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Nano-TiO_2 modifies heavy metal bioaccumulation in *Daphnia magna*: A model study

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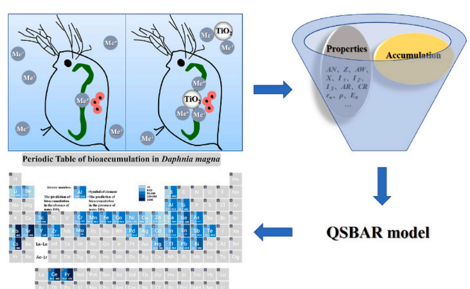
HIGHLIGHTS

- QSBAR models for predicting metal accumulation in *D. magna* were developed significantly.
- The accumulation of different metals in the absence and presence of nano-TiO_2 differ.
- Metal accumulation increases with nano-TiO_2 even at a low metal exposure concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to special properties, nano-TiO_2 will interact with heavy metals and other pollutants in water, thus affecting the environmental behavior and ecotoxicity of these pollutants. However, the exact manner in which nano-TiO_2 affects the bioaccumulation mechanisms of heavy metals is still unclear now. In the present study, quantitative structure bioaccumulation relationship (QSBAR) models were established to explore the relationships between physicochemical parameters of heavy metals and their accumulation in *Daphnia magna* in the absence and presence of nano-TiO_2 at low metal exposure concentrations. The results showed that different physicochemical parameters affected the bioaccumulation of metals in *Daphnia magna*. The metal accumulation could be described by means of a *Comprehensive Parameter* composed of seven parameters, i.e., atomic number (AN), relative atomic weight (AW), atomic radius (AR), atomic ionization potential (AN/ΔIP), covalent index (X^2r), second ionization energy (I_2) and electrochemical potential (E_0), in the absence of nano-TiO_2, whereas the metal accumulation increased with the increase in *Van Der Waals* radius (r_w) of metals in the presence of nano-TiO_2. It was demonstrated that the bioaccumulation mechanism of the metals to *Daphnia magna* changed in the presence of nano-TiO_2. Moreover, the bioaccumulation of more than 85% of the metals increased in the presence of nano-TiO_2, but it increased differently for different metals. The present study provides an alternative approach to

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understand the mechanism of heavy metal bioaccumulation at low metal exposure concentrations and the effect of nano-TiO_2 on metal bioaccumulation.

1. Introduction

Titanium dioxide nanoparticles (nano-TiO_2) are one of the most widely used nano materials in the world (Zheng and Nowack, 2021), with worldwide production estimated at over 6.2 million tons in 2020. The market is projected to register a compounded average growth rate of over 6% during the forecast period (2021–2026) (Ltd, 2021). This increased use implies that a large amount of nano-TiO_2 is likely to enter the environment, especially the aquatic environment. Indeed, it has been reported that the concentration range of nano-TiO_2 is about 3 ng/L-1.6 μg/L in global rivers (Gottschalk et al., 2013). Therefore, the toxicity and ecological risk of nano-TiO_2 in the aquatic environment are of increasing concern.

A large number of studies have found that due to its large surface area and unique photocatalytic properties (Clemente et al., 2011), nano-TiO_2 can potentially impact various organisms in the water, such as algae (Hartmann et al., 2010), water flea (Lovern and Klaper, 2010; Tan and Wang, 2014), and fish (Johnston et al., 2010). Nano-TiO_2 can also interact with other substances present in the water through adsorption, thus affecting the environmental behavior and toxic effects towards aquatic organisms of these pollutants in the aquatic environment (Gaya and Abdullah, 2008; Nagaveni et al., 2004). Heavy metals, which are common contaminants widely existing in natural waters, can interact with nano-TiO_2. This interaction affects the absorption and accumulation of heavy metals in aquatic organisms, and subsequently impacts their toxic effects on aquatic organisms (Sun et al., 2009; Torre et al., 2015). Earlier studies indicated that the uptake and accumulation of several typical heavy metals such as silver (Ag) (Rosenfeldt et al., 2014), arsenic (As) (Rosenfeldt et al., 2014; Wang et al., 2011), copper (Cu) (Fan et al., 2011; Rosenfeldt et al., 2014), cadmium (Cd) (Zhang et al., 2007), and zinc (Zn) (Tan et al., 2012), significantly increased in aquatic organisms, such as *Daphnia magna* and *Ceriodaphnia dubia*, in the presence of nano-TiO_2. Bioaccumulation is commonly regarded as a relevant endpoint of toxicity assessment. Unfortunately, the knowledge on the modification of bioaccumulation of heavy metals induced by the presence of nano-TiO_2, is still limited due to a general lack of experimental data, as experimentation is often costly and time-consuming.

