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Toxicity of copper, lead, and cadmium on the motility of two marine microalgae *Isochrysis galbana* and *Tetraselmis chui*

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Abstract

Computer assisted movement tracking was used to characterize the motility of two marine microalgae, *Isochrysis galbana* and *Tetraselmis chui*, and to investigate the toxicity of Cu, Pb, and Cd on motile percentage, curvilinear velocity, average path velocity, straight line velocity, linearity, straightness, and wobble. Except for motile percentage, all other motility parameters differed significantly between *I. galbana* and *T. chui*. Based on relative motile percentage data, the median effective concentration (EC_{50}) of Cu on the motility of *I. galbana* and *T. chui* was 31.4 and 1.3 μmol/L, respectively, while for Pb it was 37.8 and 10.9 μmol/L and for Cd it was 121.6 and 37.8 μmol/L, respectively. Compared to *I. galbana*, *T. chui* was more sensitive to all tested metals. The toxic effect of the heavy metals on motility exhibited the following decreasing order for both species: Cu > Pb > Cd. Our results indicate that *I. galbana* and *T. chui* motility is sensitive to heavy metals and can be used as an indicator for toxicology bioassays.

Key words: copper; lead; cadmium; motility; *Isochrysis galbana*; *Tetraselmis chui*

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Introduction

Anthropogenic pollutants in marine and estuarine environments have significantly increased over the last few decades. Among these pollutants, heavy metals, which tend to accumulate in bottom sediment and release slowly into water bodies, have long been recognized as major marine pollutants (Ansari et al., 2004; Depledge et al., 1994).

As primary producers, microalgae not only form the basis of life in the oceans, they also produce great amount of oxygen in the earth's atmosphere. In addition, microalgae are commercially important for their wide use as food in the aquaculture industry. Due to their ecological and commercial importance, considerable research on the effect of heavy metal pollutants on various microalgae has been conducted over the last few decades (Trevors et al., 1986; Stauber and Florence, 1987; Satoh et al., 2005).

Along with physiological traits such as growth, metabolism, and photosynthesis, motility is a key behavioral characteristic of motile cells. Previous studies on microalgae have, however, focused mainly on heavy metal bioaccumulation (Fisher et al., 1984; Sandau et al., 2004) and the toxicity impact of heavy metals on physiological traits (Break et al., 1976; Overnell, 1975; Satoh et al., 2005). To date, the effect of heavy metal toxicity on the motility of the algal species *Isochrysis galbana* or *Tetraselmis chui* has not been investigated.

Recent advances in technology and computer analysis have enabled cell motility to be measured by computer assisted movement tracking (Wilson-Leedy and Ingermann, 2007). Computer assisted sperm analysis (CASA) has, for example, been applied extensively to sperm mobility studies (Kupriyanova and Havenhand, 2005; Gage et al., 2004; Malo et al., 2005). Moreover, several parameters, including motile percentage (%MOT), curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), linearity (LIN), straightness (STR), and wobble (WOB), have been developed to characterize motility (Kawaguchi et al., 2004). Technological advances have, therefore, made it possible to investigate the toxicity effects of heavy metals on various organisms using cell motility bioassays (Abascal et al., 2007; Huang et al., 2001).

The marine microalgae *I. galbana* and *T. chui* are widely distributed along the Chinese coast. Along with the significant deterioration of Chinese marine environments, pollution poses a great threat to almost all marine organisms. In addition to major bioassays, which use physiological traits for analysis, it is useful to determine

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how common marine heavy metals such as copper, lead, and cadmium affect the flagella beating motility of *T. chui* and *I. galbana*. The results of the present study will help establish dose-response relationships between each metal and motility parameters and will contribute to the implementation of marine water quality standards.

1 Materials and methods

1.1 Microalgae

Unialgal *I. galbana* and *T. chui* cultures with densities of 7.5×10^5 and 5.0×10^5 individuals per micro-liter, respectively, were used in all experiments. The cultures were kept at (28 ± 1)°C under natural daylight. Cell counts were made prior to experiments using a haemocytometer slide and a Nikon Eclipse E600 microscope (Japan). Cultures were handled aseptically throughout the experiments.

