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Evaluation of copper toxicity using site specific algae and water chemistry: Field validation of laboratory bioassays

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ABSTRACT

Studies of metal toxicity to microalgae have predominantly been conducted using single non-target algae species and without due regard for the chemistry of the treated waters, leading to ineffective or excessive algaecide treatments. In this study, indigenous multi-algal species (*Scenedesmus quadricauda*, *and Scenedesmus subspicatus* and *Oscillatoria agardhii*) were used in laboratory toxicity bioassays under simulated field water chemistry (pH = 7.2, hardness = 196 mg L^{-1} as $CaCO_3$, and alkalinity = 222 mg L^{-1} as $CaCO_3$) to determine the optimum copper sulfate treatment dose to control algae growth in an irrigation canal. Toxicity bioassays were conducted using copper sulfate in chelated (with EDTA) and non-chelated (without EDTA) forms to assess the influence of the use of synthetic chelators in toxicity studies. Also, copper toxicity to the indigenous algae species was measured in the non-modified EPA test medium (pH = 7.5, hardness = 92 mg L^{-1} as $CaCO_3$, alkalinity = 10 mg L^{-1} as $CaCO_3$ and EDTA = 300 μg L^{-1}) to assess the impact of the water chemistry on algae inhibitory algal dosages. Under simulated water chemistry conditions, lower toxicity was measured in the test flasks with the chelated form of copper (96 h- EC_{50} = 386.67 μg L^{-1} as Cu) as compared to those with the non-chelated metal (96 h-EC_{50} = 217.17 μg L^{-1} as Cu). In addition, higher copper toxicity was measured in the test flasks prepared with the non-modified EPA medium using chelated copper (96 h-EC_{50} = 65.93 μg L^{-1} as Cu) as compared to their analogous microcosms with modified water chemistry (96 h-EC_{50} = 386.67 μg L^{-1} as Cu), the increased water hardness and alkalinity in the latter case contributing to the decrease of the metal bioavailability. Results from laboratory experiments showed good correlation with copper dosages used in a small scale field testing to control algae growth, increasing confidence in laboratory bioassays.

1. Introduction

Studies on the toxicity of copper based algaecides to microalgae have mostly used commercial species laboratory bioassays in prepared test media to determine the effective metal inhibitory dose to cultured organisms (Araújo et al., 2010; Contreras et al., 2010; Hochmuth et al., 2014). Fast growing species, easily cultured and enumerated in the laboratory have been the organisms of choice in algal inhibition tests and are usually obtained from commercial sources. These bioassays, though sensitive and highly reproducible, lack the environmental realism in using indigenous algae species of the affected water body which may have different sensitivity to the algaecide than the test organism. Also, they do not reproduce the algal-algal interactions occurring in natural ecosystems and which often affect the toxicity of the used algaecide. Single-species bioassays may over- or underestimate copper toxicity in natural waters. Franklin et al. (2004) demonstrated that the toxicity of copper to *Trachelomonas* sp. was greater in the presence of other species, with copper concentrations required to inhibit growth rate by 50% decreasing from 9.8 to 2.8 μg Cu L^{-1} in single and multi-species freshwater bioassays, respectively. In contrast, the authors reported a reduction in copper toxicity to the diatom *P. tricornutum* in marine multispecies bioassays, with an increase in the 72-h EC_{50} value from 13 μg Cu L^{-1} in single-species bioassays to 24 μg Cu L^{-1}. In their experiments on the assessment of copper toxicity to the algal species *M. Aeruginousa* in the presence and absence of *C. pyrenoidosa* and *S. obliquus*, Yu et al. (2007) found that the 24-h EC_{50} value of *M. aeruginousa* in the multispecies populations was significantly higher than those in the single species populations. Compared with *S. obliquus*, the effect of *C. pyrenoidosa* on *M. aeruginousa* was found to be more noticeable. In addition, toxicity bioassays are mostly conducted without due regard for the chemistry of the treated waters, a major factor affecting the availability of copper to biota and, consequently, the required dose of

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the used algaecide for controlling algal blooms.