Using fewer animals and making better predictions are essential elements of the future integrated testing strategies for toxicology (Hartung, 2009). In this regard, it would be helpful to gain a better understanding of the influence of nano-TiO_2 on the toxicity of heavy metals from a quantitative structure-activity relationship (QSARs) viewpoint. The relationships between the characteristics of metal ions and their biological toxicity have already been confirmed by the use of QSARs (Wang et al., 2016; Wu et al., 2013). Ochiai et al. (Ochiai, 1995) found that the toxicity mechanism of metals is closely related to the electronic structure of metal ions and their binding with biological macromolecular ligands. Wu et al., (2013) showed that the softness index (σp) was significantly and positively correlated with the log-EC_{50} (median effect concentration) of *Daphnia magna* while the covalent index (X^2r) was significantly and negatively correlated with the toxic potency of metals to mollusks. Our previous study also found that seven structural parameters, including atomic number (*AN*), relative atomic weight (*AW*), covalent radius (*CR*), Pauling ionic radius (*r*), atomic ionization potential (*AN/ΔIP*), σp and electron density (*AR/AW*), were reasonably correlated with the acute water quality criteria (WQC) of transition metals recommended by the United States Environmental Protection Agency (USEPA) (Wang et al., 2016). Moreover, the QSARs method is also widely used in nanotoxicology (Mu et al., 2016; Puzyn et al., 2011). Therefore, it is likely that the effect of nano-TiO_2 on the mechanism of toxicity of heavy metals can be determined by utilizing QSARs.

In addition, the effect of nano-TiO_2 on the toxicity of heavy metals not only depends on the interaction between nano-TiO_2 and different heavy metals, but is also related to the organisms tested. It should be because biological effects can determine the absorption behavior of nano-TiO_2 and metal ions. For example, different results on bioavailability and toxicity of copper in the presence of nano-TiO_2 for *Microcystis aeruginosa* (Chen et al., 2015) and *Daphnia magna* (Fan et al., 2011) were obtained, where the presence of nano-TiO_2 reduced the bioavailability and the toxicity of Cu in *Microcystis aeruginosa* but increase them in *Daphnia magna*. Similarly, the bioavailability of Cd to *Chlamydomonas reinhardtii* was also reduced (Yang et al., 2012). One of the main reasons for the reduced bioavailability of metals to *Microcystis aeruginosa* or *Chlamydomonas reinhardtii* is that these organisms have cell wall, leading that the uptake of nano-TiO_2 and metal ions is limited. Therefore, it is necessary to use the same tested organisms for the purpose of model development.

The preliminary results of a literature search showed that a total of 27 species of aquatic organisms were used in the previous studies of metal toxicity in the presence of nano-TiO_2 regarding bioaccumulation as the endpoint, among which *Daphnia magna* was the most commonly used organism (Table S1, Fig. S1). *Daphnia magna*, a standard model organism used for toxicity testing as recommended by USEPA and Organization for Economic Cooperation and Development (OECD), is considered as a an ecologically important “keystone” freshwater zooplankton in the mass balance and energy cycle of ecological food webs (Kim et al., 2015). It is very sensitive to toxic substances and has been widely used in the study of metal toxicity (Poynton et al., 2007). Therefore, *Daphnia magna* was used for model development in the present study.

Hence, the present study aims to assess the factors affecting metal bioaccumulation in *Daphnia magna* in the absence and presence of nano-TiO_2. Based on the modification of heavy metal bioaccumulation by nano-TiO_2, quantitative structure bioaccumulation relationship (QSBAR) models were established in the absence and presence of nano-TiO_2 to explore the quantitative relationship between physicochemical parameters of metals and their accumulation in *Daphnia magna*. The reliability and validity of the models were tested, and the accumulation of a set of 30 metals was predicted.

2. Materials and methods

2.1. Data sets

The accumulation of heavy metals in *Daphnia magna* was selected as the toxicity endpoint. The present study used all historical studies in the Web of Science up to the July 14, 2020, that have reported the joint toxicity of nano-TiO_2 and eleven heavy metals (Cu, Cd, Pb, Zn, As, Ag, Hg, Ni, Cr, Mn and Co) to *Daphnia magna* (Fig. S1). Accuracy, consistency and reliability of data were evaluated by use of standard methods (Klimisch et al., 1997). In order to make a more accurate comparison, the screening standards of data used in the study were based on the strict rules as follows: (1) the accumulation tests must be carried out strictly follow standard methods; (2) a blank control experiment is required, in which no abnormalities were observed in terms of mortality of illness; (3) the exposure time of the accumulation test for *Daphnia magna* must be in between 48 and 96 h; (4) the experimental data from the same references were used to report accumulation both in the absence and presence of nano-TiO_2 (Table S2).

Based on the results of several previous studies, twenty-five physicochemical parameters of the metals were obtained (Haynes, 2013;

Information 2019; Mathematics 2018; Miessler G. et al., 2014; Wang et al., 2016), including AN, ionic charge (Z), AW, electronegativity index (X), first ionization energy (I_1), second ionization energy (I_2), third ionization energy (I_3), atomic radius (AR), CR, *van der Waals* radius (r_w), r, density (ρ), electrochemical potential (E_0), first hydrolysis constant (K_{OH}), X^2r, polarization force parameters (Z/r、Z/r^2 and Z^2/r), ionization potential (IP), AR/AW, similar polarization force parameters (Z/AR and Z/AR^2), difference in ionization potentials between the ion oxidation numbers OX and OX^{-1} (ΔIP), AN/ΔIP, and σp (Table 1). Some parameters such as AN/ΔIP, Z/AR、Z/rx and Z^2/r were recalculated to fit the model. These parameters are commonly used physicochemical parameters of the metals to predict the relative toxicity or sublethal effects of metal ions to organisms. Many of these characteristics reflect the binding tendencies of metals to ligands. Such tendencies are notionally linked to metal binding of biomolecules and consequent toxic effects (McCloskey et al. 1996). For example, the covalent index X^2/r, which is composed of two fundamental ionic characteristics X and r, quantifies the relative importance of covalent over ionic interactions. Alternatively, polarizing power parameters such as ΔIP, E_0, Z/r, Z^2/r, AN/ΔIP, Z/AR, are used to determine ionic metal binding to biological ligands (Newman et al., 1998).