1.2 Metal solutions and heavy metal bioassays

Experiments were performed using the following analytical grade salts: $Cu(NO_3)_2 \cdot 3H_2O$, $Pb(NO_3)_2$, and $Cd(NO_3)_2 \cdot 4H_2O$. Stock solutions were prepared in deionized water at concentrations (1 mol/L) high enough to prevent weighing errors and salinity changes (Wang et al., 2009). Experimental concentrations were chosen on the basis of preliminary trials. In the first series of assays, *I. galbana* and *T. chui* were exposed to different concentrations of the three tested metal salts. Control tests were conducted with 0 μmol/L of heavy metals. Three replicates were used for each concentration and for the control tests. Sterile polystyrene six-well cell culture clusters with a total volume of 10 mL (Costar®, USA) were used as test chambers for all toxicity bioassays. A one hour treatment time was applied to all trials.

1.3 Motility analysis

One drop of microalgae suspension with a volume of 30 μL was used to make a wet mount (depth approximately 0.25 mm) (Everett et al., 2004). Thirty second video clips (100 frames per second) of motile microalgae were captured using NTS-Elements D3.0 through a Nikon Eclipse E600 microscope at 100× magnification. Percentage of motile cells was calculated as the number of motile algae divided by the total number of algae in the field of view. Movement tracking was performed using the manual tracking function of Image-J (Daniel Marsh, http://rsb.info.nih.gov/ij/plugins/avi-reader.html). The parameter calculation of curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), larger linearity (LIN), straightness (STR), and wobble (WOB) was conducted according to the algorithm introduced by Wilson-Leedy and Ingermann (2007).

1.4 Statistical analysis

Relative values of all the parameters including MOT, VCL, VAP, VSL, LIN, STR, and WOB incubated at different heavy metal concentrations were obtained by dividing their values by that of the control tests (Young and Nelson, 1974). The EC_{50} values and their 95% confidence intervals were determined according to the probit method (Newman, 1995) using the percentage data of relative motile cells (MOT), VCL, VAP, and VSL. In the present study, the EC_{50} values and their 95% confidence intervals were not estimated for LIN, STR, and WOB.

Data are presented as mean ± SD. One-way analysis of variance (ANOVA) was performed on all data using "R" statistic package (R Development Core Team, 2009). The values of motile cells (MOT), LIN, STR, and WOB were arcsine-transformed prior to ANOVA and presented in tables as non-transformed percentages (Zar, 1996). A p-value < 0.05 was accepted as statistically significant for all tests.

2 Results

2.1 Motility of *I. galbana* and *T. chui*

The movement tracks of *I. galbana* and *T. chui* in the control tests are illustrated in Fig. 1. As shown in Table 1, without heavy metal treatment, most *I. galbana* and *T. chui* (> 99%) were motile and there was no significant difference between the two marine microalgae species in the percentage of MOT. However, *T. chui* swam faster than *I. galbana* as VCL, VAP, and VSL were significantly greater than those of *I. galbana* ($p < 0.05$). In addition, with significantly LIN, STR, and WOB, *T. chui* swam straighter and wobbled more frequently than *I. galbana*.

2.2 Copper

When *I. galbana* was exposed to increasing Cu concentrations for one hour, the relative values of all motility parameters showed a significant decrease (Table 2). This indicates that increasing metal concentrations affected *I. galbana* motility in a dose-dependent manner. Similarly, as shown in Table 3, the relative values of MOT, VCL,

Table 1 Motility comparisons between *T. chui* and *I. galbana* controls using one way ANOVA

	I. galbana (n = 30)	*T. chui* (n = 30)	ANOVA	p value
MOT (%)	99.26 ± 0.48	99.39 ± 0.30	F1,58 = 1.61	0.21
VCL (μm/sec)	276.85 ± 37.30	426.65 ± 143.63	F1,58 = 32.83	< 0.01*
VAP (μm/sec)	109.45 ± 16.94	246.47 ± 113.44	F1,58 = 46.81	< 0.01*
VSL (μm/sec)	70.46 ± 30.60	150.64 ± 72.18	F1,58 = 34.29	< 0.01*
LIN (%)	16.94 ± 7.02	34.05 ± 14.92	F1,58 = 35.00	< 0.01*
STR (%)	40.10 ± 17.39	68.43 ± 17.84	F1,58 = 43.07	< 0.01*
WOB (%)	24.82 ± 5.84	51.58 ± 19.70	F1,58 = 56.56	< 0.01*

MOT: motile cells; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight line velocity; LIN: linearity; STR: straightness; WOB: wobble.
* Significant difference between the values of *I. galbana* and *T. chui*.

