Environ Sci Pollut Res (2018) 25:3170–3182
DOI 10.1007/s11356-017-9428-0

Water–effect ratio of copper and its application on setting site-specific water quality criteria for protecting marine ecosystems of Hong Kong

Vivien W.W. Bao[1] · Kevin K.Y. Ho[1] · Kenneth K.Y. Lai[1] · Yanny K.Y. Mak[1] · Erica P.Y. Mak[2] · Guang-Jie Zhou[1] · John P. Giesy[1,3,4] · Kenneth M.Y. Leung[1,3]

Received: 2 March 2017 / Accepted: 1 June 2017 / Published online: 28 June 2017

Abstract Generic water quality criteria (WQC) of a chemical are usually set based on results generated from toxicity tests which were conducted using standard laboratory water with well-controlled physiochemical properties. However, in natural aquatic environments, physiochemical characteristics, such as salinity, total suspended solid, total organic carbon and the co-existence of chemical contaminants, often vary spatially and temporally. These parameters can, in turn, alter the bioavailability of target chemicals and, thus, influence their toxicity to marine organisms. To account for site specificity, the US Environmental Protection Agency's water–effect ratio (WER = site water-LC50 / laboratory water-LC50) procedure can be applied to derive site-specific WQC. Most past studies, however, were conducted for freshwater systems. Here, for the first time, the WER of copper (Cu) was determined for three marine water control zones (WCZs) in Hong Kong: Victoria Harbour, Deep Bay and Southern WCZs. Samples of water were collected from three locations within each WCZ, while acute toxicities to the marine diatom *Skeletonema costatum*, intertidal copepod *Tigriopus japonicus* and larvae of marine medaka *Oryzias melastigma* were determined in site or laboratory (artificial seawater) waters. Results of this study showed that conservative final WER relative coefficients for Cu ranged from 0.57 to 0.73 for the three WCZs, and water from some locations caused >30% mortality in the fish larvae in the controls (without Cu addition). These results suggested that current generic WQC for Cu are likely under-protective for marine organisms in the three areas, and it should be tightened by multiplying it with site-specific WER to offer better protection to marine biodiversity and integrity of the ecosystem.

Keywords Metal · Asia · Diatom · Copepod · Medaka fish · Environmental quality standard · Hazard · Risk

Responsible editor: Philippe Garrigues

Highlights
- Water–effect ratio (WER) of copper (Cu) was derived for three marine areas in Hong Kong.
- Acute toxicity of Cu to three marine species was determined in site and lab waters.
- Conservative WER values for Cu ranged from 0.57 to 0.73 for the three marine areas.
- Site waters from some locations alone induced >30% mortality in fish larvae.
- Based on WER, water quality criteria of Cu should be tightened for better protection.

✉ Kenneth M.Y. Leung
kmyleung@hku.hk

[1] The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China

[2] Department of Earth Sciences, University of Ottawa, Ottawa, Canada

[3] The State Key Laboratory in Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

[4] Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon S7N 5B3, Canada

Introduction

Generic water quality criteria (WQC) are commonly established as a tool to regulate water quality and provide a balance between optimizing economic benefits and minimizing local water pollution. Generic standards are simple and unambiguous to apply and allow for standardization within and among jurisdictions. Generic WQC of chemicals are usually set based on results of toxicity tests conducted with standard procedures in which standard laboratory waters, such as artificial water or filtered natural water collected from a clean reference site, are utilized as test media. Physiochemical properties such as temperature and pH

are well controlled (Heijerick et al. 2005). However, in natural ecosystems, a variety of physical and chemical characteristics of water bodies have been shown to alter bioavailabilities of environmental contaminants, and thus, their toxicities to aquatic organisms (MESL 1997; Rosen et al. 2005a). These parameters include temperature, pH, conductivity, dissolved oxygen, nutrient, total suspended solid (TSS), dissolved organic carbon (DOC) and availability of other contaminants to the water body, as well as hardness, alkalinity and Ca/Mg ratio specific for freshwater systems and salinity for saltwater systems (CCME 2002; Li et al. 2014; Ytreberg et al. 2011; Wang et al. 2016; Zhou et al. 2014). Some of these factors have been considered in derivation of generic WQC of chemical contaminants; however, the presence of atypical water quality parameters, such as TSS or total organic carbon, has potential to influence protectiveness of WQC, leading to over- or under-protection of aquatic ecosystems (MESL 1997).

It is necessary to derive site-specific WQC to account for effects of physical and chemical parameters on the toxicity of a chemical substance (MESL 1997). For metals, the chemical form or "speciation" is important in determining toxicities to aquatic organisms. General, free, divalent cations are considered to be the most readily available and toxic forms of metals, while metals bound into complexes by inorganic or organic ligands are less available (Giesy et al. 1983; Giesy 1987). Site-specific criteria for metals can be predicted by use of geochemical speciation modes (Giesy 1983; Alberts et al. 1984). This concept has been developed into the "biotic ligand model" where competition of metals for ligands in solution and on the gills of organisms is used to predict toxic potentials of various surface waters (Tompsett et al. 2014; Vardy et al. 2014). This technique allows prediction of the free ions of metals in the presence of inorganic and organic ligands. While the biotic ligand model has been successful in accounting for the activities of metals and predicting apparent reactive or biologically available forms of metals in solution, it has not been successful in predicting toxicities in waters where the presence of additive or synergistic chemical constituents affected toxicities of metals.

To be able to account for both infra-additive and supra-additive effects of constituents in surface waters, in 1984, the Environmental Protection Agency of the USA (USEPA) proposed three main procedures to derive site-specific WQC (USEPA 1984, 1996), in which the water–effect ratio (WER) procedure (formerly referred to as the indicator species procedure) has been used as the primary basis and regarded as a useful tool for modifying generic WQC to account for unique characteristics of the site under investigation since then (MESL 1997; CCME 2002). The USEPA further derived an interim guidance on the determination and the use of WERs for metals in 1994 (USEPA 1994). In the WER procedure, side-by-side LC50s are determined in toxicity tests with the same species, using site-specific dilution water and standard laboratory water respectively. WER is calculated as the quotient of the LC50 in site water (site water-LC50) divided by the LC50 determined in standard laboratory water (laboratory water-LC50). The final site-specific WQC is determined by multiplying the generic WQC with the WER (Diamond et al. 1997a; Rand and Clark 2000; Welsh et al. 2000; CCME 2002). When a WER is not significantly different from the unity, it indicates that there is no difference in the toxicity of a chemical between the site water and laboratory water, and thus no change of the generic WQC is required (Rand and Clark 2000). The WER is a coefficient that indicates relative toxicities in the two types of water and can be greater or less than 1.0. When WER values are greater than 1.0, it means that there are characteristics in surface waters that are reducing the apparent reactive concentration (activity) of metals like Cu. When WER values are less than 1.0, there are factors in the site water that are potentiating or adding to toxicities of metals. In the case of metals, this might be due to the presence of other ions acting through the same mechanism of action. For metals, the WER is like an activity coefficient (γ) that when multiplied by the effect endpoint, such as the LC50, corrects the value upwards or downwards depending on whether in site water the toxic potency of the metal is greater or less. If γ is less than 1.0, it decreases the value of the LC50 (more potent) and when it is greater than 1.0, it increases the value of the LC50 (less potent).

As the importance of setting site-specific WQC became more apparent in 1994, the USEPA developed guideline procedures for applying the WER to metals, which promoted application of the WER approach in various water bodies (USEPA 1994). Most uses of WER have been for freshwaters, such as streams, rivers, creeks and lagoons, and almost all studies were focused on transition metals, including copper (Cu), cadmium (Cd), lead (Pb), silver (Ag) and zinc (Zn) (Carlson et al. 1986; Brungs et al. 1992). Relevant studies of WER on marine systems are relatively scarce.