2.2. Bioaccumulation modification

Considering the differences in experimental conditions in different studies, the net accumulation was used in the present study, which refers to the accumulation of the experimental group minus the control group (Eq. (1)).

$$C_B = C_{experiment} - C_{control} \tag{1}$$

In addition, different concentrations of metals will affect their accumulation in aquatic organisms, so it is necessary to scale the accumulation at different metal exposure concentrations to the same metal exposure concentration along with the linear fit for every metal (Biesinger et al., 1982; Ling et al., 2017). Bioaccumulations and metal exposure concentrations were used as independent and dependent variables, respectively. Moreover, in the case of nano-TiO_2 being present, data obtained at a low concentration of nano-TiO_2 (1–4 mg/L) were merged. The accumulation of metals in the absence and presence of nano-TiO_2 was modified respectively. Original data, logarithmic transformation based on 10 and inverse transformation data were used to establish linear regression models. In order to obtain more acceptable models, the model was considered sufficient when the P value (the level of statistical significance) was less than 0.1. The model with minimum P value and maximum regression coefficient (R^2) values was deemed the best model.

2.3. Quantitative structure bioaccumulation relationship (QSBAR)

Twenty-five physicochemical parameters of metals were correlated to the net accumulation by use of *Pearson* correlations analysis. The QSBAR models were developed based on linear regressions of those parameters with the greatest correlations and predictive power. Selected parameters and the net accumulation were used as independent and dependent variables, respectively. Principal component analysis (PCA), an important unsupervised learning method, was used to create several new variables expressed as principal components (PCs) to optimally represent the dynamic and interactive relationships among the original variables (Jolliffe, 2005). Then linear regression was also used to fit those PCs whose total explained variance exceeds 70% of the total variance, also called "comprehensive parameters with key information", and the net accumulation. The goodness-of-fit of each QSBAR model was evaluated with the coefficient of determination (R^2), residual standard error (*RSE*), the value of the *F*-test statistic using analysis of linear regression fit and the level of Type I error (P) set to $\alpha < 0.05$.

2.4. Internal validation and applicability domain

The robustness and effectiveness of the model were evaluated with the leave-one-out (LOO) cross-validation correlation coefficient (Q^2_{LOO}) (Eq. (2)), for which the recommended minimum acceptable value is 0.5, and with the cross-validated root mean square error of prediction

Table 1
Physicochemical properties of five metals and extent of metal accumulation in *Daphnia magna* (unit: μg/g dry weight) when metal concentration equals to 0.0185 μmol/L.

Conditions/properties	Abbreviation	Metals				
		Cu	Cd	Pb	Ag	As(V)
In the absence of nano-TiO_2	/	12.5	7.07	48.2	0.3	0.052
In the presence of nano-TiO_2	/	−30.8	1.78	105.8	1	51.7
Atomic number	AN	29	48	82	47	33
Ionic charge	Z	2	2	2	1	5
Relative atomic weight	AW	63.546	112.414	207.2	107.868	74.922
Electronegativity index	X	1.9	1.69	1.87	1.93	2.18
First ionization energy	I_1	745.5	867.8	715.6	731	947
Second ionization energy	I_2	1957.9	1631.4	1450.5	2070	1798
Third ionization energy	I_3	3555	3616	3081.5	3361	2735
Atomic radius	AR	128	151	175	144	119
Covalent radius	CR	132	144	146	145	119
Van der Waals radius	r_W	140	158	202	172	185
Pauling ionic radius	r	73	95	119	115	46
Density	ρ	8.96	8.65	11.34	10.49	5.727
Electrochemical potential	E_0	0.159	0.4	0.13	0.8	0.56
First hydrolysis constants	K_{OH}	8	10.1	7.7	12.4	2.22
Covalent index	X^2r	2.635	2.713	6.46	4.284	2.186
Polarization force parameters	Z/r	2.74	2.105	1.681	0.87	10.87
Polarization force parameters	Z^2/r	5.48	4.21	3.361	0.87	54.348
Polarization force parameters	Z/r^2	3.75	2.22	1.412	0.76	23.629
Ionization potential	IP	36.836	37.48	31.937	21.486	127.555
Electron density	AR/AW	0.0201	0.0134	0.00845	0.0134	0.0159
Similar polarization force parameters	Z/AR	1.563	1.325	1.1429	0.694	4.202
Similar polarization force parameters	Z/AR^2	1.221	0.877	0.653	0.482	3.531
Difference in ionization potentials between the ion oxidation numbers OX and OX^{-1}	ΔIP	16.544	20.572	16.905	13.911	64.926
Atomic ionization potential	AN/ΔIP	1.753	2.333	4.851	3.379	0.508
Softness index	σ_p	0.121	0.0972	0.118	0.144	0.0308

($RMSE_{CV}$) (Wang et al., 2018) (Eq. (3)).