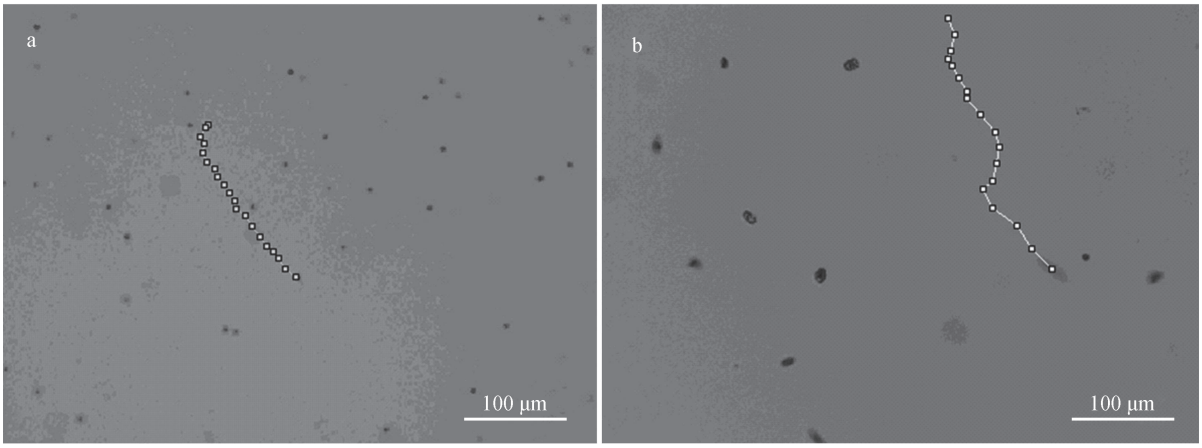

Fig. 1 Image-J reconstructed movement track of *I. galbana* (a) and *T. chui* (b).

Table 2 Effect of Cu on relative MOT, VCL, VAP, VSL, LIN, STR, and WOB of *I. galbana*

	Cu concentration							
	0 (control)	12.59 μmol/L	19.95 μmol/L	31.62 μmol/L	50.12 μmol/L	79.43 μmol/L	125.89 μmol/L	199.53 μmol/L
MOT (%)	100 ± 3.2	97.3 ± 8	82. 7 ± 10.7*	31. 7 ± 11.6*	21 ± 9.2*	13 ± 6.2*	1 ± 1*	0 ± 0*
VCL (%)	100 ± 5.2	87 ± 4.6	79.3 ± 3.5*	49 ± 5.6*	43 ± 7.2*	17.3 ± 3.5*	3 ± 2*	0 ± 0*
VAP (%)	100 ± 2.9	83.3 ± 2.1*	71.4 ± 4.5*	42.7 ± 7.2*	37.3 ± 8.1*	13.4 ± 4*	2 ± 2*	0 ± 0*
VSL (%)	100 ± 4.6	73.3 ± 4.7*	64.7 ± 4.2*	34.8 ± 9*	22.3 ± 3.1*	4.7 ± 1.2*	0.6 ± 0.5*	0 ± 0*
LIN (%)	100 ± 7.4	84.5 ± 8.5	81.1 ± 3.4*	70.1 ± 10.8*	53 ± 12.6*	26.9 ± 2.9*	16.3 ± 14.6*	n.a.
STR (%)	100 ± 6.9	88.1 ± 7	90.3 ± 4.1	80.5 ± 6.7*	62 ± 18.5*	35.4 ± 1.8*	33.8 ± 16*	n.a.
WOB (%)	100 ± 1.1	95.9 ± 1.3*	89.9 ± 1.7*	86.8 ± 4*	86.4 ± 4.7*	76.3 ± 10.7*	73.3 ± 9.4*	n.a.

Values shown are the mean ± SD (n = 3); * Significant difference with respect to the values of control test ($p < 0.05$, ANOVA). n.a.: not available.