Algae susceptibility to copper varies widely between species. Blue-green algae are the most sensitive to the effect of copper, whereas in other algae groups copper toxicity is reduced through a decrease in the bioavailability of the metal caused by algal excretion of metal-binding compounds or the production of intercellular metal-binding peptides (Huff and Angel, 1989; Cooke et al., 1993; Hullebusch et al., 2002; Bossuyt and Janssen, 2004; Wang et al., 2017). Hence, laboratory toxicity testing using commercial algae species, reflect the susceptibility of the monitored species to direct chemical effects and exclude potential effects of the algaecide on indigenous coexisting algal organisms in the affected water body (Franklin et al., 2004; De Laender et al., 2009).

In addition, the chemistry of the treated water plays a major role in defining copper speciation in natural ecosystems and consequently its algaecidal effect. Copper is less toxic in waters with high pH, hardness, and alkalinity due to its precipitation into insoluble forms and to competition with calcium and magnesium for binding sites on the algal cells membrane (Button and Hostetter, 1977; Cook et al., 1997; Hullebusch et al., 2002). Also, adsorption of copper on colloid and particulate components within the treated water body, and its complexation by natural dissolved organic carbon (DOC) reduces in general its availability for biological uptake (Cook et al., 1997; Hullebusch et al., 2002). Synthetic chelators such as ethylenediaminetetraacetic acid (EDTA) and citrate commonly used in toxicity bioassays to prevent the precipitation of ions in the test media, could thus underestimate copper toxicity in field waters.

The determination of correct copper dosages for a given lake or water supply reservoir is a key factor in ensuring success in controlling undesirable algae growth. In this study, it is our purpose to define optimum copper dosages for the treatment of an irrigation canal suffering from excessive algae proliferation with consequent negative impact on the irrigation scheme, mainly the obstruction of drip irrigation systems and sprinkler nozzles. For this aim, algal toxicity bioassays using indigenous algal species representative of the natural algal community and accounting for the site specific water characteristics were conducted in laboratory microcosms. Copper sulfate in chelated and non-chelated forms was used to account for the effect of synthetic chelators in toxicity bioassays. In addition, toxicity bioassays were conducted in the standard non-modified EPA test medium to account for the effect of water chemistry, namely pH, hardness and alkalinity, on the copper speciation and toxicity. The results from laboratory experiments were compared to copper dosages used in small scale field testing to assess the accuracy of laboratory predictions of responses of algae in site waters.

2. Materials and methods

2.1. Water sampling and testing

Water samples were collected from Canal 900, an open concrete-lined irrigation channel that extends over 18.5 km of the south central portion of Lebanon's Bekaa Valley. The Canal extracts water from Lake Qaraoun in the Litani River Basin and serves the surrounding agricultural lands through distribution reservoirs and irrigation networks (Litani River Authority, 2018). The Canal is subject to algae proliferation during the summer causing water flow retardation, clogging of irrigation drippers, and foul odors. As a result, the Canal is operating at around 30% of its capacity, serving 1900 ha out of the originally planned 7000 ha of irrigated land (BAMAS, 2005). Algae proliferation in the Canal is essentially due to agricultural runoff and domestic wastewater discharge in the upper Litani Basin leading to water quality degradation in the River and the built up of nutrients in the Qaraoun Lake (LRBMS, 2011). The high temperature, long daylight duration, low flow rate and closed end of the Canal are further contributing to algae growth. A total of eight water samples were collected along the water stretch in 1 L polyethylene bottles and transported to the laboratory in a portable cooler (4 °C) for subsequent analysis and algae identification. Fig. 1 shows the study area along with the field sampling and testing sites, and exact coordinates are provided in Supplementary information, SI (Table S1). Aliquots of 50 mL of the collected samples were acidified using nitric acid (HNO_3) to pH < 2 and stored at 4 °C for subsequent copper analysis. Samples were analyzed, and exhibited, pH 7.14 (sd = 0.097), alkalinity 221 mg L^{-1} as $CaCO_3$ (sd = 13.82), hardness 198 mg L^{-1} as $CaCO_3$ (sd = 8.91), total phosphorous 85 µg L^{-1} (sd = 18), total nitrogen 3.94 mg L^{-1} (sd = 0.43), DOC < 0.5 mg L^{-1}, and background copper concentration 4.65 µg Cu L^{-1} (sd = 3.65). The measurements were respectively performed according to the following standard methods: SM 4500-H^+ B, SM 2320B, SM 2340C,