$$Q_{LOO}^2 = 1 - \frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{\sum_{i=1}^{n} (y_i - \bar{y})^2} \quad (2)$$

$$RMSE_{CV} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}_i)^2} \quad (3)$$

In addition, the William plot was used to evaluate the applicability domains (Wang et al., 2016). The model can be applied for predicting, if their calculated leverage values are not higher than the critical value of warning h^* (Puzyn et al., 2011). Models with good predictive ability were applied to predict the net accumulation for metals not included in

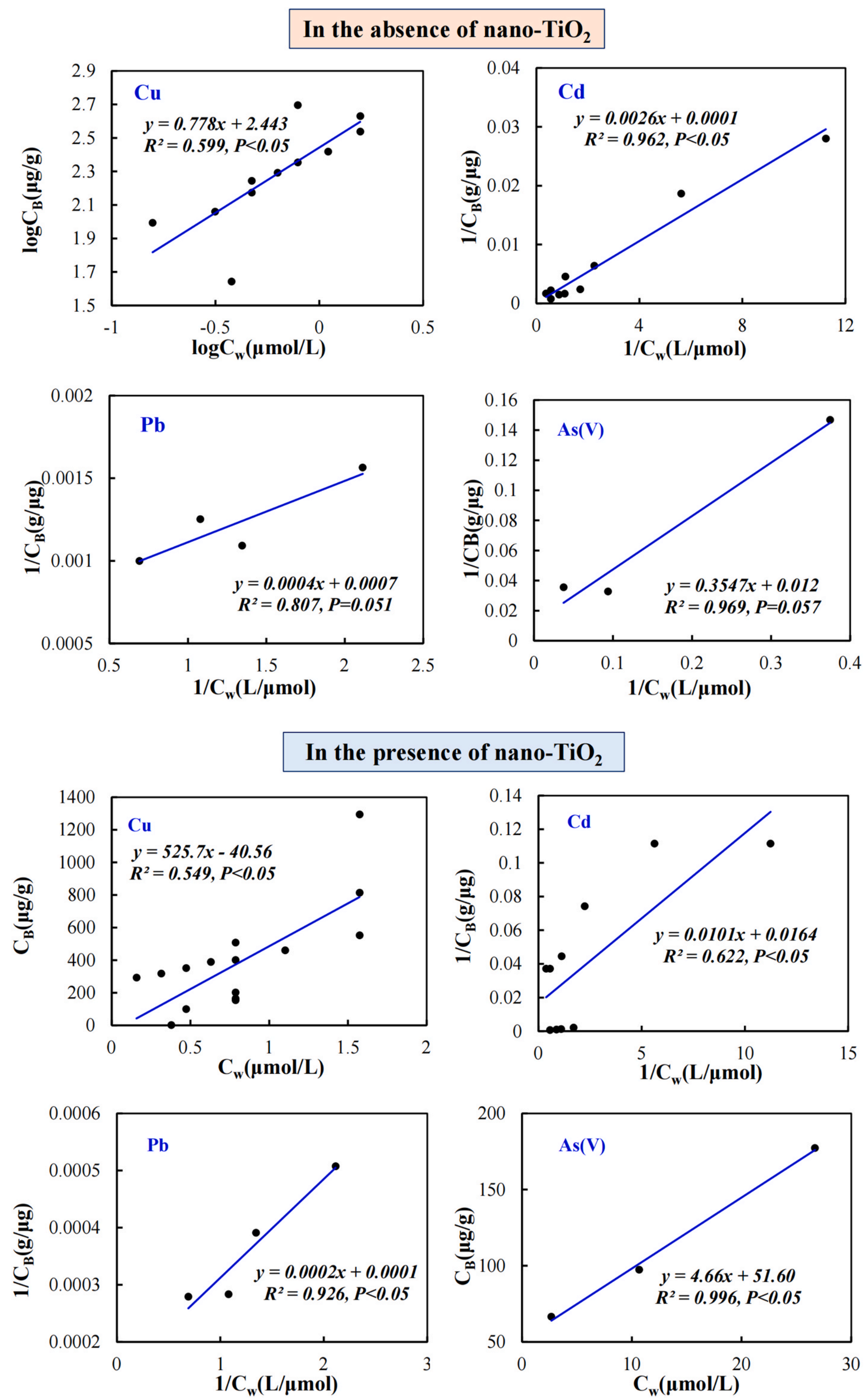

Fig. 1. Modified accumulation models of Cu, Cd, Pb and As(V) in the absence and presence of 1–4 mg/L nano-TiO_2, where *Cw* is metal exposure concentrations and C_B is the corresponding bioaccumulations of metal in *Daphnia magna*.

the data set used for model development.

3. Results and discussion

3.1. Accumulation modification of metals in Daphnia magna

According to the principle of data screening, 69 sets of data were obtained for five metals, including Cu, Cd, Pb, As (V) and Ag (Table S2). There was only one set of data for Ag, where its concentration was 0.0185 μmol/L. Such a low exposure concentration may be related to the strong toxicity of silver ions to aquatic organisms. The corrected accumulation models of different metals in the presence and absence of nano-TiO_2 were established (Table S3). After comparison, the best models were finally chosen (Fig. 1) and the accumulation of metals in the absence and presence of nano-TiO_2 was modified according to the exposure concentration used in the accumulation experiments with Ag, that is 0.0185 μmol/L. After examination of the leverage value and standard residual, the best models exhibited statistically robust, demonstrated that accuracies of models could be acceptable. The metal accumulation in *Daphnia magna* increased with the increase in metal concentration whether in the absence or presence of nano-TiO_2 (Fig. 1).