Table 3 Effect of Cu on relative MOT, VCL, VAP, VSL, LIN, STR, and WOB of *T. chui*

	Cu concentration							
	0 (control)	0.79 μmol/L	1.26 μmol/L	2.00 μmol/L	3.16 μmol/L	5.01 μmol/L	7.94 μmol/L	12.59 μmol/L
MOT (%)	100 ± 4.7	62 ± 6.6*	50. 3 ± 8.3*	39. 3 ± 6.5*	23 ± 2*	32 ± 3.6*	20.3 ± 2.1*	0 ± 0*
VCL (%)	100 ± 4.2	92.7 ± 5.7	95 ± 3.6	96.7 ± 3.2	94 ± 4.6	93.7 ± 3.1	95.3 ± 3.2	0 ± 0*
VAP (%)	100 ± 3.9	96.4 ± 4.3	94.6 ± 4.6	90.3 ± 1.1*	92.1 ± 2.1*	90.3 ± 0.6*	89.7 ± 2.4*	0 ± 0*
VSL (%)	100 ± 3.6	91.9 ± 1.3*	87 ± 2.7*	86.3 ± 3.1*	87 ± 6*	86 ± 6.1*	85.3 ± 5.1*	0 ± 0*
LIN (%)	100 ± 5.4	94.4 ± 4.9	91.6 ± 6	89.4 ± 5.6	92.8 ± 8.8	91.9 ± 7	89.7 ± 8.6	n.a.
STR (%)	100 ± 5.9	89.4 ± 6.7	92.2 ± 7.3	95.5 ± 6	94.4 ± 7.5	95.1 ± 7.8	88.1 ± 9.8	n.a.
WOB (%)	100 ± 2.1	94.8 ± 4.7	99.8 ± 8.7	101 ± 5.7	96.5 ± 4.7	93.8 ± 7	98.4 ± 10.4	n.a.

Values shown are the mean ± SD (n = 3). * Significant difference with respect to the values of control test ($p < 0.05$, ANOVA). n.a.: not available.

VAP, and VSL for *T. chui* significantly decreased with increasing Cu concentrations. However, compared to the gradual decrease of *I. galbana* motility with increasing Cu incubating concentrations, a significant reduction in relative VCL, VAP, and VSL at concentrations between 7.94 and 12.59 μmol/L of Cu were noted for *T. chui*. In addition, when exposed to various Cu concentrations, the values of relative LIN, STR, and WOB of *T. chui* did not show a significant change compared to that of the control.

Motility was completely arrested at 199.53 μmol/L of Cu for *I. galbana* and 12.59 μmol/L of Cu for *T. chui*. However, the lowest Cu concentration that significantly affected motility was 12.59 μmol/L for *I. galbana* and 0.79 μmol/L for *T. chui*.

2.3 Lead

After one hour exposure to increasing Pb concentrations, all relative motility parameters of both *I. galbana* and *T. chui* significantly decreased (Tables 4 and 5), indicating concentration-dependent motility inhibition.

2.4 Cadmium

Dose-dependent motility inhibition was also observed for Cd (Tables 6 and 7). The motility of *I. galbana* was significantly retarded at 100 μmol/L of Cd. At 10 μmol/L, Cd significantly inhibited the relative values of MOT, VSL, and LIN for *T. chui*. All other motility traits of *T. chui* were significantly reduced at 31.62 μmol/L of Cd. Motility was completely arrested at 1000 and 316.23 μmol/L for *I. galbana* and *T. chui*, respectively.

Table 8 shows the EC_{50} values and their 95% confidence intervals. According to the EC_{50} values calculated from the MOT data, Cu, Pb, and Cd were 24, 4 and 3 times more toxic to *T. chui* than to *I. galbana*. Based on the motility of *I. galbana*, Cu was slightly more toxic than Pb and 4 times more toxic than Cd. Based on the motility of *T. chui*, Cu was about 10 times more toxic than Pb and 30 times more toxic than Cd. From these results, Cu was the most toxic metal tested, with toxicity ranking from highest to lowest in the following order: Cu > Pb > Cd.

Table 4 Effect of Pb on relative MOT, VCL, VAP, VSL, LIN, STR, and WOB of *I. galbana*