Concentrations of Cu observed in coastal waters and sediments especially in marinas and harbours are frequently greater than historical background concentrations. Recently, due to the partial ban of organotin compounds in antifouling products in 1980–1990s, and a subsequent global ban of these compounds in 2008, there has been a dramatic increase in applications of Cu-based antifouling paints and co-applications of Cu (e.g. Cu_2O) with booster antifouling biocides (Schiff et al. 2007), which may also possess high toxicity to marine organisms. For instance, the toxicity of copper pyrithione (CuPT) to marine organisms was higher or comparable to that of tributyltin (TBT) (Bao et al. 2011). Variations in environmental conditions like temperature and salinity had significant effects on toxicity of CuPT or $CuSO_4$ to marine organisms (Kwok and Leung 2005; Li et al. 2014). Moreover, widespread contamination of coastal marine environments might be intensified if Cu acted additively with other metals or synergistically with other biocides (Bao et al. 2008, 2013, 2014). Therefore, contamination of the marine environment with Cu has been a rising concern around the world. For instance, the Department of

Water of the Government of Western Australia (2009) found that total concentrations of Cu ranged from <1 to a maximum of 12,000 μg/L in coastal waters of Perth, Western Australia and samples from six out of eight sites exceeded the marine water quality guideline of 1.3 μg Cu/L, stipulated by the Australian and New Zealand Environment and Conservation Council. In Hong Kong, SAR, China, Cu commonly persists in marine sediments. The greatest concentration was detected in central Victoria Harbour (140 mg Cu/kg dry mass (dm); EPD 2014a), which was greater than the Upper Chemical Exceedance Level set by the Hong Kong SAR Government (110 mg Cu/kg, dm; ETWB 2002). Concentrations of Cu were relatively less in sediments from Deep Bay in western waters (32–57 mg Cu/kg dm), southern waters (9–35 mg Cu/kg dm) and eastern waters (9–35 mg Cu/kg dm) of Hong Kong (EPD 2014a). At the same time, western waters also exhibited lesser salinity and greater concentrations of TSS and total organic carbon (TOC) compared to those in southern and eastern waters, especially during the summer (EPD 2014a). Currently, no numerical WQC (or called water quality objective) of Cu has been set for protecting the marine ecosystem of Hong Kong. This study, for the first time, (1) determine WER for Cu in coastal marine water from three areas, Deep Bay, Victoria Harbour and Southern water control zones (WCZs) in Hong Kong based on toxicities to three marine species representing three trophic levels and (2) investigate the effects of TSS and salinity on the WER of Cu for the three WCZs.

Materials and methods

Collection of water from various sites

Samples of water were collected from three locations in each of the three selected marine WCZs, Deep Bay, Victoria Harbour and Southern WCZs of Hong Kong (Fig. 1; Table 1). At each sampling location, approximately 15 L of water was collected from 1 m below the water surface by use of a Van Dorn water sampler. Samples were stored in three acid-washed polyethylene containers, leaving no air space inside the containers. Samples were then immersed in ice and transferred within 2 h to the Swire Institute of Marine Science, Cape D'Aguilar, Hong Kong. All toxicity tests with site waters were conducted within 36 h of collection.

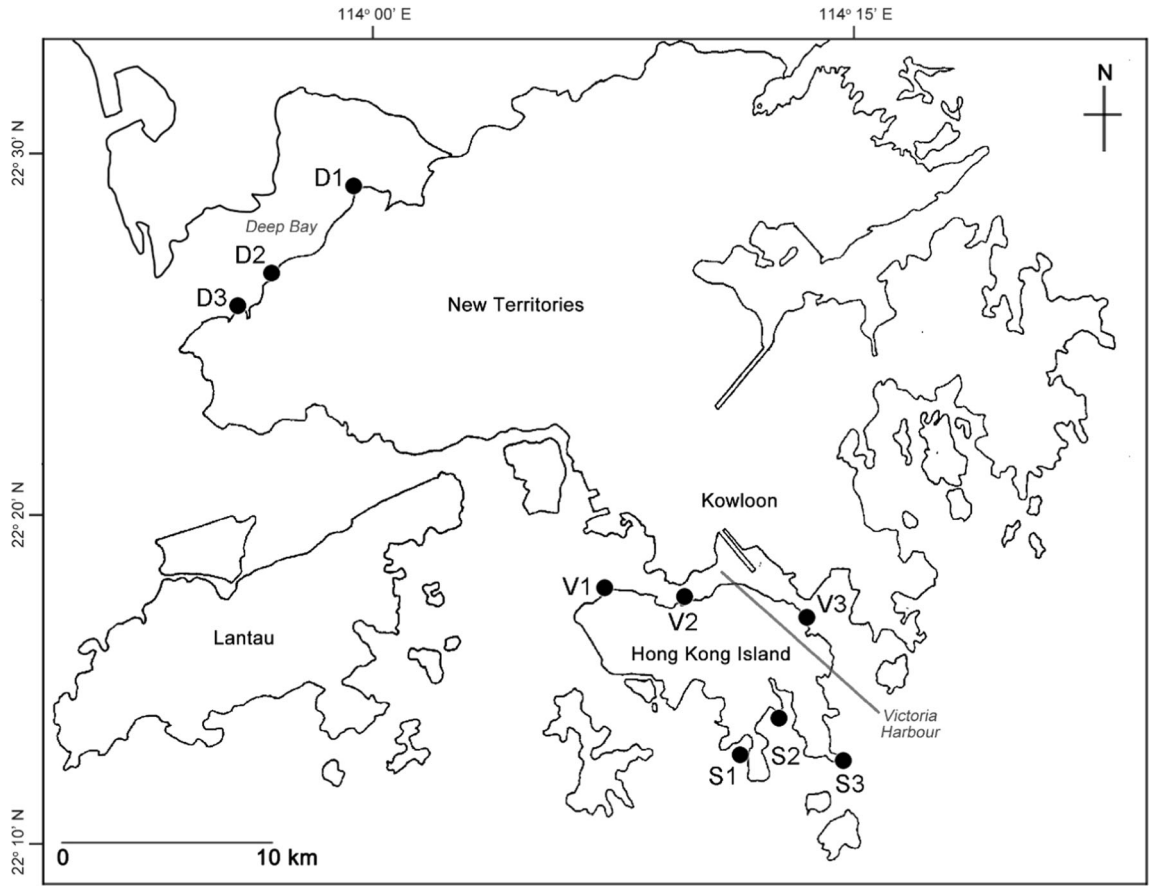

Fig. 1 Sampling sites in three marine water control zones (WCZs) of Hong Kong, including Deep Bay (*D1*: Lau Fau Shan; *D2*: Sheung Bak Nai; *D3*: Lung Kwu Tan landfill site), Victoria Harbour (*V1*: Belcher Bay; *V2*: Wan Chai; *V3*: Shau Kei Wan) and Southern (*S1*: Stanley Bay; *S2*: Tai Tam Bay; *S3*: Cape D'Aguilar) WCZs

Table 1 Locations of sampling sites and their coordinates in Deep Bay, Victoria Harbour and Southern water control zones in Hong Kong

Water control zone	Sampling time	Site	Coordinates
Deep Bay	December 2010–January 2011	D1 (Lau Fau Shan)	22° 29.345′ N, 113° 59.863′ E
		D2 (Sheung Bak Nai)	22° 26.472′ N, 113° 56.980′ E
		D3 (Lung Kwu Tan landfill site)	22° 25.566′ N, 113° 56.155′ E
Victoria Harbour	June–July 2011	V1 (Belcher Bay)	22° 17.088′ N, 114° 07.705′ E
		V2 (Wan Chai)	22° 17.080′ N, 114° 10.437′ E
		V3 (Shau Kei Wan)	22° 16.990′ N, 114° 13.834′ E
Southern Water	June–July 2011	S1 (Stanley Bay)	22° 12.578′ N, 114° 12.516′ E
		S2 (Tai Tam Bay)	22° 13.225′ N, 114° 13.636′ E
		S3 (Cape D'Aguilar)	22° 12.349′ N, 114° 15.470′ E

Treatment of site waters

Filtered artificial seawater (laboratory water; pH 8.1–8.4; salinity 32 ± 0.5‰ or adjusted to be the same as that of the site water depending on experimental setup) was prepared by dissolving sea salt (Tropic Marine, Germany) in de-ionized water, and then filtered through a 0.45-μm membrane filter.

Two experiments were conducted for each WCZ. Experiment I was used to determine the WER for Cu in the selected WCZs based on results for three marine species representing three trophic levels, including the diatom *Skeletonema costatum*, the copepod *Tigriopus japonicus* and the marine medaka fish *Oryzias melastigma*. Experiment II investigated effects of characteristics of water treatment, such as TSS or salinity on Cu toxicity using the adult copepod *T. japonicus* as the test species.

In experiment I, site water was filtered through Whatman glass microfiber filter (pore size 0.7 μm) to collect suspended solids for TSS and volatile suspended solid (VSS) quantification, and then further filtered through 0.45-μm membrane filter. pH and salinity of filtered site water was adjusted with autoclaved 1 M HCl or NaOH solution and pure NaCl (Sigma-Aldrich, >99.8% USA), respectively, to match the laboratory water with pH 8.1–8.4 and salinity of 32 ± 0.5‰. Site water and laboratory water used for testing toxicity to the diatom *S. costatum* were filtered through an autoclaved glass filter system with 0.45-μm membrane filter to decrease the chance of microbial infection, and stocks for f/2 + Si culture medium were added accordingly before using in toxicity tests. For each WCZ, four sets of 96-h Cu toxicity tests were conducted side-by-side, i.e. using the three pre-treated site water samples from each WCZ, and the laboratory water with the three selected test organisms.