By comparing the metal corrected accumulation between the two conditions of presence and absence of nano-TiO_2 (Table 1), it was found that the accumulation of Cu and Cd in *Daphnia magna* in the presence of nano-TiO_2 was less than that in the absence of nano-TiO_2. This is because the presence of nano-TiO_2 reduces the concentration of free metal ions in water; at low exposure concentrations of metal, mainly free ions enter the organism (Miao et al., 2012). Thus, the presence of nano-TiO_2 resulted in the reduction of the total accumulation of Cu and Cd. However, the accumulation of Pb, Ag and As(V) increased in the presence of nano-TiO_2. This is because nano-TiO_2 can adsorb metals and organic contaminants because of their large surface areas by means of physical sorption through electrostatic force and chemical sorption through chemical bonding, and then facilitate the entry of pollutants adsorbed on the nanoparticles themselves into aquatic organisms. Comparing the increased accumulation of Pb, Ag and As(V), it showed that the effect of nano-TiO_2 on different metals was different. Among the metals studied, the increase in Pb accumulation was the most, which is consistent with the results of previous studies that indicated higher sorption capacity and sorption affinity of TiO_2 NPs for Pb (Ling et al., 2017). In fact, the difference of influence of nano-TiO_2 on the metal bioaccumulation may be due to the difference of the relative uptake rate of NPs to different ions. For example, comparing adsorptions results of Cu^{2+}, Cd^{2+} and Pb^{2+} onto TiO_2 at 10^{-4} M, the adsorption affinity follows the order Pb^{2+} (log K_{Pb} = 1.2) > Cu^{2+} (log K_{Cu} = 0.63)> Cd^{2+}(log K_{Cd} = -1.2) (Yang and Davis, 1999). And the study of Tan et al., (2012) found that the uptake constant (k_u) adsorbed on nano-TiO_2 by *Daphnia magna* of Cd was 5.07 ± 0.79 L/g/h and that of Zn was 8.37 ± 0.97 L/g/h (values in 95% confidence intervals), which were 80.6 and 185 times higher than the k_u of Cd and Zn based on dissolved uptake in a previous study (Yu and Wang, 2002). Furthermore, the net accumulation of Cu was corrected to −30.8 μg/g dry weight in the presence of nano-TiO_2, which means that the total accumulation of Cu in *Daphnia magna* decreased by 30.8 μg/g when the exposure concentration of Cu was 0.0185 μmol/L in the presence of nano-TiO_2. This result is in agreement with our previous study (Fan et al., 2011), and may be related to the low exposure concentration of Cu.

3.2. Development of QSBAR models in the absence of nano-TiO_2

To develop canonical and effective models to predict accumulation of heavy metals in *Daphnia magna* under different conditions, *Pearson* product-moment parametric correlation analysis was used. The relationships between 25 physicochemical properties of five heavy metals and the corrected net accumulation of these metals were investigated in the absence and presence of nano-TiO_2, respectively. Seven physicochemical parameters, including atomic number (AN), relative atomic weight (AW), atomic radius (AR), atomic ionization potential (AN/ΔIP), covalent index (X^2r), second ionization energy (I_2) and electrochemical potential (E_0), were found to be reasonably correlated with the corrected net accumulation of five heavy metals in the absence of nano-TiO_2 (*Correlation*> 0.5 and $P < 0.1$; Table S4). The results showed that the physicochemical properties affecting the net accumulation of different metals in *Daphnia magna* were similar to the properties identified in our previous studies on metal toxicity in the absence of nano-TiO_2 (Wang et al. 2016, 2020).

The interaction between metals and *Daphnia magna* is complex. The parameters AW, AN, AR, AN/ΔIP and X^2r were found to be significantly and positively correlated with the net accumulation. AR and AW could affect the reactivity of metals with other substances and the change in energy during the reaction (Szarek et al., 2019). As a result, the metal accumulation in *Daphnia magna* increased with the increase of metal AN, AW and AR, which may lead to increase in metal toxicity. Similarly, AN/ΔIP describes the ability of covalent binding and complexation of metal ions due to configurations of their electrons and subsequent crystalline structures. Hence, increased AN/ΔIP can increase the accumulation of metals in organisms, thus increasing the toxicity of metal ions. A previous study also indicated that AN/ΔIP was negatively correlated with log-EC_{50} of *Lymnaea acuminata* (Wu et al., 2013) and CMCs recommended by USEPA (Wang et al., 2016). X^2r represents the covalent binding ability of metal-ligand complexes with cations (Nieboer and Richardson, 1980). A larger value of X^2r indicates a softer ion which binds more easily to nitrogen and sulfur containing functional groups on the surface of the organism. This increases the accumulation of metal ions and results in a stronger toxic potency of the metal (Wang et al., 2018). The parameters I_2 and E_0 were significantly and negatively correlated with the net accumulation. This result showed that the smaller the values of I_2 and E_0, the easier the metal loses electrons and the stronger its reactivity. As a result, the more stable the corresponding metal ions are, the easier they can combine with biomacromolecules and remain in *Daphnia magna*, thus increasing the accumulation. This is consistent with the result of our previous study which showed that ions of metals with stronger ionization energies have less toxic potency to aquatic organisms (Wang et al., 2018).

