt(IV) (Figure SI-9).

The simple regression models for *O. mykiss* were developed based on the -log EC_{50} values of Ag, Cd, Cu, Mn, Na, Ni, Pb and Zn as no values were available for Ca, Co, K or Mg (data not shown). The model OM using log $\square_m^2 r$ as the entry variable was the best descriptor of the toxicity of the TCE toward *O. mykiss* with an adjusted r^2 of 0.63 and a RMSE of 1.334 (Figure SI-10A). The few TCEs for which toxicity values were available fell within the rather wide prediction interval bands of the model (Figure SI-10B).

The simple linear regression model for *P. promelas* was built with -log EC_{50} values of the eleven training metals but without Ca, for which no data were available (data not shown). Among the studied metal characteristics, log $\square_m^2 r$ was the best variable to describe the toxicity of the training metals (Figure SI-11A). The obtained model PP has an adjusted r^2 of 0.87 and a RMSE of 0.661. Since no EC_{50} values were available for *P. promelas* exposed to the TCEs, the ability of the model to predict their toxicity could not be not tested (Figure S11-B).

Discussion

Quality of the toxicological database

The quality of toxicological models such as those developed in the present work is intrinsically linked to the quality of the database used to construct them. Here, the toxicological database was based on acute toxicity data that complied with the pre-set conditions: (i) that the total dissolved metal concentrations had been measured, (ii) that the toxicological data were obtained, as much as possible, from several sources and (iii)

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that the tested species were among those recommended by OECD and USEPA in their guidance documents for aquatic toxicity testing to predict EC_{50} values.

In the algal toxicological database, copper had the highest number of available tests (62) with 38 performed with the recommended OECD and US EPA algal species *P. subcapitata*. In contrast, the Ni database (n=25) included a variety of algal species, 11 in total, the highest number among the algal databases. The least extensive databases were those for the major cations (Ca, K, Mg, Mn and Na). Although these elements have been studied over the years, they were rarely measured in the exposure medium (i.e., nominal concentrations or indirect estimates based on conductivity measurements were used – Santos et al. (2007), Venancio et al. (2017)) and thus many of the earlier studies were not useful for the present study. The daphnia database included many more tests than the algal database. The largest individual database was again for Cu with 239 data points and seven different daphnia species. The Ni database had the highest number of studied species (13 in total) whereas in contrast EC_{50} values for Co were only available for *C. dubia*. Although the database for the major cations was again the least extensive in terms of the number of experiments, one large data set was found in the literature, thanks especially to the work of Mount and colleagues who did extensive work on *C. dubia* (Mount et al. 2016). The Ag database contained only nine EC_{50} values but they came from eight different sources, suggesting good representativeness of the data. The toxicological data concerning fish are numerous for Cd, Cu, Ni, Pb and Zn, with a total of 201 results for Cu with eight species represented. The US EPA (2016) report from which the Cd data were extracted provided responses for 25 different species, and a total of 100 data points. In contrast, the Co database had only two available results for *P. promelas*

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since three of the five EC_{50} values were reported as “higher than a certain value” and thus were not usable for the present study. Data for the major cations were again challenging to find in the literature, mostly because the total dissolved concentrations in the exposure media were again rarely measured.

Statistical analyses of the EC_{50} values for each class of organism revealed that the incidence of outliers was linked to the use of additional species other than *P. subcapitata* for algae, such as *C. reinhardtii* and *C. vulgaris* for Cd, *Chlorella sp.* for Ni and *C. kessleri* for Pb, and additional fish species other than *P. promelas* and *O. mykiss*, such as *Danio rerio*, *Lepomis macrochirus* and *Perca flavescens* for Cd. In contrast, outliers in the daphnia database were not related to the use of particular species. Note that the outliers in the algal and fish databases were removed before the data were used in the QICAR modeling.