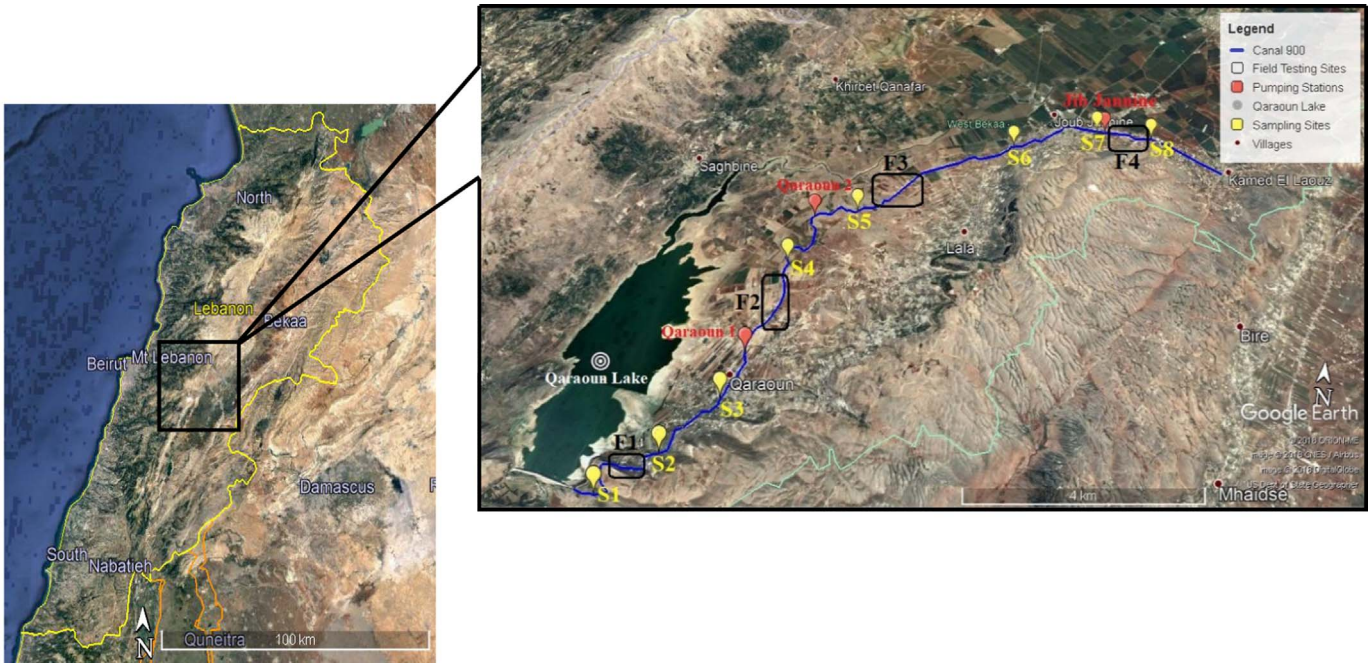
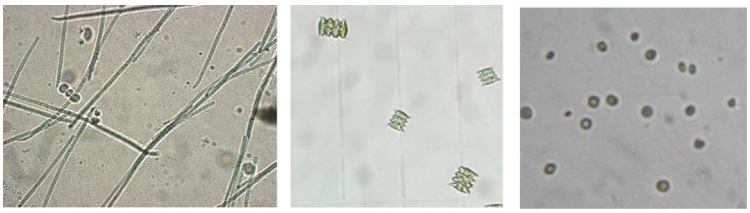


Fig. 1. Study area including field sampling and testing sites along Canal 900.



Oscillatoriaagardhii *Scenedesmus.quadricauda* *Scenedesmus subspicatus*

Fig. 2. Identified algae species in the Canal water (Algae taxonomy books of Stevenson et al., 1996 and Bellinger and Sigee, 2015 were used for algae identification).

Table 1
Concentrations of the elemental nutrients in the different test media.
Source: EPA, United States Environmental Protection Agency, 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms. Fourth edition, EPA-821-R-02–013, p. 202.