	Pb concentration						
	0 (control)	12.59 μmol/L	19.95 μmol/L	31.62 μmol/L	50.12 μmol/L	79.43 μmol/L	125.89 μmol/L
MOT (%)	100 ± 3.2	95.4 ± 5	93.7 ± 6.8	75.9 ± 7.2*	19.3 ± 1.7*	0 ± 0*	0 ± 0*
VCL (%)	100 ± 5.2	93.7 ± 4.9	92.3 ± 5.6	86.1 ± 3.2*	59.2 ± 5.5*	0 ± 0*	0 ± 0*
VAP (%)	100 ± 2.9	87.2 ± 1.3*	90.9 ± 2.7*	84.3 ± 7.1*	41.5 ± 10.8*	0 ± 0*	0 ± 0*
VSL (%)	100 ± 4.6	86.2 ± 1.4*	84.1 ± 3.5*	74.0 ± 5.3*	25.2 ± 5.2*	0 ± 0*	0 ± 0*
LIN (%)	100 ± 7.4	92.6 ± 4.3	91.7 ± 8.2	85.5 ± 7.6	29.4 ± 8.3*	n.a.	n.a.
STR (%)	100 ± 6.9	98.8 ± 4.7	92.9 ± 8.3	81.8 ± 17.4	42.0 ± 7*	n.a.	n.a.
WOB (%)	100 ± 1.1	93.7 ± 4.5	98.7 ± 1.7	73.6 ± 14.3*	72.8 ± 12.8*	n.a.	n.a.

Values are presented as mean ± SD (n = 3). * Significant difference with respect to the values of control test ($p < 0.05$, ANOVA). n.a.: not available.

Table 5 Effect of Pb on relative MOT, VCL, VAP, VSL, LIN, STR, and WOB of *T. chui*

	Pb concentration							
	0 (control)	7.94 μmol/L	12.59 μmol/L	19.95 μmol/L	31.62 μmol/L	50.12 μmol/L	79.43 μmol/L	125.89 μmol/L
MOT (%)	100 ± 4.7	98.3 ± 1.7	4 ± 1.3*	1 ± 0.7*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
VCL (%)	100 ± 4.2	92.9 ± 7.7	32.5 ± 11.5*	15.3 ± 3.8*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
VAP (%)	100 ± 3.9	93.3 ± 6.7	13.9 ± 3*	5.7 ± 1.2*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
VSL (%)	100 ± 3.6	69.5 ± 9.3*	5.5 ± 3.5*	0.8 ± 0.4*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
LIN (%)	100 ± 5.4	74.9 ± 7.5*	19 ± 13.4*	5.7 ± 3.1*	n.a.	n.a.	n.a.	n.a.
STR (%)	100 ± 5.9	75 ± 11.5*	40.5 ± 26.6*	14.3 ± 5.2*	n.a.	n.a.	n.a.	n.a.
WOB (%)	100 ± 2.1	100.4 ± 5.3	44.3 ± 6.1*	37.9 ± 8.1*	n.a.	n.a.	n.a.	n.a.

Values are presented as mean ± SD (n = 3) * Significant difference with respect to the values of control test ($p < 0.05$, ANOVA). n.a: not available.

Table 6 Effect of Cd on relative MOT, VCL, VAP, VSL, LIN, STR, and WOB of *I. galbana*

	Cd concentration						
	0 (control)	10 μmol/L	31.62 μmol/L	100 μmol/L	316.23 μmol/L	1000 μmol/L	3162.28 μmol/L
MOT (%)	100 ± 3.2	97 ± 3.2	96.9 ± 3.1	47.8 ± 7.7*	17.9 ± 3.9*	0 ± 0*	0 ± 0*
VCL (%)	100 ± 5.2	97.3 ± 4.9	96.9 ± 3	80.1 ± 3.5*	33.6 ± 4*	0 ± 0*	0 ± 0*
VAP (%)	100 ± 2.9	95.9 ± 3.3	94.1 ± 4.7	83.3 ± 6.9*	20 ± 9.6*	0 ± 0*	0 ± 0*
VSL (%)	100 ± 4.6	95.5 ± 4.4	94.8 ± 5.6	56.6 ± 10.5*	9.54 ± 3.6*	0 ± 0*	0 ± 0*
LIN (%)	100 ± 7.4	98.6 ± 5.3	97.9 ± 3.8	70.2 ± 10.1*	29.5 ± 13.8*	n.a.	n.a.
STR (%)	100 ± 6.9	97 ± 2.7	91.4 ± 11.9	68.5 ± 14.9*	49.8 ± 16.4*	n.a.	n.a.
WOB (%)	100 ± 1.1	98.6 ± 1.7	94.5 ± 5.2	76.7 ± 3.1*	72.8 ± 6.8*	n.a.	n.a.

Values are presented as mean ± SD (n = 3). * Significant differences with respect to the values of control test ($p < 0.05$, ANOVA). n.a.: not available.