There are far fewer EC_{50} values for the TCEs than for the training metals. In total, one or two algal EC_{50} values were available for each of the TCEs (n=12), with a maximum of five data points for Pd(II); *P. subcapitata* was again the most studied algal species. *Ceriodaphnia dubia*, *D. magna* and *D. magna straus* were the only daphnia species for which toxicological data were available for the TCEs, but the database did include 10 elements; Pd(II) and Pt(IV) had the highest number of data points (n=6) whereas only one test result was available for Au(I), Ir(III), Ge(IV) and Ru(III). Few EC_{50} values were available for fish exposed to TCEs since for several experiments, the results were reported as “higher than a certain value” and thus could not be used in the present study (Table SI-18). Nonetheless, seven elements were represented with one or

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two EC_{50} values; *O. mykiss* was the most studied species but no *P. promelas* data were available.

Best QICAR models

Among the studied metal characteristics and based on the EC_{50} values for the training metals, the best predictor of the metal acute toxicity towards algae, daphnids and fish is the composite value $\chi_m^2 r$ or its log transformed value (Figures 2, 3 and 4). The χ_m term represents the electronegativity of the metal, e.g. its ability to attract electrons when it interacts with a ligand in solution or a membrane-bound metal transport site, whereas r is its ionic radius. Their combination as the composite $\chi_m^2 r$ parameter was introduced in the early 1980's by Nieboer and Richardson in order to classify metals into three sub-categories, i.e., *class A*, *class B* and *borderline* metals (Nieboer and Richardson 1980). This classification was carried out in the context of trying to understand and predict metal toxicity. At that time, Nieboer and Richardson demonstrated that "*the index $\chi_m^2 r$ is an estimate of the quotient obtained by dividing the valence orbital energy by the ionic energy*". In other words, $\chi_m^2 r$ represents the importance of covalent interaction in metal-ligand binding, relative to ionic interaction. For elements with low $\chi_m^2 r$ values (class A) such as Na (0.88) and Ca (1.00), the interaction with ligands is predominantly an ionic interaction between the cation and the donor atom(s) in the ligand, whereas metals with high $\chi_m^2 r$ values (class B) tend to form bonds with covalent character. This classification was completed with the use of z^2/r, which describes "*the ability of cations to form ionic bonds*" and takes into account the charge on the cation and the ability of ions with small radii to approach ligands donor groups closely. Since publication of their landmark 1980

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paper, $\square_m^2 r$ has been a recurrent parameter used in QICAR models, either alone or in combination with z^2/r or log K_{OH} (Walker et al. 2013).

In the present study, the best models used $\square_m^2 r$ as the sole parameter to describe the toxicity of total dissolved metals to freshwater aquatic organisms, without the further need to add another characteristic or index such as z^2/r. Moreover, this latter index showed very poor correlations with metal toxicity for all the studied organisms. Note that log K_{OH} was not tested due to the lack of values for some *data-poor* elements.

Quality of the QICARs prediction

Very good correlations were found between $\square_m^2 r$ and the toxicity of the training metals, with r^2_{adj} values higher than 0.8 for all organisms except for *O. mykiss* (r^2_{adj} = 0.62) (Figure SI-10). However, predictions for the TCEs were not as good, in particular for Au (algae, daphnia, fish), Pd(II) (fish), Pt(II) (algae, daphnia) and for Rh and Ru (daphnia). Several factors may be contributing to the poorer predictive power of the models for these elements. First, the toxicity data for the TCEs were extracted from very few experimental tests, meaning that the EC_{50} values are not as robust as those for the training metals. Secondly, the $\square_m^2 r$ values for the training metals that were used to construct the models ranged between 0.88 (Na) and 4.28 (Ag). As a consequence, for elements for which the $\square_m^2 r$ values lie outside this range, such as Au(I) (7.89) and Au(III) (4.90), the model predictions necessarily involve an extrapolation, rather than an interpolation, and this may contribute to the poorer performance of the model. Note, however, that the $\square_m^2 r$ values of Pd, Pt, Rh and Ru do fall within the range within which the models have been constructed. Thirdly, the toxicity testing of some TCEs were often performed with compounds having a chemical structure much more complex than those