	Elemental nutrients	Concentration		
		Standard EPA medium with EDTA	*Reconstituted canal water with EDTA*	*Reconstituted canal water without EDTA*
Macroelements (mg/L)	N	4.20	4.20	4.20
	P	0.186	0.186	0.186
	Mg	2.90	27.20	27.20
	Ca	1.20	41.20	41.20
	S	1.91	1.91	1.91
	Na	11	34	34
	K	0.469	39.56	39.56
	C	2.14	2.14	2.14
Microelements (µg/L)	B	32.5	32.5	32.5
	Mn	115	115	115
	Zn	1.57	1.57	1.57
	Co	0.354	0.354	0.354
	Cu	0.004	0.004	0.004
	Mo	2.88	2.88	2.88
	Fe	33.1	33.1	33.1
	Se	0.91	0.91	0.91
	$Na_2EDTA{\cdot}2H_2O$	300	300	–

SM 5220 D, SM 4500-P B (5) E, Hach 10071–10072, 5310 D, and SM 3500-Cu^{2+} B. The total phosphorous and total nitrogen were indicative of hypereutrophic state of the canal. The detailed canal water analysis including additional water quality parameters is provided in SI, Table 2.

2.2. Algae identification

For algal identification, 1-mL aliquot of collected samples was pipetted into an Improved Neubauer counting chamber of 0.1 mm depth and a total volume of 0.0018 mL. Microscopic examination of algae was carried out using a Zeiss fluorescence microscope (Axiovert 200) equipped with a digital camera (Zeiss AxioCam HRC). Algae were observed under 100X oil immersion objective allowing an overall visual magnification of 1000 times (eyepiece magnification 10×). Identified algal species included the colonial green algae *Scenedesmus quadricauda and Scenedesmus subspicatus*, and the filamentous cyanobacteria *Oscillatoria agardhii. Scenedesmus species* were dominant in all examined water samples. Identified algae species are shown in Fig. 2.

2.3. Stock algal cultures and algal inoculum for toxicity testing

Algae collected from the irrigation canal were cultured in the laboratory over a period of six weeks in order to establish healthy, reproducible growing algal cultures, and to achieve a starter culture with at least 10^6 cells mL^{-1}. Algal cultures were maintained in autoclaved freshwater nutritive medium prepared according to the EPA 96-h static chronic algal toxicity test (2002). The composition of the standard EPA medium is provided in Table S3 in SI. The cultures were incubated in a controlled environmental chamber at 25 ± 1 °C under continuous illumination provided with cool white-type fluorescent lamps (Orsam daylight L36W/765, 120 cm, 76 Hz) permitting an energy level output varying between 55.48 and 64.24 µmol photons m^2/s. Transfer of stock algal cultures to fresh nutritive medium was performed once weekly to maintain a supply of cells in the logarithmic growth phase. Details about the initiation of laboratory algae cultures and biomass increase are provided in SI in Section S4. Microscopic observation showed a prevalence of Scenedesmus species in the stock algal cultures as in the original samples extracted from the Canal. Algal inoculum used in the toxicity bioassays was prepared by centrifuging four-day stock algal cultures with exponentially growing algae, and re-suspending the sediment algal cells in a 15 mg L^{-1} $NaHCO_3$ solution, for three consecutive times. All operations were carried out under sterile conditions.

2.4. Algae growth inhibition tests

2.4.1. Culture medium

Two culture media were used in algae growth inhibition tests as dilution water to prepare the different test copper concentrations. The standard non-modified EPA nutritive medium (Table S3) used to maintain algal cultures, was adopted in a first set of experiments and exhibited pH 7.5, hardness 92 mg L^{-1}as $CaCO_3$, and alkalinity 10 mg L^{-1} as $CaCO_3$. Reconstituted water with chemical characteristics representative of the irrigation canal was used in a second set of experiments. To reconstitute field water, the standard EPA nutritive medium was supplemented with additional chemicals to match the canal water chemistry and exhibited pH 7.2, hardness 196 mg L^{-1}as $CaCO_3$, and alkalinity 222 mg L^{-1} as $CaCO_3$ (Table S5 in SI). Table 1 presents the concentrations of the elemental nutrients in the EPA nutritive medium and the reconstituted canal water culture media. N and P levels in culture media are indicative of hypereutrophic conditions and comparable to N and P concentrations in the Canal water, making possible the comparison of the results from laboratory bioassays and field testing experiments.