Moreover, QSBAR models were constructed using the single-parameter linear regression method in the absence of nano-TiO_2 based on the seven descriptors. Only AW, AN and X^2r were selected as having sufficient goodness-of-fit in the absence of nano-TiO_2 (R^2>0.5,$P < 0.1$) (Table 2, Fig. S2). However, as there is a strong correlation (0.75–0.86) between the seven structural parameters and the corrected accumulation of metals, and considering that choosing multiple structural characteristics can effectively represent different properties of metals (Wang et al., 2018), the PCA regression method was used to reduce the dimension and establish a prediction model by use of a *Comprehensive Parameter* based on all seven structural parameters ($R^2 = 0.697$, $F = 12.724$, $P = 0.079$) (Eqs. (1) and (2)).

$$CP = 0.989\ AW + 0.988\ AN + 0.97\ AR + 0.926\ X^2r + 0.914\ AN/\triangle IP - 0.734\ I_2 - 0.431\ E_0 \quad (1)$$

$$C_B = 0.0633 \times CP + 77.156 \quad (2)$$

Where: *CP* is the first principal component of the best combination of parameters obtained by using PCA, in which 76% of the cumulative contribution of variance was accounted for.

The QSBAR model based on PCA regression is an acceptable and predictive model, since it passed the internal validation ($Q^2_{LOO} = 0.806 > 0.5$, $RMSE_{CV} = 10.087$) and its optimal prediction is in the application domain ($h < h^*$). The result of PCA regression is consistent with the result of the *Pearson* product-moment parametric correlation analysis, which showed that the accumulation of metals in *Daphnia magna* increased with the increase in AW, AN, AR, X^2r and AN/ΔIP. On the other hand, the metal accumulation in *Daphnia magna* decreased with

Table 2
QSBAR regression models, where R^2 is the regression coefficient, RSE is the residual standard error, *F* is the value of *F*-test statistic and *P* is the level of statistical significance. *Comprehensive Parameter (CP)* is the first principal component of the best combination of seven structural parameters (AW, AN, X^2r, AR, AN/ΔIP, I_2 and E_0) obtained by using PCA.

Conditions	Properties	Regressions	R^2	RSE	F	P
In the absence of nano-TiO_2	AW	$C_B = 0.305 \times AW - 20.913$	0.744	11.695	8.704	0.060[b]
	AN	$C_B = 0.812 \times AN - 25.180$	0.717	12.289	7.602	0.070[b]
	X^2r	$C_B = 9.384 \times X^2r - 20.690$	0.679	13.083	6.353	0.086[b]
	AR	$C_B = 0.732 \times AR - 91.415$	0.633	13.994	5.175	0.107
	AN/ΔIP	$C_B = 9.100 \times AR/\Delta IP - 9.726$	0.560	15.320	3.821	0.146
	I_2	$C_B = -0.0597 \times I_2 + 119.998$	0.550	15.505	3.660	0.152
	E_0	$C_B = -52.396 \times E_0 + 35.081$	0.542	15.641	3.544	0.156
	CP	$\mathbf{C_B = 0.0633 \times CP + 77.156}$	**0.697**	**12.724**	**6.888**	**0.079**[b]
In the presence of nano-TiO_2	$\mathbf{r_W}$	$\mathbf{C_B = 2.136 \times r_W - 340.143}$	**0.910**	**18.574**	**30.237**	**0.012**[a]
	AW	$C_B = 0.742 \times AW - 58.060$	0.614	38.415	4.770	0.117
	AR/AW	$C_B = 9834.97 \times AR/AW + 166.047$	0.612	38.503	4.734	0.118
	AN	$C_B = 1.970 \times AN - 68.237$	0.589	39.614	4.307	0.130
	I_2	$C_B = -0.160 \times I_2 + 311.745$	0.554	41.289	3.726	0.149
	I_3	$C_B = -0.105 \times I_3 + 368.461$	0.508	43.349	3.102	0.176

[a] Means $p < 0.05$.
[b] Means $0.05 < p < 0.1$.

the increase of I_2 and E_0. These findings supported the conclusions of previous QSAR studies on toxicity in some ways. For instance, Wu et al., (2013) found that there is a positive relationship between E_0 and log EC_{50} of metals to *Lemna minor*, which means that the stronger the ability of hydrolysis and ionization of metal ions, the weaker the toxicity to aquatic organisms.

3.3. Development of QSBAR models in the presence of nano-TiO_2

Six physicochemical parameters, including atomic number (AN), relative atomic weight (AW), electron density (AR/AW), *Van Der Waals* radius (r_w), second ionization energy (I_2) and third ionization energy (I_3), were found to be reasonably well correlated with the corrected net accumulation of five heavy metals in the presence of nano-TiO_2 (*Correlation* > 0.5 and $P < 0.1$; Table S5). Except for the parameters AN, AW and I_2, the results were different from those obtained in the absence of nano-TiO_2. The parameter r_w was significantly and positively correlated with the net accumulation, while the opposite trend was observed for AR/AW and I_3. This indicated that the metal reactivity and the interaction mechanism of metal ions change in the presence of nano-TiO_2. r_w, a basic parameter of metals, is obtained from the distance between nuclei when atoms interact by means of *Van Der Waals* interactions. This parameter affects and determines various important properties of metals that might be involved in the adsorption and migration of ions (Walker et al., 2003).