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of the training metals, which were usually added as dissociable chloride or nitrate salts, and this might have contributed to the deviations from the QICAR predictions. For example, Pt(II) was added as tetraammineplatinum hydrogen carbonate and tetrammineplatinum diacetate whereas Pt(IV) was added as dihydrogen hexahydroxoplatinate and platinum chloride. Similarly, Rh was added as diammonium sodium hexakis(nitrito-N) rhodate and as rhodium nitrate hydrate. In the present study, we have expressed the EC_{50} values as total dissolved metal concentrations, but to know to what extent these complex forms remain intact in the aqueous exposure media within the timeframe of the toxicity test would be very helpful. This is also true for the training metals, the speciation of which can vary as a function of pH, carbonate and DOM concentrations in exposure media. Given the central role of the free metal ion activity in the prediction of metal toxicity towards aquatic organisms (Campbell 1995), ongoing projects are now focusing on developing QICAR models based on the free metal ion activity to determine if this improves the prediction of TCE toxicities.

CONCLUSION

Eight 'best' models ($r^2_{adj} > 0.6$) were constructed to predict acute EC_{50} values for twelve *data-rich* metals and ten *data-poor* TCEs, using toxicity testing data for freshwater algae, daphnids and fish (Table 1). All of these models used the covalent index, $\square_m^2 r$, in simple linear regressions, illustrating that a metal's ability to form covalent bond is a good proxy for its toxicity.

QICAR models show potential as a screening tool that could be used to review existing data and identify 'outliers', i.e. toxicity values that lie outside the QICAR prediction intervals. These outliers might be values that are abnormally low (and driving

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regulation setting), or values that are unusually high; in both cases, the outliers might reflect inaccurate toxicity test results or inadequate predictive tools. The QICARs could be used to flag values that merit further scrutiny and possible retesting.

In the case of TCEs, QICARs might also be used as an interpolation or extrapolation tool, to estimate their toxicity. Indeed, both parameters, $\square_m$ (electronegativity) and r (ionic radius) forming the covalent index are easily retrieved from the basic *Handbook of physics and chemistry* (Rumble 2018). Improvement in QICAR predictions of TCE toxicity should include consideration of metal speciation in the exposure media (for both training and prediction sets of metals) and concurrent measurements of metal accumulation in the test species. Since metal-ion specific probes are generally not available for the TCEs, consideration of metal speciation will necessarily involve the use of chemical equilibrium models to calculate the major metal species present in the exposure media. Such models are inherently sensitive to the quality of the thermodynamic data that are available for the metal, and this may well be a limiting factor for some of the TCEs, which are *data-poor* not only in the case of toxicity data, but also with respect to their behavior in aqueous solution. Concurrent measurements of metal bioaccumulation, with a distinction between surface bound and internalized metal, would help establish links between metal speciation, metal accumulation and toxicity. The lack of such information currently hinders attempts to explain the mode of action of the TCEs.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data Accessibility Statement--We have compiled an extensive Supplementary Materials (SM) file that is included with this submission. The toxicological data for the training metals are available in the open literature and the sources are indicated in the SM file. The comparable data for the TCEs are plotted in the figures in the manuscript and in the SM file. Readers are invited to contact the corresponding author for any additional information (severine.le-faucheur@univ-pau.fr).