Experiment II determined effects of water on toxicity of Cu. Water was collected from one site in each WCZ (i.e. D2 for Deep Bay WCZ, S1 for Southern WCZ and V3 for Victoria Harbour WCZ), and four sets of tests of toxicity of Cu were conducted with the copepod *T. japonicus* using the original (raw), settled (the top 1 L of site water settled in a 2-L beaker for 1 h) and filtered site water (0.45-μm membrane filter) samples as well as laboratory water. pH and salinity of raw, settled and filtered site water samples were adjusted to match the laboratory water with pH 8.1–8.4 and salinity of 32 ± 0.5‰. To determine effects of salinity on toxicity of Cu, tests using the copepod were conducted at the same time with the above four sets of tests of toxicity using site water from Stanley Bay (i.e. S1 in Southern WCZ) without adjustment of salinity (i.e. 28‰) and laboratory water with salinity adjusted to 28‰.

To determine TSS and VSS, suspended solids collected on the microfibre filter paper were rinsed with de-ionized water and dried at 80 °C until constant weight was obtained for TSS measurement, and then burned at 500 °C (Thermolyne 47900, Thermo Scientific, USA) for 3 h before quantification of VSS. For each WCZ, there were two to three replicates for measurement of TSS and VSS.

Test chemicals

A stock solution of Cu (1 g Cu/L) was prepared by dissolving copper(II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$, formula weight 249.7; purity ≥99.5%; BDH Chemicals Ltd. Pode, England) in distilled water.

Toxicity tests

Inhibition of growth of diatoms

Growth inhibition tests were conducted for Cu to *S. costatum* following the methods reported previously (Bao et al. 2008, 2011). Pure culture of the diatom, *S. costatum* (CCAP 1077/3; CCAP, UK) was maintained in autoclaved f/2 + Si medium (Guillard's medium for diatoms, 32 ± 0.5‰, pH 8.1–8.4; Guillard and Ryther 1962) under controlled laboratory conditions (25 ± 2 °C, 16:8 h light/dark photoperiod) and was manually shaken twice every day (Bao et al. 2008, 2011).

Cell density for a fresh diatom culture of *S. costatum* in exponential growth phase (<1 week old) was determined by

use of a haemocytometer. An appropriate amount of diatom culture and Cu stock were added to each 10-mL autoclaved test vial (with autoclaved plastic lids), with f/2 + Si medium made from site water or laboratory water, to make a final test solution volume of 5 mL and an initial algal density of 10^4 cells/mL with Cu levels ranged from 0 (control) to 5000 μg/L. Test vials were randomly positioned in an environmental chamber (25 ± 1 °C, 16:8 h light/dark photoperiod) and tested for 96 h, with manually shaken twice every day. There were three to five replicates for the control and three replicates for the treatments.

At 48 and 96 h, 200 μL test solution from each vial was transferred into a 96-well microplate, fixed with 66.7 μL of 1 M HCl and stored in dark at 4 °C for subsequent cell counting within 48 h using the haemocytometer (Bao et al. 2008).

A relative growth rate (RGR) of each treatment was calculated as the toxicity endpoint, which was defined (Eq. 1)

$$\mathrm{RGR} = (N_t - N_0)_{Treatment} / (N_t - N_0)_{Control} \quad (1)$$

where N_t: the cell density at time t; N_0: the initial cell density; and t: exposure duration.

Acute toxicity to adult copepods

Standard 96 h acute toxicity tests were conducted for Cu to adult copepods to determine mortality of copepods and median lethal concentration (LC50) for Cu, following the methods reported previously (Bao et al. 2011, 2013, 2014). Adults of the copepod *T. japonicus* were collected from rock pools in the splash zone of the rocky shore in the Cape D'Aguilar Marine Reserve, Hong Kong. They were fed the green alga *Tetraselmis* sp. for at least 2 weeks before the experiment, and maintained under controlled conditions at 25 ± 2 °C, 32 ± 0.5‰, pH 8.1–8.4 and 16:8 h light/dark photoperiod.

Acute toxicity tests with adult copepods were conducted in 50-mL glass beakers with 25-mL test solutions of Cu concentrations ranging from 0 (control) to 20,000 μg/L. There were 2–3 replicates per treatment, and 20 copepods per replicate. Beakers were randomly positioned in an environmental chamber (25 ± 1 °C, 16:8 h light/dark photoperiod) for 96 h. Dead copepods were defined as when the urosome was at a right angle to the prosome (Finney 1979) and counted at the end of 48 and 96 h.

Acute toxicity to larval medaka

Acute toxicity tests were conducted for Cu to fish larvae to determine mortality of larvae and LC50 for Cu (Bao et al. 2011), with details shown as below. Embryos of the marine medaka fish *O. melastigma* were obtained from an established fish culture at School of Biological Sciences, the University of Hong Kong. Newly hatched larvae were cultured in filtered artificial seawater (FASW; 32 ± 0.5‰, pH 8.1–8.4) under controlled laboratory conditions (25 ± 2 °C; 16:8 h light/dark photoperiod) and fed with aquarium fish food powder (Azoo, Taiwan) for 7–10 days before the experiment.

There were two to three replicates per treatment group, and each replicate consisted of ten larvae in 100 mL FASW with a designated Cu level ranging from 0 (control) to 20,000 μg/L in a test glass bowl (Pyrex). All test glass bowls were incubated in the environmental chamber (25 ± 1 °C, 16:8 h light/dark photoperiod) for 96 h. Mortality was monitored at the end of 48 and 96 h under a stereomicroscope. Fish larvae that had no body movement (e.g. tail and fins stopped flapping) and without obvious heartbeat were defined as dead.

Analysis of total recovery Cu in water samples from Deep Bay

Concentrations of Cu in site water were determined following instructions in 3030 regulation of APHA (1992). Acid digestion of suspended solids in unfiltered water samples with heating process was done for total recovery Cu measurement by Atomic Absorption Spectroscopy (AAS; PerkinElmer AAnalyst 800). During acid digestion, 3 mL of 69% HNO_3 was added into 100 mL site water with suspended solids and concentrated to 5–10 mL. Then, 1.5 mL of 37% HCl was added to the solution and heated for further digestion. Finally, 2% HNO_3 was added to make up a 14- to 15-mL solution for Cu measurement by the AAS. Site samples spiked with and without a known amount of Cu were also prepared and measured so as to determine the background concentration of Cu and calculate recovery of Cu.

Statistical analyses and calculation of water–effect ratio

Effective concentrations at 10 and 50% (EC10 and EC50, respectively) for Cu on the diatom *S. costatum* and lethal concentrations at 10 and 50% (LC10 and LC50, respectively) for Cu on the copepod and fish larvae, as well as their respective 95% confidence intervals were calculated using sigmoidal dose–response (variable slope) non-linear regression based on Hill's model (four parameters logistic regression) with GraphPad Prism version 5.00 (GraphPad Software, CA). For tests using mortality as an endpoint, when control mortality reached >5%, the treatment mortality rates were corrected with control mortalities using Abbott's formula (Abbott 1925) before the Hill's model analysis. For each WCZ, data of daily algal growth of the diatom in the control treatments or the control mortalities of the copepod and the medaka fish were checked for homogeneity of variances using Levene's test, and then the values were compared between the laboratory water and the site waters using one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons between the laboratory water and each site water.