QSBAR models in the presence of nano-TiO_2 based on the six

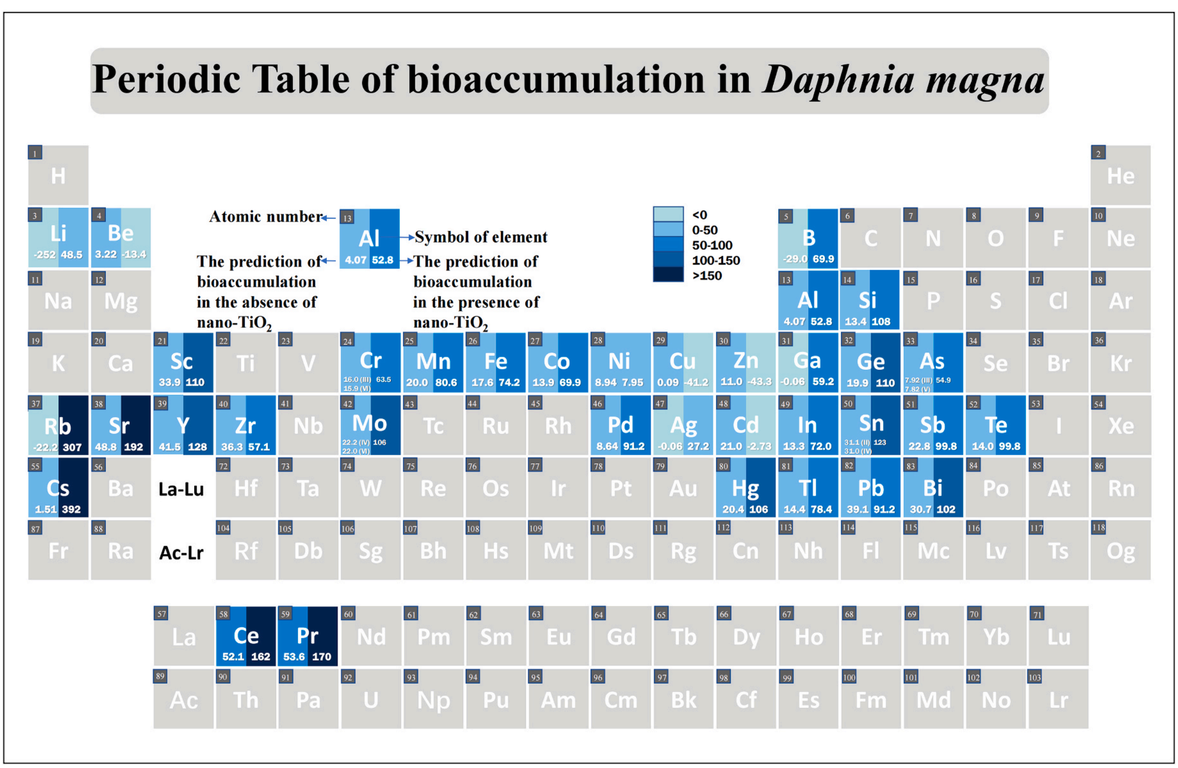

Fig. 2. Periodic Table of bioaccumulation in *Daphnia magna* in the absence and presence of nano-TiO_2. The colors on the left- and right-hand sides of each element represent the extent of bioaccumulation in *Daphnia magna* in the absence and presence of nano-TiO_2, respectively. The depth of each color represents the amount of accumulation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

descriptors were then constructed by use of the single-parameter linear regression method. Only r_w was selected as having a higher goodness-of-fit in the absence of nano-TiO_2 ($R^2 = 0.91$, $F = 30.237$, $P = 0.012$) (Table 2, Fig. S2). The single regression QSBAR model is considered to be an acceptable and predictable model ($Q^2_{LOO} = 0.743 > 0.5$, $RMSE_{CV} = 25.717$, $h < h^*$). The model considers that the *Van Der Waals* interactions are inversely proportional to the sixth power of the interaction distance, which means that the larger the *Van Der Waals* radius of the metal, the smaller the *Van Der Waals* interactions. This weakens the intensity of adsorption caused by *Van Der Waals* interactions. Therefore, the metal accumulation in *Daphnia magna* increased with the increase in *Van Der Waals* radius.