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FIGURES

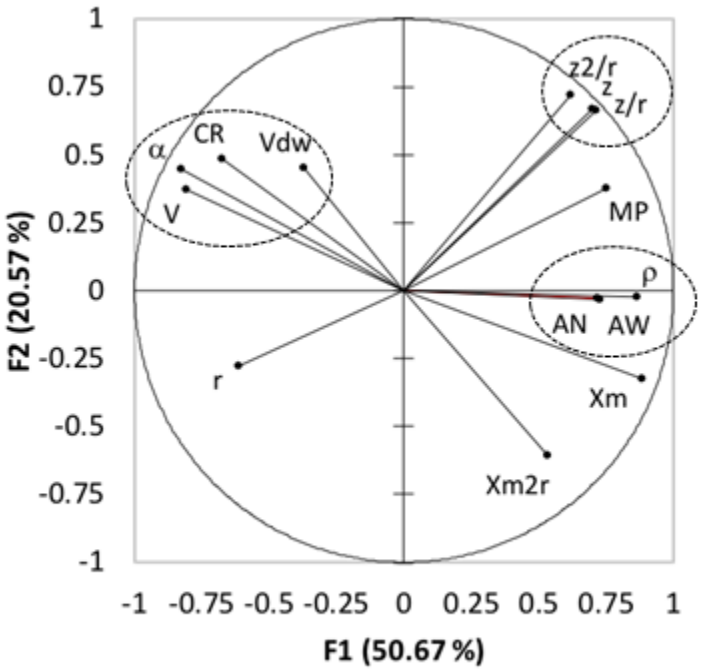

Figure 1: Principal component analysis of the metal characteristics considered for testing in QICAR modeling, excluding IP, ΔIP, E^0 and ΔE^0. The dashed ellipses represent groups of strongly-correlated variables.

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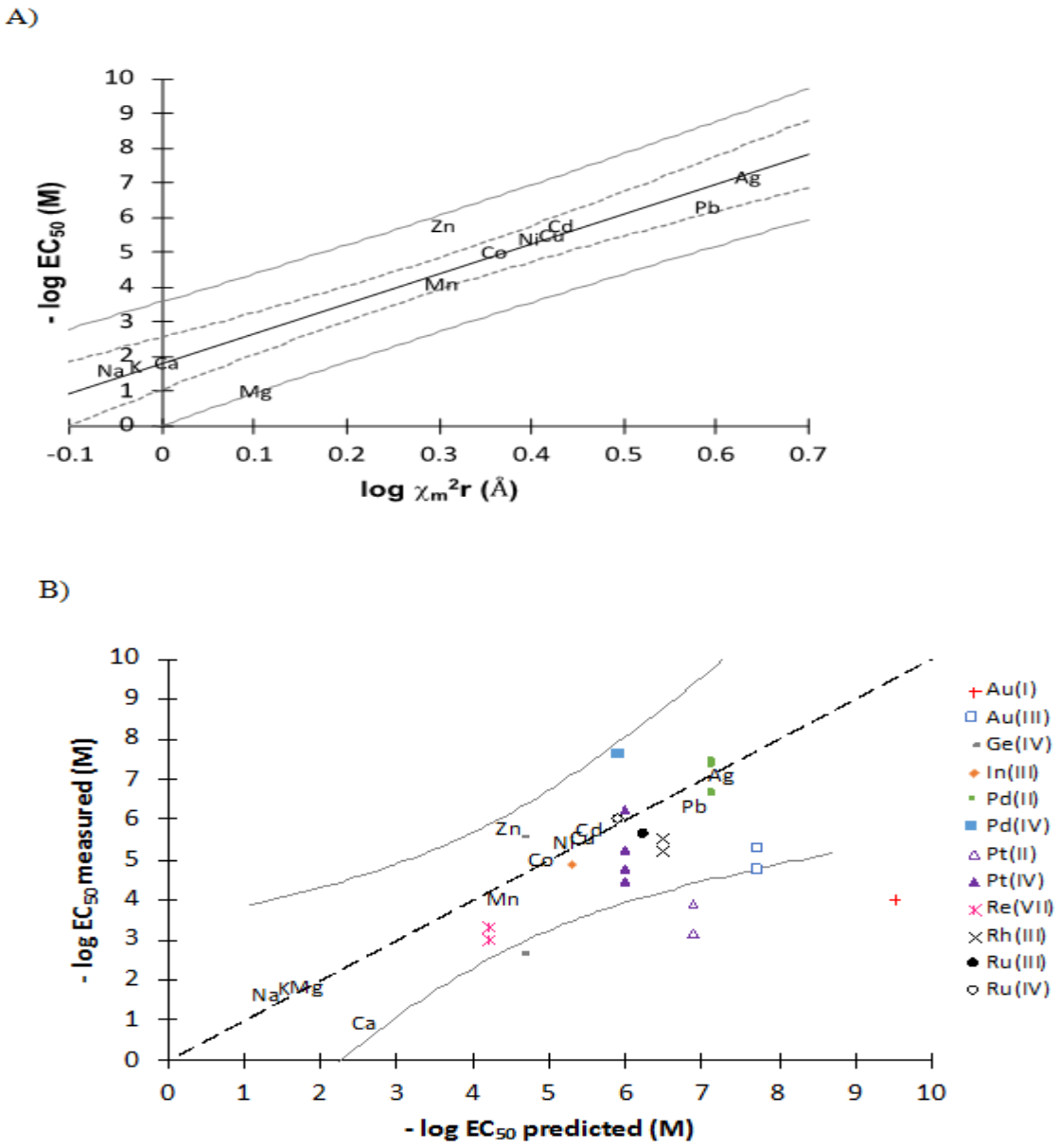