Table 2 Ninety-six-hour EC50 and LC50 values (95% confidence interval, C.I., in parentheses) of copper (Cu) estimated for the three test organisms in experiment I

Water control zone	Water sample	96-h EC50 or LC50 of Cu (95% C.I.) (μg/L)		
		Skeletonema costatum	*Tigriopus japonicus*	*Oryzias melastigma*
Deep Bay	Laboratory water	252 (153–414)	870 (NA)	1773 (1319–2384)
	D1 (Lau Fau Shan)	560 (NA)	595 (437–810)	1347 (894–2030)
	D2 (Sheung Bak Nai)	624 (494–789)	881 (598–1300)	1593 (1124–2258)
	D3 (Lung Kwu Tan landfill site)	117 (56–242)	667 (513–867)	1626 (1149–2300)
Victoria Harbour	Laboratory water	593 (489–718)	1101 (923–1314)	1433 (1046–1964)
	V1 (Belcher Bay)	583 (497–684)	788 (698–891)	2209 (1267–3850)
	V2 (Wan Chai)	631 (474–840)	859 (712–1035)	1088 (815–1450)
	V3 (Shau Kei Wan)	614 (426–884)	794 (713–884)	2179 (1506–3152)
Southern Water	Laboratory water	595 (492–720)	608 (513–722)	856 (747–982)
	S1 (Stanley Bay)	586 (499–687)	532 (335–844)	1782 (1061–2992)
	S2 (Tai Tam Bay)	636 (480–842)	291 (218–387)	1079 (854–1363)
	S3 (Cape D'Aguilar)	618 (430–890)	223 (169–293)	1109 (751–1638)

NA data not available

WERs were calculated as the quotient of the site water-LC50 divided by the laboratory water-L(E)C50. A WER for each site, within each WCZ, was determined with two ways: one simply used the geometric mean of the three WER values among the three test species; another employed the smallest WER among the three test species (i.e. the conservative WER). The final WER (fWER) was calculated as the geometric mean of the WERs of the three sites within each WCZ. WERs and conservative WERs were compared respectively among the three WCZs using one-way ANOVA. All statistical analyses were performed using IBM SPSS Statistics 20.0 (International Business Machines Corporation, USA), and alpha was set as 0.05.

Results

Cu toxicity to marine organisms in site and laboratory waters

Based on the 96-h L(E)C50 values from experiment I, the diatom *S. costatum* and the copepod *T. japonicus* were less tolerant (i.e. lesser L(E)C50 values) to Cu than larvae of *O. melastigma* (Table 2), while the 96-h L(E)C50s ranged from 117 to 1101 μg/L for the former two species, and the 96-h LC50s were from 856 to 2209 μg/L for larvae of *O. melastigma*. Based on the 96-h LC50 values from experiment II, Cu in settled site water was more toxic to *T. japonicus*

Table 3 Ninety-six-hour EC50 and LC50 values (95% confidence interval, C.I., in parentheses) of copper (Cu) to *Tigriopus japonicus* in the laboratory water and site waters with various water treatments (i.e. data from experiment II)

Site	Water Sample (salinity) (‰)	96-h LC50 of Cu (95% C.I.) (μg/L)
D2 (Sheung Bak Nai)	Laboratory water (32.5)	644 (502–827)
	Filtered site water (32.5)	863 (535–1390)
	Settled site water (32.5)	502 (296–852)
	Raw site water (32.5)	961 (670–1378)
V3 (Shau Kei Wan)	Laboratory water (32.5)	544 (422–669)
	Filtered site water (32.5)	365 (278–478)
	Settled site water (32.5)	284 (221–366)
	Raw site water (32.5)	554 (500–614)
S1 (Stanley Bay)	Laboratory water (32.5)	600 (473–761)
	Filtered site water (32.5)	532 (335–844)
	Settled site water (32.5)	466 (389–559)
	Raw site water (32.5)	205 (156–271)
	Laboratory water (28)	581 (387–871)
	Filtered site water (28)	383 (297–493)

than in filtered site water, but Cu was less toxic to *T. japonicus* in raw site water than settled/filtered site waters except in S1 (Stanley Bay) (Table 3).

Toxicity of the site water alone (without added Cu)

Site waters from most of locations inhibited growth of *S. costatum* measured at 48- or 96-h exposure (Fig. 2, upper panel). Site water was also toxic to larvae of *O. melastigma*, with 96-h mortality reaching >30% for water from some locations (e.g. V1 and S1; Fig. 2, lower panel). However, no significant effects were observed for any of the site waters on the copepod (Fig. 2, middle panel).

Water–effect ratio

Geometric means of 96-h WERs for Cu, based on toxicity tests with the three species, ranged from 0.69 to 1.31 among nine sites (Table 4). There were no significant differences of WER (based on geometric means) among the three WCZs (one-way ANOVA $F_{0.05,2,6} = 0.059$, $p > 0.05$). fWERs of the three WCZs, calculated as the geometric mean of the WERs in the respective WCZ, were only slightly less than 1.0 ranging from 0.96 to 0.98 (Table 4).

However, conservative WERs of Cu from all nine sites were less than 1.0, ranging from 0.37 to 0.90, and they did not significantly differ among WCZs (one-way ANOVA

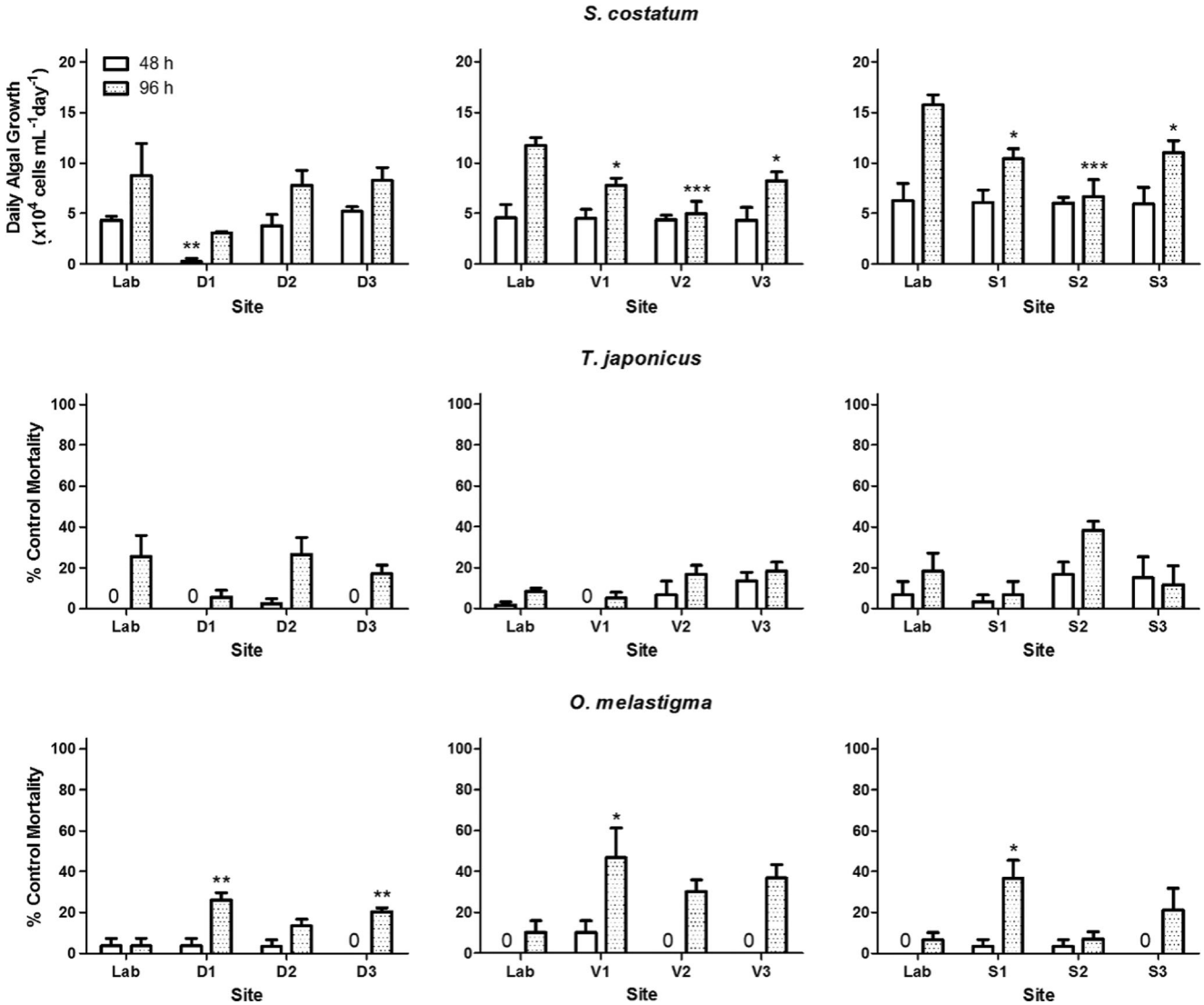

Fig. 2 Daily algal growth (mean + SEM; $n = 3$ in tests for Deep Bay water control zone, WCZ, or $n = 5$ in tests for Victoria Harbour and Southern WCZs) of the diatom *Skeletonema costatum* in the control treatments (i.e. Cu = 0 μg/L) during 96-h algal growth inhibition tests and mortality (mean + SEM; $n = 3$) of the adult copepod *Tigriopus japonicus* and the larvae of medaka fish *Oryzias melastigma* in control treatments during 96-h toxicity tests. "0" means no mortality. *Bars with the asterisks on the top* indicate that the control daily algal growth in the site water is significantly lower than that in the lab water for the diatom, or the control mortality in the site water is significantly greater than that in lab water for copepods and larvae of the fish (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; Dunnett's test)