Table 7 Effect of Cd on relative MOT, VCL, VAP, VSL, LIN, STR, and WOB of *T. chui*

	Cd concentration							
	0 (control)	1 μmol/L	3.16 μmol/L	10 μmol/L	31.62 μmol/L	100 μmol/L	316.23 μmol/L	1000 μmol/L
MOT (%)	100 ± 4.7	99.1 ± 1.6	98.2 ± 2.3	81 ± 5.1*	66.7 ± 5*	26.1 ± 4.4*	0 ± 0*	0 ± 0*
VCL (%)	100 ± 4.2	98.8 ± 3.8	96.9 ± 3.5	96.2 ± 3.6	91 ± 2.2*	59.9 ± 3.5*	0 ± 0*	0 ± 0*
VAP (%)	100 ± 3.9	98.7 ± 3.5	96.4 ± 3	93.3 ± 6.8	87.6 ± 3.1*	55.4 ± 6.8*	0 ± 0*	0 ± 0*
VSL (%)	100 ± 3.6	98.7 ± 3.9	98.1 ± 5.5	85.4 ± 5*	73.5 ± 5.1*	45.2 ± 5.7*	0 ± 0*	0 ± 0*
LIN (%)	100 ± 5.4	96.9 ± 4.4	90.9 ± 8.4	83.9 ± 7.1*	78.4 ± 5.2*	45.6 ± 5*	n.a.	n.a.
STR (%)	100 ± 5.9	97 ± 4.3	91.6 ± 9.6	92.6 ± 6.2	76.3 ± 9.8*	50 ± 5.8*	n.a.	n.a.
WOB (%)	100 ± 2.1	99.5 ± 6.3	98.7 ± 5.3	95.6 ± 4.1	82.6 ± 5.7*	92.4 ± 8	n.a.	n.a.

Values are presented as mean ± SD (n = 3). * Significant differences with respect to the values of control test ($p < 0.05$, ANOVA). n.a.: not available.

Table 8 EC_{50} and 95% confidence intervals of Cu, Pb and Cd (μmol/L) on MOT, VCL, VAP, and VSL of *I. galbana* and *T. chui*

	EC_{50} on *I. galbana*				EC_{50} on *T. chui*			
	MOT	VCL	VAP	VSL	MOT	VAP	VCL	VSL
Cu	31.4 (18.3–52.0)	36.1 (20.4–54.3)	31.7 (18.8–49.7)	24.5 (17.3–32.8)	1.3 (0.5–4.3)	13.2[a] n.c	12.1[a] n.c	9.9[a] n.c
Pb	37.8 (17.0–51.4)	44.8 (19.6–56.1)	40.7 (22.3–49.3)	35.7 (25.4–40.3)	10.9[a] n.c	12.6[b] (3.7–74.2)	11.2[b] (4.3–63.4)	8.1[a] n.c.
Cd	121.6 (104–201.1)	190.0 (131–245.5)	168.4 (122.1–221.9)	124.0 (106–205.7)	37.8 (17.8–62.1)	70.2 (43.8–135.7)	58.5 (22.4–124.5)	42.2 (17.9–102.3)

The 95% confidence intervals are indicated in brackets.
[a] EC_{50} was calculated based on the data from exposure to 7.94 and 12.59 μmol/L heavy metal solutions;
[b] EC_{50} was calculated based on the data from exposure to 7.94, 12.59, 19.95, and 31.62 μmol/L heavy metal solutions.
n.c: confidence intervals not calculated.

3 Discussion

3.1 Motility

Motility parameters of the microalgae in the present study were grouped into three categories, percentage of motile (MOT), movement velocity (VCL, VAP, and VSL), and movement pattern (LIN, STR, and WOB). Based on our results, the movement velocity and pattern for the two microalgae were species specific. The curvilinear velocity was (276.85 ± 37.3) μm/sec for *I. galbana* and (426.65 ± 143.63) μm/sec for *T. chui*, which are comparable to results obtained for Arctic green algae *Pyramimonas gelidicola* (Burch and Marchant, 1983), marine bacteria *Pseudoalteromonas haloplanktis* and *Shewanella putrefaciens* (Barbara and Mitchell, 2003), and protozoa *Euplotes aediculatus* (Salvadó et al., 1997).