Figure 2: (A) Simple linear regression of the negative logarithm of the mean measured EC_{50} values of training metals (without algal species distinction), as a function of log $\chi_m^2 r$ (-log EC_{50} predicted = 1.816 + 8.607 × log $\square_m^2 r$; r^2_{adj} = 0.89). The dotted and black lines represent the 95% confidence interval and prediction interval bands, respectively. (B) Linear regression between

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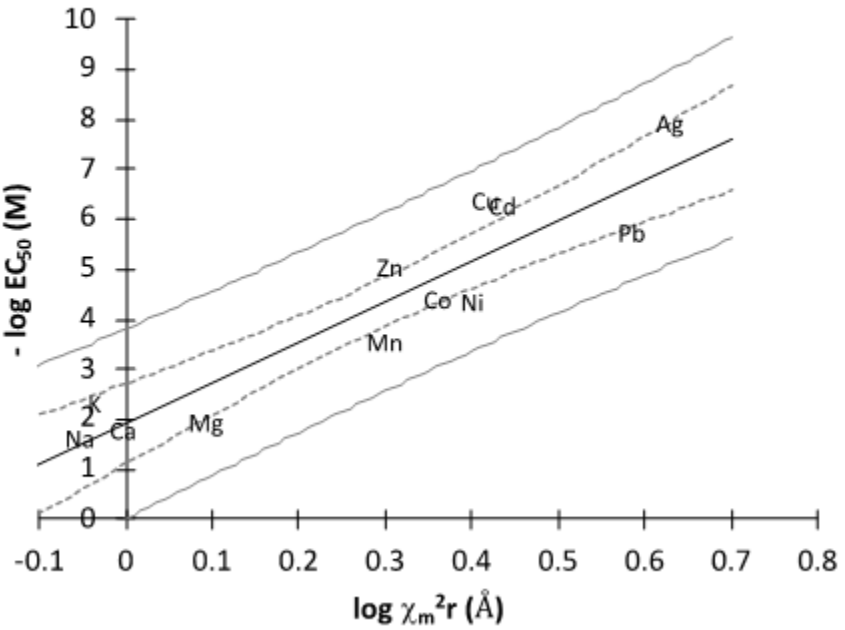
the -log EC_{50} predicted with the model presented in (A) and the negative logarithm of the mean measured EC_{50} values. The training metals are represented as single letters in black whereas for the TCEs, the individual EC_{50} values are presented as dots in several colors. The two gray lines indicate the 95% prediction interval bands.

A)