Table 4 Water–effect ratio (WER) and final WER (fWER) of Cu based on 96-h toxicity tests for the three sampling sites in (A) Deep Bay water control zone (WCZ), (B) Victoria Harbour WCZ and (C) Southern WCZ

A. WER for Deep Bay WCZ	**Site D1**	**Site D2**	**Site D3**
Algae (*Skeletonema costatum*)	2.22	2.48	0.46
Copepod (*Tigriopus japonicus*)	0.68	1.01	0.77
Larvae of medaka fish (*Oryzias melastigma*)	0.76	0.90	0.92
WER (geometric mean)	1.05	1.31	0.69
fWER	***0.98***		
Conservative WER	0.68	0.90	0.46
Conservative fWER	***0.68***		
B. WER for Victoria Harbour WCZ	**Site V1**	**Site V2**	**Site V3**
Algae (*Skeletonema costatum*)	0.98	1.06	1.04
Copepod (*Tigriopus japonicus*)	0.72	0.78	0.72
Larvae of medaka fish (*Oryzias melastigma*)	1.54	0.76	1.52
WER (geometric mean)	1.03	0.86	1.04
fWER	***0.98***		
Conservative WER	0.72	0.76	0.72
Conservative fWER	***0.73***		
C. WER for Southern WCZ	**Site S1**	**Site S2**	**Site S3**
Algae (*Skeletonema costatum*)	0.98	1.07	1.04
Copepod (*Tigriopus japonicus*)	0.87	0.48	0.37
Larvae of medaka fish (*Oryzias melastigma*)	2.08	1.26	1.30
WER (geometric mean)	1.21	0.86	0.79
fWER	***0.96***		
Conservative WER	0.87	0.48	0.37
Conservative fWER	***0.57***		

$F_{0.05,2,6} = 0.506$, $p > 0.05$). Conservative fWERs for the three WCZs ranged from 0.57 to 0.73 for the three WCZs (Fig. 3; Table 4).

Effects of water treatment and salinity on toxicity of Cu

Effects of water treatments on toxicity of Cu to the adult copepod *T. japonicus* varied among WCZs (Fig. 4). For water samples from D2 in Deep Bay and V3 from Victoria Harbour, 96-h WERs were greatest in the original (i.e. raw) water sample, and least in settled water. This result indicated that Cu added during the acute test exerted the greatest toxicity to adult copepods in settled water and least toxicity in original water samples from D2 and V3. Results of S1 from Southern WCZ showed a different pattern; 96-h WERs were relatively similar in both filtered and settled waters, which were much greater than that in the original water sample. This result indicated that toxicity of Cu to the copepod was the greatest in the original water sample from S1. Alternatively, WERs obtained from filtered, settled and raw waters from Deep Bay WCZ were generally greater than those obtained from Victoria Harbour and Southern WCZs (Fig. 4). Deep Bay also contained greater concentrations of TSS and VSS than those in Victoria Harbour and Southern WCZs (Table 5). Both the WER values for 96-h acute toxicity tests of Cu to the adult copepod at 28.0 and 32.5‰ were less than 1.0, indicating that toxicity of Cu to the copepod in site waters was consistently greater than that in laboratory water (Fig. 5). Toxicity of Cu to the copepod was greater in site water with lesser salinity (i.e. 28.0‰; smaller LC50 and WER value) than in site water with salinity adjusted to 32.5‰.

Discussion

Toxicity of Cu in site or laboratory water

Acute toxicities of Cu determined in both experiments I and II (Tables 2 and 3) were consistent with those reported previously (Bao et al. 2011), except that the 96-h LC50 for newly hatched larvae of *O. melastigma* 7300 μg Cu/L, reported by Bao et al. (2011), was greater than that of the current study in which larvae of 7–10 days old were used. Since there was no food supplied for the fish larvae during the whole 96-h test period, newly hatched medaka fish larvae relied on egg yolk as the sole source of nutrients and energy. However, 7- to 10-day-old larvae no longer had egg yolk; thus, they were likely more vulnerable to Cu toxicity.

Toxicity of site waters without addition of Cu

Survival in controls without addition of Cu, in the experiment I, indicated that all nine site waters were toxic to test organisms, especially to the diatom *S. costatum* and larvae of *O. melastigma*. Metals might be contaminants in site waters. In this study, total recoverable Cu in the site waters from Deep Bay (D1-D3) were relatively great with concentrations of 46–59 μg Cu/L (Table 6). Concentrations of metals, especially Cu and Ag greater than expected historical background concentrations in sediments of Victoria Harbour and Deep Bay, were due to previous industrial pollution from the 1960s to 1980s (EPD 2014a). In addition to metals, in the Cape D'Aguilar marine reserve in Hong Kong (i.e. site S3 in this study), due to effluent discharge of the nearby sewage treatment plants and surface runoff, concentrations of the endocrine disrupting chemical (EDC), 4-nonylphenol, exceeded a hazard quotient (HQ) of 1.0 (Xu et al. 2014). Also, EPD's water monitoring program reported concentrations of the degradation product of synthetic, industrial surfactants, nonylphenol in sediment near effluent discharge points of preliminary treatment plants in central Victoria Harbour, while concentrations of polychlorinated biphenyls (PCBs) in sediment in the western portion of Victoria Harbour were greater than those in more remote locations (EPD 2014a). A systematic quantification of chemicals would therefore be necessary to determine

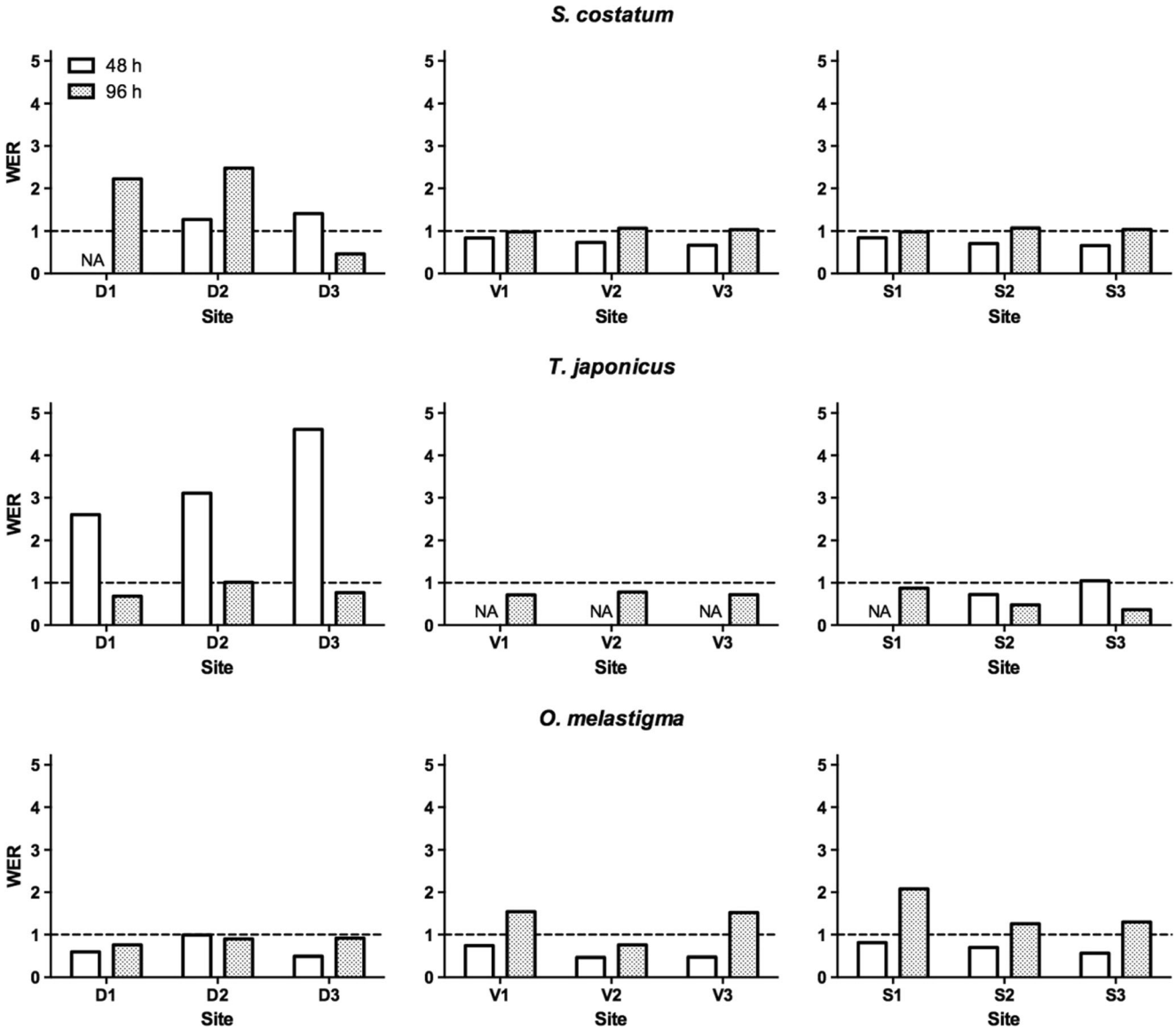

Fig. 3 Water–effect ratio (WER) for Cu at sampling sites in three marine water control zones (WCZs) in Hong Kong, including Deep Bay (*D1*, *D2* and *D3*), Victoria Harbour (*V1*, *V2* and *V3*) and Southern (*S1*, *S2* and *S3*) WCZs

possible toxicants in site waters. Alternatively, exposures during tests with raw site water could be interpreted by measuring concentrations of a range of contaminants accumulated by algae, copepod and fish to show the rate of uptake.