With a few exceptions (such as LIN, STR, and WOB of *T. chui* incubated in Cu), dose-dependent motility inhibitions by Cu, Pb, and Cd were evident in the present study, which is consistent with toxicity data for pollutants affecting the motility of other organisms (Tanaka et al., 2005; Salvadó et al., 1997). Compared to lengthy toxicity investigations based on physiological traits, investigating toxicity based on motility is a rapid and convenient bioassay to test acute toxicity of environmental pollutants.

3.2 Toxicity of copper, lead, and cadmium

Without information from other studies investigating heavy metal toxicity on the motility of algae, the results of the present study can only be compared with data on the growth toxicity for various algal species. There is, therefore, an unavoidable interpretation problem since most toxicity tests have been carried out using different toxicity criteria, algal species, and experimental conditions compared to the present study. Based on the EC_{50} values obtained in our experiments, Cu had the most toxic effect on the motility of *I. galbana* and *T. chui*, followed by Pb and Cd. This result is in accordance with toxicity data based on IC_{50} (concentrations of metals estimated to inhibit 50% growth relative to the control) of *Prasinococcus* sp. (Satoh et al., 2005), and *I. galbana*, *Chaetoceros calcitrans*, *Tetraselmis tetrahele*, and *Tetraselmis* sp. (Ismail et al., 2002). A similar order in relative toxicity for the three heavy metals was also reported in sperm cell toxicity tests of *Paracentrotus lividus* (Novelli et al., 2003).

In the present study, Cu showed a significant reduction in motility for *I. galbana* and *T. chui* at 12.59 and 0.79 μmol/L, respectively. The EC_{50} (based on relative MOT data) was 1.3 and 31.4 μmol/L for *I. galbana* and *T. chui*, respectively. These findings are consistent with previous research where the growth of *Chlorella pyrenoidosa* and *Asterionella glacialis* were significantly arrested by Cu at 1.0 and 1.6 μmol/L (Stauber and Florence, 1987) and the growth of *Scenedesmus subspicatus* was inhibited by 50% at 5.4 μmol/L of Cu (Ma et al., 2003). Similarly, a Cu concentration of EC_{50} = 33 μmol/L was reported based on the growth of *S. acuminatus* (Lombardi et al., 2007).

The MOT data EC_{50} values obtained for the effect of Pb on *I. galbana* and *T. chui* motility were 37.8 and 10.9 μmol/L (equivalent to 2.4 and 0.69 mg/L), respectively. These values are comparable to IC_{50} values reported for *I. galbana* (2.5 mg/L), *Chlorococcum littorale* (10.6 mg/L), *Chlorococcum* sp. (21.4 mg/L), *Prasinococcus* sp. (6.4 mg/L), *Tetraselmis tetrathele* (8.6 mg/L), *Heterocapsa* sp. (14.4 mg/L), and *Synechococcus* sp. (5.4 mg/L) (Satoh et al., 2005). Similarly, the EC_{50} values for Cd obtained in the present study (equivalent to 13.67 and 4.25 mg/L for *I. galbana* and *T. chui*, respectively) are also comparable to the IC_{50} values (2.9–11.2 mg/L) reported for the same series of species tested for Pb (Satoh et al., 2005).

In the present study, *I. galbana* and *T. chui* showed different sensitivities to trace metals. These differences can be attributed to variations in metal handling strategies, sizes of the two organisms, or differences in their in ecology and biology. The toxicity mechanisms of Cu, Pb, and Cd on microalgae motility may be directly related to cellular respiration processes that provide energy for motility. Research has shown that intracellular ATP concentration decreases as Cu concentration increases in *Phaeodactylum tricornutum* (Cid et al., 1995). Similarly, Cd appears to affect protein synthesis and cellular organelles such as mitochondria (Trevors et al., 1986). Moreover, the toxicity of Pb on green alga *Stichococcus bacillaris* is related to inorganic phosphate concentration (Monahan, 1976; Pawlik-Skowroska, 2002). Schulze and Brand (1978) suggested the toxicity effect of Pb on mortality of green algae is caused by depriving the cells of phosphate. Thus, Pb may lead to toxicity effects on motility by depriving phosphate needed for cellular respiration.

4 Conclusions

Using computer assisted movement tracking, we investigated the sensitivity of *I. galbana* and *T. chui* cell motility to Cu, Pb, and Cd. Most values of the relative motility parameters were shown to be suitable for toxicity tests. Information obtained in the present study contributes to knowledge on how such toxicity bioassays can assist in the environmental quality assessment of marine environments.

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