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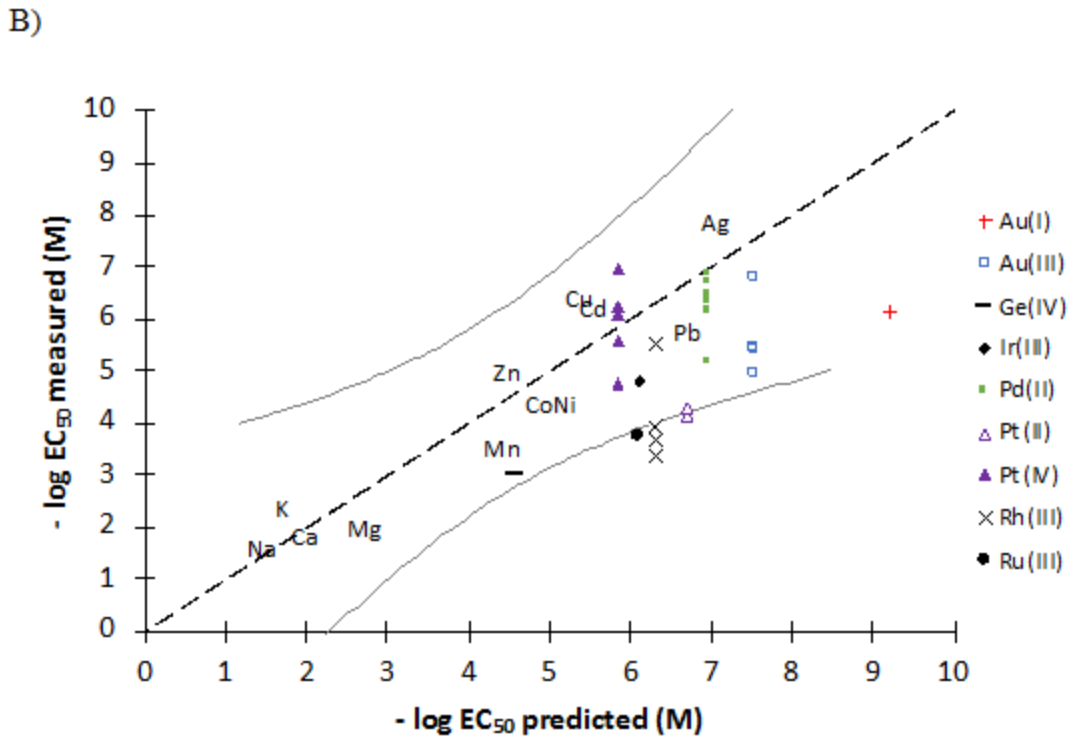

Figure 3: (A) Simple linear regression of the negative logarithm of the mean measured EC_{50} values of training metals (without daphnid species distinction), as a function of log $\chi_m^2 r$ (-log EC_{50} predicted = 1.912 + 8.154 × log $\square_m^2 r$; r^2_{adj} = 0.86). The dotted and black lines represent the 95% confidence interval and prediction interval bands, respectively. (B) Linear regression between the -log EC_{50} predicted with the model presented in (A) and the negative logarithm of the mean measured EC_{50} values. The training metals are represented as single letters in black whereas for the TCEs, the individual EC_{50} values are presented as dots in several colors. The two gray lines indicate the 95% prediction interval bands.

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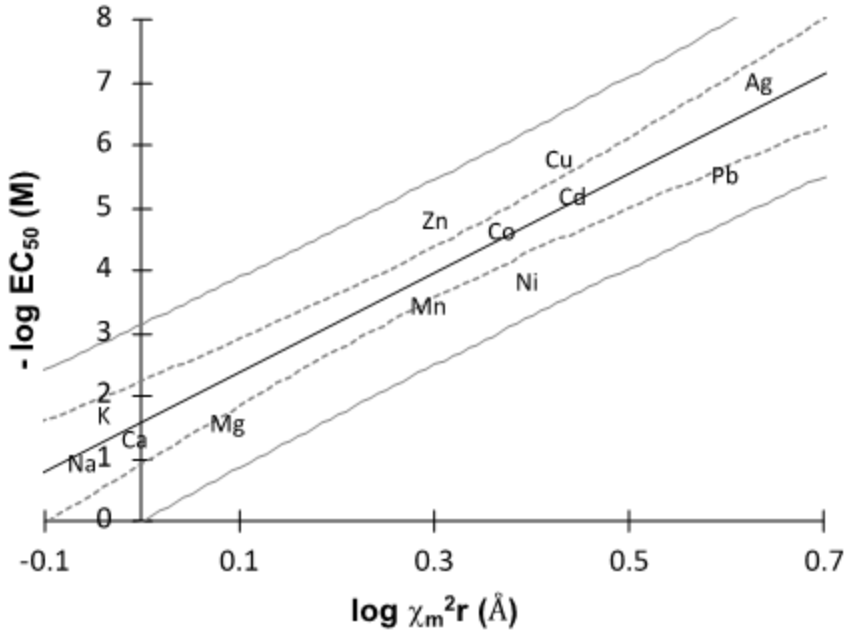
A)