WER

Since 1994, the WER approach has been applied in various waters. Most applications of WER have been to freshwater systems such as streams, rivers, creeks and lagoons, and almost all studies were focused on certain heavy metals, including Cu, Cd, Pb, Ag and Zn. In freshwater systems, the least WER-geometric mean (WER-GM) values determined were 2.61 and 2.2, while the greatest WER-GM values were 11.6 and 15.21, based on total recoverable Cu and dissolved Cu respectively (CH2MHILL 2002; Jop et al. 1995; LWA 2007). The maximum WER value reported for Cu was 22.92 for the Lower Boise River water to development of embryos of *Ceriodaphnia dubia* (CH2MHILL 2002). Values for WER-GM based on total recoverable Cd, Pb, Ag or Zn have been reported to be in ranges of 2.5–4.6, 0.8–3.7, 2.1–3.7 and 1.6–7.24, respectively, in freshwater systems (Jop et al. 1995; Diamond et al. 1997b; CH2MHILL 2002; Coughlin et al. 2009). WER-GM values based on concentrations of dissolved Pb were in the range of 1.8–14.7 (Diamond et al. 1997b; CH2MHILL 2002).

There have been fewer applications of WER in saltwater systems such as marinas and harbours, and all of those studies were for Cu. WER-GM of total recoverable Cu or dissolved Cu were 2.08 and 1.54, respectively, for San Diego Bay,

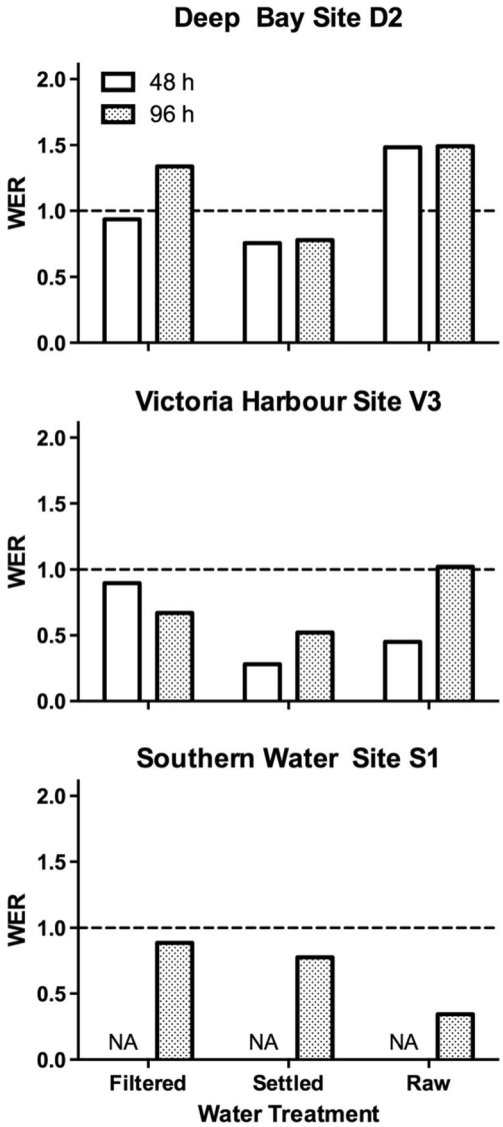

Fig. 4 Effect of treatments to water on water–effect ratio (WER) of water collected from three water control zones (WCZs) of Hong Kong, including Sheung Bak Nai (*D2* in Deep Bay WCZ), Shau Kei Wan (*V3* in Victoria Harbour WCZ) and Stanley Bay (*S1* in Southern WCZ), based on 48- and 96-h acute toxicity tests with the adult copepod *Tigriopus japonicus*. *NA* means data not available

California (Rosen et al. 2005b). WER-GM of dissolved Cu was 1.44 in Sinclair Inlet (Rosen et al. 2005a), and the WER of Cu was in the range of 1.5–3.2 for various test organisms in New York Harbour (Allen and Hansen 1996). All the above-mentioned studies had WER values greater than one, and were greater than those determined in the present study. Using the biotic ligand model, it was found that the WER values of Cu were positively correlated with concentrations of DOC in site waters (Arnold 2005), which provided an indirect method of predicting WERs by measuring concentrations of DOC in water. Models based on concentrations of DOC have been used to for site-specific criteria of Cu in San Francisco Bay and Chesapeake Bay, USA (Arnold and Warren-Hicks 2007a, b). In order to better explain WERs of the site waters in the present study, a further study should include the measurement of DOC and measurements of nutrients and other co-existing chemical contaminants such as other heavy metals, PCBs and EDCs in site water samples.

Effects of water treatment and salinity on Cu toxicity

Results of experiment II demonstrated that various water treatments (i.e. filtered, settled, raw) and salinity can affect toxicity of Cu in site water samples. Results of previous studies have demonstrated that both DOC and particular organic matters decreases bioavailability of Cu, and thus reduce its toxicity to aquatic organisms (Grassi et al. 2000; Arnold 2005; Arnold and Warren-Hicks 2007a, b). Results of the present study showed that, except the 96-h WERs for Cu in filtered and raw waters from site D2 in Deep Bay, all other 96-h WERs determined in experiment II were near or less than 1.0. Those results implied that although the organic matters in the site waters could decrease Cu toxicity to the copepod, there were other factors such as co-existing chemical contaminants as well as the interactions among other ions and contaminants with Cu (e.g. synergistic effect) in the site waters that could overrule effects of organic matter and increase net toxicity of Cu.

Salinity can affect both amounts of free, ionic metals and complexes (organic or inorganic) of Cu (Hall and Anderson 1999). Results of the present study showed that toxicity of Cu was inversely proportional to salinity in the filtered site waters. These results were consistent with those of other studies, which demonstrated that toxicity of Cu in both natural water (Verslycke et al. 2003) and artificial seawater (Kwok and Leung 2005) was inversely proportional to salinity.

Implications for setting WQC for Cu

In Hong Kong, the current numerical WQC only cover parameters such as dissolved oxygen, chlorophyll-a, total inorganic nitrogen, *E. coli* count and TSS and have yet to include metals. There is no numerical WQC for Cu in Hong Kong (EPD 2010). Only sediment quality criteria are available for Cu, which are 65 and 110 mg Cu/kg dm, for the Lower and Upper Chemical Exceedance Level, respectively (EPD 2014a).