3.4. Prediction and comparison of predicted metals accumulation by established QSBAR models

The net accumulation *in Daphnia magna* of another set of 30 metals at an exposure concentration of 0.0185 μmol/L was predicted by means of the two established QSBAR models (Fig. 2). The results showed that except for Be, Ni, Cu, Zn and Cd, the accumulation of all other metals increased in the presence of nano-TiO_2. This result showed that the metal exposure concentration of 0.0185 μmol/L was too low to induce significant accumulation of some free metal ions, such as nutrients (Cu and Zn), into *Daphnia magna*. However, nano-TiO_2 combined with some other metal ions and the smaller nano-TiO_2 particles entered *Daphnia magna* through ingestion, which called carrier effect. It allowed the adsorbed metal ions to also accumulate in *Daphnia magna* through the release of the metal from the ingested nano-TiO_2 particles (Fan et al., 2012). These metal ions may accumulate in *Daphnia magna* and increase the bioaccumulation of metals. In addition to nano-TiO_2 acting as carriers, nano-TiO_2 may also provide potential binding or adsorption sites for any incoming/ingested metals (Tan and Wang, 2014), which would produce joint toxicity combined with metals. The existence of nano-TiO_2 can also lead to competitive adsorption that makes other substances unable to adsorb heavy metals. Thus, nano-TiO_2 can promote the enrichment of metals and then enhance their toxicity. Moreover, the order of accumulation of transition metals in the fourth period in the periodic table in *Daphnia magna* in the presence of nano-TiO_2 was approximately consistent with the Irving-Williams stability series (Irving and Williams, 1953), i.e., $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$, which suggests that there is a relationship between metal accumulation and the stability of doubly charged cations to form a complex with a biotic ligand in the presence of nano-TiO_2. It is probably because nano-TiO_2 such as P25 has a negative zeta potential in the test medium.

However, the extent of accumulation of different metals in *Daphnia magna* differed a lot in the presence of nano-TiO_2 (Figs. S3A and S3B). This may be related to the different mechanisms through which nano-TiO_2 promotes the uptake of metals, such as Cd and Zn (Tan and Wang, 2014). Interestingly, for the metals in the fourth period of the periodic table, except for Ga (1060-fold increase in accumulation) and metals with reduced accumulation (Ni, Cu, Zn), the fold increase in metal accumulation increased strictly with the increase in AN in the presence of nano-TiO_2 (Sc < Cr (III)<Cr (V) < Mn < Fe < Co < Ge < As (III)<As (V)). However, the same trend was not found in other periods. Besides, for the elements within the same group of the periodic table, the fold increase in metal accumulation in groups IVA, VA and IIIB (Fig. S3C) was inversely proportional to AN. It is necessary to further investigate the mechanisms of such increase in the accumulation of different metals induced by nano-TiO_2.

3.5. Importance and uncertainties

Unique QSBAR models were established for prediction of metal accumulation in *Daphnia magna* in the absence and presence of nano-TiO_2. It was found that the presence of nano-TiO_2 changes the accumulation of metals in *Daphnia magna* at low metal exposure concentrations. For example, in the absence of nano-TiO_2, the accumulation is related to the reactivity of metals, while in the presence of nano-TiO_2, accumulation is related to the intensity of the *Van Der Waals* interactions. This is of great significance for a better understanding of the bioaccumulation mechanisms of metals and the effect of nano-TiO_2 on metal bioaccumulation. It also provides a preliminary theoretical basis for the prediction of nano-TiO_2 on the toxic effect of metals in the future.

Although the models are able to properly predict metal accumulation in *Daphnia magna*, it is deemed necessary to further improve the models in order to obtain even more reliable predictions in future studies. The improvements needed are related to the following aspects: (1) using consistently measured data such as employing the same instruments and recording data in the same way by different laboratories as this issue was not used as a selection criterion in the present study; (2) using more consistent exposure conditions, such as limiting the exposure time of metals to a certain time period, for instance 24–96 h (3) differentiating for the crystalline forms (e.g., rutile, anatase, and brookite), and the different properties (such as hydrophilicity and hydrophobicity) of nano-TiO_2 used in the sorption experiments since the crystalline phase of nano-TiO_2 might influence the interaction of the nanoparticles and metal ions; (4) expanding the database, especially for data-poor metals such as As(V), which will help to decrease the uncertainty related to the net accumulation correction; (5) increasing the number of metals used for developing the QSBAR model as this will increase the accuracy of the models. Nevertheless, this study is still an important advancement of the understanding of metal bioaccumulation in the absence and presence of nano-TiO_2.

4. Conclusions

The presence of nano-TiO_2 impacts metal accumulation. For more than 85% of the studied metals, metal accumulation in *Daphnia magna* increases in the presence of nano-TiO_2, as shown by the model predictions. Different descriptors affecting heavy metal bioaccumulation could be identified in the absence and presence of nano-TiO_2. The main conclusion drawn from the data generated and the models developed is that the environmental risk of heavy metals in the natural water environment is underestimated when the impact of nano-TiO_2 is not properly taken into account. This implies that more attention should be paid to the effects of nanomaterials (such as nano-TiO_2) on the toxicity of heavy metals in natural conditions. Moreover, it can be concluded that it is of great significance to better understand the environmental impacts of the coexistence of nanomaterials and contaminants, since this will allow to properly assess the actual risk of heavy metals in aquatic environments.

Author contributions statement

Ying Wang: Conceptualization, Writing – original draft, Methodology, Validation. Xiang Gao: Data curation, Methodology, Formal analysis. Yinghao Cheng: Data curation, Visualization; Zhaomin Dong: Software, Validation. Willie J. G. M. Peijnenburg: Writing-reviewing & editing; Wenhong Fan: Conceptualization, Supervision, Writing-reviewing & editing.

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.

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Appendix A. Supplementary data

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