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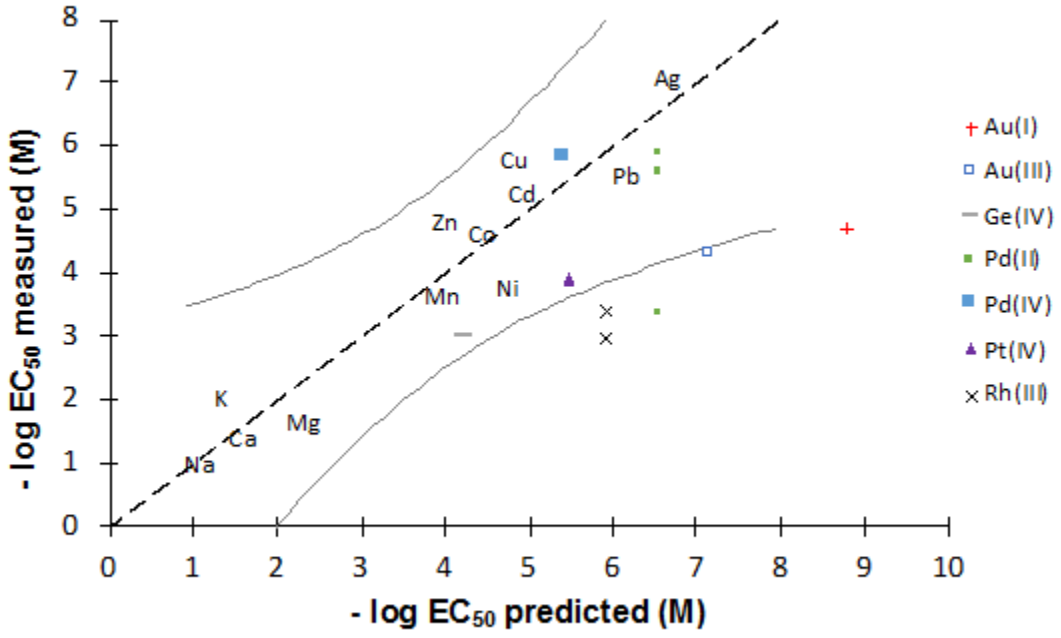

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Figure 4: (A) Simple linear regression of the negative logarithm of the mean measured EC_{50} values of training metals (without fish species distinction), as a function of log $\chi_m^2 r$ (-log EC_{50} predicted = 1.578 + 7.979 × log $\square_m^2 r$; r^2_{adj} = 0.89). The dotted and black lines represent the 95% confidence interval and prediction interval bands, respectively. (B) Linear regression between the -log EC_{50} predicted with the model presented in (A) and the negative logarithm of the mean measured EC_{50} values. The training metals are represented as single letters in black whereas for the TCEs, the individual EC_{50} values are presented as dots in several colors. The two gray lines indicate the 95% prediction interval bands.

Table 1: Compilation of the best models to predict metal acute toxicity towards freshwater algae, daphnids and fish, with and without species-specificity (models based on total dissolved metal concentrations).

	Model (-log EC_{50})	**r^2_{adj}**
All algal species	1.816 + 8.607 × log $\chi_m^2 r$	0.89
P. subcapitata	2.119 + 8.348 × log $\chi_m^2 r$	0.94
All daphnia species	1.912 + 8.154 × log $\chi_m^2 r$	0.86
D. magna	1.654 + 8.614 × log $\chi_m^2 r$	0.86
C. dubia	1.953 + 8.808 × log $\chi_m^2 r$	0.86
All fish species	1.578 + 7.979 × log $\chi_m^2 r$	0.89
O. mykiss	1.776 + 8.387 × log $\chi_m^2 r$	0.62
P. promelas	1.572 + 7.741 × log $\chi_m^2 r$	0.87

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