The WQC for Cu varies among jurisdictions, depending on designated uses and protection levels. In China, WQC for Cu range from 5 to 100 μg Cu/L, depending on designated uses of marine waters. In Australia, WQC (i.e. marine water quality guidelines) for protection of ecosystem range from 0.3 to 8 μg Cu/L, while for human consumption the criterion of Cu is 1000 μg/L. In Canada and the USA, the WQC for protection of aquatic organisms range from 3.1 to 4.8 μg Cu/L, while for human consumption, the criterion is 1300 μg Cu/L (EPD

Table 5 Concentrations of total suspended solid (TSS) and volatile suspended solid (VSS) (mean with 95% confidence interval (C.I.) in parentheses; $n = 3$) in three water control zones

Water control zones	Site	TSS (95% C.I.) (mg/L)	VSS (95% C.I.) (mg/L)
Deep Bay	D1 (Lau Fau Shan)	98.83 (86.38–107.28)	4.69 (3.81–5.58)
	D2 (Sheung Bak Nai)	122.19 (84.82–159.57)	10.46 (6.05–14.9)
	D3 (Lung Kwu Tan landfill site)	16.16 (14.76–17.57)	1.63 (0.81–1.51)
Victoria Harbour	V1 (Belcher Bay)	15.50 (14.93–16.08)	3.48 (3.37–3.58)
	V2 (Wan Chai)	14.35 (13.69–14.37)	2.33 (2.28–2.38)
	V3 (Shau Kei Wan)	11.09 (10.43–11.78)	2.31 (2.25–2.37)
Southern Water	S1 (Stanley Bay)	16.54 (0.71–32.4)	5.14 (2.62–7.65)
	S2 (Tai Tam Bay)	17.57 (2.67–32.47)	5.33 (2.89–7.77)
	S3 (Cape D'Aguilar)	14.51 (2.19–26.83)	5.75 (4.31–7.19)

2010). In establishing WQC for Cu in Hong Kong's marine waters, site-specific beneficial uses of marine waters, compliancy rate of the WQC with a view to meeting the management objective, and the past contamination history of the area of concern should be considered. Since both fWERs and conservative fWERs from all of the three WCZs were less than 1.0, the generic WQC using site waters alone could be underprotective of marine organisms in these WCZs in Hong Kong. Therefore, it is suggested that local, site-specific WQC be derived for WCZs by use of WERs.

In this study, three marine species, including an alga, an invertebrate and a vertebrate, were used to derive WERs. It was shown that the relative toxicities of Cu between the site and the laboratory water were species-dependent. The range of toxicities could be great if only a few species and taxonomic groups were used. Variability of WER could be minimized if more species would be included, though the workload for increased number of toxicity tests could be inevitably large. The range of WER values should be provided so that policy makers could judge whether they should use the average value or a more conservative value of WER to derive the site-specific WQC accordingly for sustainable development (i.e. a balanced economic growth with adequate ecosystem protection).

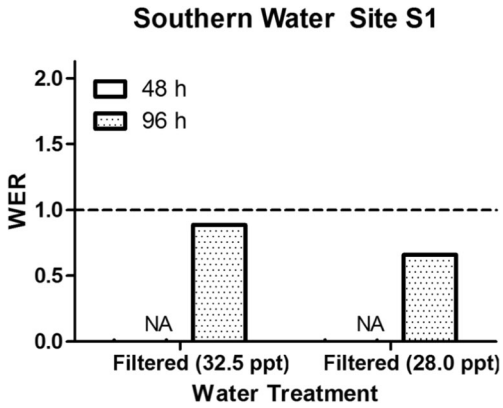

Fig. 5 Effect of salinity on water–effect ratio (WER) for copper (Cu) to adult copepods (*Tigriopus japonicus*) based on 48- and 96-h acute toxicity tests using water samples collected from Stanley Bay (*S1* in Southern water control zone). *NA* means data not available

Future studies

This study investigated only three of the ten WCZs in Hong Kong (EPD 2014b). Further studies should cover more WCZs, in particular those with more anthropogenic disturbances, or those with high biodiversity and conservation value like marine protected areas. Some examples of these areas are Tolo Harbour and Channel (rich in fish fry resources and where Hoi Ha Wan Marine Park is located; ERM 1998; EPD 2010), North Western (home to the Indo-Pacific humpback dolphins *Sousa chinensis* and where Sha Chau and Lung Kwu Chau Marine Park is situated in; Morton 1996) and Mirs Bay (with wide range of coral coverage and fish culture zones, also where Tung Ping Chau and Yan Chau Tong Marine Parks are situated in; Morton 1994; EPD 2010). Within each WCZ, more sampling sites should be included in order to account for the finer scale of site specificity and also allow sound statistical comparisons of the site-specific WERs against the unity which could indicate whether the generic WQC is under- or over-protective to marine life. Seasonal variation and tidal effect may also be taken into consideration when deriving fWERs with high confidence.

Table 6 Total recoverable copper (Cu) concentration (with standard deviation, SD) in water collected from Deep Bay water control zone

Site	Total recoverable Cu[a] (μg/L)	SD (μg/L)
D1 (Lau Fau Shan)	54	4
D2 (Sheung Bak Nai)	59	5
D3 (Lung Kwu Tan landfill site)	46	3
Average	53	1

[a] This is based on the assumption that the total recoverable Cu in distilled water is 0 μg/L

Conclusion

For the first time, water–effect ratio (WER) values for Cu were derived for three water control zones in marine waters of Hong Kong. Acute toxicity tests were conducted by use of a marine diatom, a copepod and larvae of a marine fish using the site and laboratory waters, respectively. Conservative, final WER values for Cu varied spatially, ranging from 0.57 to 0.73, which indicated that the generic water quality criteria of Cu could be under-protective to marine life. Results also showed that toxicities of Cu in site waters could be affected by various factors such as salinity and suspended solid. Therefore, the current site-specific WER approach is useful for setting more protective, site-specific, WQC of Cu as demonstrated in this study.

Acknowledgements This work was supported by the Research Grants Council (RGC) of the Hong Kong SAR Government through a competitive General Research Fund (Project No. HKU 703407P). GJ Zhou was supported by the Hong Kong Scholars Program (Project No. XJ2012050) and RGC via Collaborative Research Fund (project no. C7044-14G) and Theme-based Research Scheme (project no. T21-711/16-R). Prof. Giesy was supported by the Canada Research Chair program, the 2012 "High Level Foreign Experts" (#GDT20143200016) program, funded by the State Administration of Foreign Experts Affairs, the P.R. China to Nanjing University and the Einstein Professor Program of the Chinese Academy of Sciences and a Distinguished Visiting Professorship in the School of Biological Sciences of the University of Hong Kong. We thank Cecily Law and Helen Leung for their technical support.

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Ent 18:265–267

Alberts JJ, Giesy JP, Evans DW (1984) Distribution of dissolved organic carbon and metal binding capacity among ultrafilterable fractions isolated from selected surface waters of the southeastern United States. Environ Geol Water Sci 6:91–101

Allen HE, Hansen DJ (1996) The importance of trace metal speciation of water quality criteria. Water Environ Res 68:42–54

APHA (1992) Standard methods for the examination of water and wastewater, 18th edition, method 2540 D. American Public Health Association and American Water Works Association and Water Environment Federation, Washington, D.C.

Arnold WR (2005) Effects of dissolved organic carbon on copper toxicity: implications for saltwater copper criteria. Integr Environ Assess Manag 1:34–39

Arnold WR, Warren-Hicks WJ (2007a) Assessment of aquatic ecological risk and site-specific criteria of copper in San Francisco Bay, California, USA. Integr Environ Assess Manag 3:32–48

Arnold WR, Warren-Hicks WJ (2007b) Probability-based estimates of site-specific copper water quality criteria for the Chesapeake Bay, USA. Integr Environ Assess Manag 3:101–117

Bao VWW, Leung KMY, Kwok KWH, Zhang AQ, Lui GCS (2008) Synergistic toxic effects of zinc pyrithione and copper to three marine species: implications on setting appropriate water quality criteria. Mar Pollut Bull 57:616–623

Bao VWW, Leung KMY, Qiu JW, Lam MHW (2011) Acute toxicities of five commonly used antifouling booster biocides to selected subtropical and cosmopolitan marine species. Mar Pollut Bull 62:1147–1151

Bao VWW, Leung KMY, Lui CGS, Lam MHW (2013) Acute and chronic toxicities of Irgarol alone and in combination with copper to the marine copepod *Tigriopus japonicus*. Chemosphere 90: 1140–1148

Bao VWW, Lui GCS, Leung KMY (2014) Acute and chronic toxicities of zinc pyrithione alone and in combination with copper to the marine copepod *Tigriopus japonicus*. Aquat Toxicol 157:81–93

Brungs WA, Holderman TS, Southerlan MT (1992) Synopsis of water-effect ratios for heavy metals as derived for site-specific water quality criteria. EPA contract 68-CO-0070. U.S. EPA, Washington, D.C

Carlson AR, Elson H, Hammermeister D (1986) Development and validation of site-specific water quality criteria for copper. Environ Toxicol Chem 5:997–1012

CCME, Canadian Council of Ministers of the Environment (2002) Guidance on the site-specific application of water quality guidelines in Canada: procedures for deriving numerical water quality objectives. Winnipeg. <http://ceqg-rcqe.ccme.ca/download/en/221>

CH2MHILL (2002) Boise City Water-Effect Ratio Project: final report. Prepared for Boise City, Boise, Idaho, USA. <https://www.deq.idaho.gov/media/445303-boisecity_watereffectratio_project_report.pdf>

Coughlin DP, Looney BB, Millings MR (2009) Chronic zinc screening water effect ratio for the H-12 outfall, Savannah River Site. Savannah River National Laboratory. Prepared for the U.S. Department of Energy under contract number DE-AC09-08SR22470. <http://sti.srs.gov/fulltext/SRNS-STI-2009-00012.pdf>

Department of Water, the Government of Western Australia (2009) Antifouling biocides in Perth coastal waters: a snapshot at select areas of vessel activity. Water Science technical series. Report no. WST 1. <https://www.water.wa.gov.au/__data/assets/pdf_file/0005/5477/84012.pdf>

Diamond JM, Gerardi C, Leppo E, Miorelli T (1997a) Using a water-effect ratio approach to establish effects of an effluent-influenced stream on copper toxicity to the fathead minnow. Environ Toxicol Chem 16:1480–1487

Diamond JM, Koplish DE, McMahon J III, Rost R (1997b) Evaluation of the water-effect ratio procedure for metals in a riverine system. Environ Toxicol Chem 16:509–520

EPD, Environmental Protection Department, The Government of the Hong Kong Special Administrative Region (2010) Review and development of marine water quality objectives. First stage public engagement. Technical notes: Review of local condition and overseas practices. <http://www.epd.gov.hk/epd/wqo_review/en/pdf/Technical%20Note_Website.pdf>

EPD, Environmental Protection Department, The Government of the Hong Kong Special Administrative Region (2014a) Marine water quality in Hong Kong in 2014. <http://wqrc.epd.gov.hk/pdf/water-quality/annual-report/MarineReport2014eng.pdf>

EPD, Environmental Protection Department, The Government of the Hong Kong Special Administrative Region (2014b) Environmental Protection Interactive Centre: Marine water quality monitoring data. <http://epic.epd.gov.hk/EPICRIVER/marine/?lang=en>

ERM, Environmental Resources Management (1998) Fisheries resources and fishing operations in Hong Kong waters: final report. Environmental Resources Management, Hong Kong, p 255

ETWB, Environment, Transport and Works Bureau (2002) Technical circular (works) no. 34/2002. Management of dredged/ excavated sediment. <http://www.devb.gov.hk/filemanager/technicalcirculars/en/upload/124/1/C-2002-34-0-1.pdf>

Finney CM (1979) Salinity stress in harpacticoid copepods. Estuaries 2:132–135

Giesy JP (1983) Metal binding capacity of soft acid organic rich waters. Toxicol Environ Chem 6:203–224

Giesy JP (1987) Current status of metal speciation studies. In: Patterson JW, Passino R (eds) Metal, speciation, separation and recovery. Lewis Publishers, Chelsea, pp 225–242

Giesy JP, Newell A, Leversee GJ (1983) Copper speciation in soft, acid humic waters: effects on copper bioaccumulation by and toxicity to *Simocephalus serrulatus* (Daphnidae). Sci Total Environ 28:23–36

Grassi MT, Shi B, Allen HE (2000) Partition of copper between dissolved and particulate phases using aluminum oxide as an aquatic model phase: effect of pH, solids and organic matter. J Braz Chem Soc 11: 516–524

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Hall LW, Anderson RD (1999) A deterministic ecological risk assessment for copper in European saltwater environments. Mar Pollut Bull 38: 207–218

Heijerick DG, Bossuyt BTA, De Schamphelaere KAC, Indeherberg M, Mingazzini M, Janssen CR (2005) Effect of varying physiochemistry of European surface waters on the copper toxicity to the green alga *Pseudokirchneriella subcapitata*. Ecotoxicology 14:661–670

Jop KM, Askew AM, Foster RB (1995) Development of a water-effect ratio for copper, cadmium, and lead for the Great Works River in Maine using *Ceriodaphnia dubia* and *Salvelinus fontinalis*. Bull Environ Contam Toxicol 54:29–35

Kwok KWH, Leung KMY (2005) Toxicity of antifouling biocides to the intertidal harpacticoid copepod *Tigriopus japonicus* (Crustacea, Copepoda): effects of temperature and salinity. Mar Pollut Bull 51: 830–837

Li AJ, Leung PTY, Bao VWW, Yi AXL, Leung KMY (2014) Temperature-dependent toxicities of four common chemical pollutants to the marine medaka fish, copepod and rotifer. Ecotoxicology 23:1564–1573

LWA, Larry Walker Associates (2007) Los Angeles river copper water-effect ratio (WER) study. Draft final report. Submitted to the Los Angeles regional water quality control board. Prepared by Larry Walker Associates, Davis

MESL, Macdonald Environmental Sciences Ltd. (1997) Methods for deriving site-specific water quality objectives in British Columbia and Yukon. Prepared by Macdonald Environmental Sciences Ltd., Ladysmith, BC for BC Ministry of Environment, Victoria

Morton B (1994) Hong Kong's coral communities: status, threats and management plans. Mar Pollut Bull 29:74–83

Morton B (1996) Protecting Hong Kong's marine biodiversity: present proposals, future challenges. Environ Conserv 23:55–65

Rand GM, Clark JR (2000) Hazard/risk assessment of pyridaben: II. Outdoor aquatic toxicity studies and the water-effect ratio. Ecotoxicology 9:169–177

Rosen G, Rivera-Duarte I, Johnston RK (2005a) Sinclair inlet toxicity assessment: Puget Sound Naval Shipyard & Intermediate Maintenance Facility surface water copper bioavailability and toxicity study. Draft Report. Environmental Sciences & Applied Systems Branch, Code 2375 Space and Naval Warfare Systems Center, U.S. Navy San Diego, CA

Rosen G, Rivera-Duarte I, Kear-Padilla L, Charwick DB (2005b) Use of laboratory toxicity tests with bivalve and echinoderm embryos to evaluate the bioavailability of copper in San Diego Bay, California, USA. Environ Toxicol Chem 24:415–422

Schiff K, Brown J, Diehl D, Greenstein D (2007) Extent and magnitude of copper contamination in marinas of the San Diego region, California, USA. Mar Pollut Bull 54:322–328

Tompsett AR, Vardy DW, Higley E, Doering JA, Allan M, Liber K, Giesy JP, Hecker M (2014) Effects of Columbia River water on early life-stages of white sturgeon (*Acipenser transmontanus*). Ecotox Environ Safe 101:23–30

USEPA, U.S. Environmental Protection Agency (1984) Guidelines for deriving numerical aquatic site-specific water quality criteria by modifying national criteria. EPA-600/3-84-099, Duluth

USEPA, U.S. Environmental Protection Agency (1994) Interim guidance on determination and use of water-effect ratios for metals. EPA-823-B-94-001, Washington, D.C

USEPA, U.S. Environmental Protection Agency (1996) Water quality-based permitting for trace metals fact sheet. Washington, D.C. <http://webapp1.dlib.indiana.edu/virtual_disk_library/index.cgi/6530961/FID48/tmfact.pdf>

Vardy DW, Santore R, Ryan A, Giesy JP, Hecker M (2014) Acute toxicity of copper, lead, cadmium, and zinc to early life stages of white sturgeon (*Acipenser transmontanus*) in laboratory and Columbia River water. Environ Sci Pollut Res 21:8176–8187

Verslycke T, Vangheluwe M, Heijerick D, De Shampelaera K, Van Sprang P, Janssen CR (2003) The toxicity of metal mixtures to the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) under changing salinity. Aquat Toxicol 64:307–315

Wang Z, Meador JP, Leung KMY (2016) Metal toxicity to freshwater organisms as a function of pH: a meta-analysis. Chemosphere 144: 1544–1552

Welsh PG, Lipton J, Chapman GA (2000) Evaluation of water-effect ratio methodology for establishing site-specific water quality criteria. Environ Toxicol Chem 19:1616–1623

Xu EGB, Liu S, Ying GG, Zheng GJS, Lee JHW (2014) The occurrence and ecological risks of endocrine disrupting chemicals in sewage effluents from three different sewage treatment plants, and in natural seawater from a marine reserve of Hong Kong. Mar Pollut Bull 85: 352–362

Ytreberg E, Karlsson J, Ndungu K, Hassellöv M, Breitbarth E, Eklund B (2011) Influence of salinity and organic matter on the toxicity of Cu to a brackish water and marine clone of the red macroalga *Ceramium tenuicorne*. Ecotoxicol Environ Saf 74:636–642

Zhou GJ, Wang Z, Lau ETC, Xu XR, Leung KMY (2014) Can we predict temperature-dependent chemical toxicity to marine organisms and set appropriate water quality guidelines for protecting marine ecosystems under different thermal scenarios? Mar Pollut Bull 87:11–21