2.4.2. Toxicity tests procedure

A stock solution of copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) was used to prepare the test solutions with copper concentrations ranging from 50 to 2000 µg Cu L^{-1} in culture flasks. Algae growth inhibition bioassays were carried out in 200 mL Erlenmeyer glass flasks and were thoroughly rinsed with acetone and a 10% solution of reagent grade hydrochloric acid, followed by two times rinsing with MILLI-Q water according to the EPA 96-h static chronic algal toxicity test (EPA, 2002). Three sets of algal inhibition tests were conducted. In one set, the non-modified EPA culture medium including EDTA ($Na_2EDTA{\cdot}2H_2O$, 300 µg/L^{-1}), a chelating agent preventing the complexation of essential trace metals, was used. In the two other sets, reconstituted canal water with modified chemistry was used with and with no EDTA addition. In the first case, EDTA was added at the same concentration used in the standard non-modified EPA medium ($Na_2EDTA{\cdot}2H_2O$, 300 µg/L^{-1}). The exclusion of EDTA in the latter case was intended to better simulate the field conditions where low levels of naturally occurring ligands (DOC < 0.5 mg/L) were measured in the canal water. Results from the

Table 2
Toxicity test conditions.

Test conditions	*Description*
Temperature	25 ± 1 °C
Light intensity	55.48–64.24 μmol photons m^2/s
Photoperiod	Continuous illumination
Initial cell density in test flasks	10,000 cells/mL
Copper test concentrations	Seven copper concentrations (50; 100; 200; 350; 500; 1000; and 2000 μg Cu L^{-1}) and a control
Number of replicate flasks per concentration per test	3
Test duration	96 h
Endpoint	Growth rates (cell counts), EC_{50}, EC_{10}, NOEC, LOEC
Test acceptability criteria	Mean cell density of at least 10^6 cells/mL in the controls and a variability among control replicates ≤ 20% Temperature and pH measured at the end of each 24 h exposure period in at least one test flask at each concentration and in the control of every test Temperature must not deviate by more than 3 °C during the tests pH should not increase more than 1 unit in the controls of the test

bioassays conducted in the standard EPA medium and reconstituted canal water with EDTA were used to assess the impact of water chemistry, namely pH, hardness, and alkalinity, on copper toxicity. The impact of the addition of chelating agent on copper toxicity in the irrigation canal was evaluated through comparison of the results from the bioassays carried out in reconstituted canal waters with and without EDTA to the field testing results. Table 2 describes the test conditions and acceptability for the three different media studied.

For each of the three treatments, seven copper concentrations (50; 100; 200; 350; 500; 1000; and 2000 μg Cu L^{-1}) were tested in triplicate cultures and were prepared by serial dilution of the stock copper solution. Nominal copper exposure concentrations were verified analytically in the different test solutions using atomic absorption spectrometry with G95 graphite furnace (Thermo Labsystems, SOLAAR) and are provided in Table S6 in SI. The test cultures were aseptically prepared by adding algal inoculum to 40 mL copper test solutions to provide an initial cell density of 10^4 cells mL^{-1} in the test flasks. In addition, triplicate control cultures were prepared in test medium with no added copper. The same algal inoculum was used in the preparation of test flasks of the three toxicity bioassays conducted with the different test media. This ensured similar initial relative abundance of the algae species in all tests, and allowed subsequent comparison of toxicity results between the different conducted bioassays. Test flasks were incubated under continuous illumination (55.48–64.24 μmol photons m^2/s) at 25 ± 1 °C, and were shaken twice daily by hand to keep the algae in suspension and facilitate the transfer of CO_2. Flasks positions in the incubator were randomly rotated to minimize potential spatial differences in illumination and temperature on algal growth rate. Algae growth was measured at the end of 24, 48, 72, and 96 h of incubation, by performing algae cell counts using direct optical microscopic observation and Improved Neubauer counting chamber (0.1 mm depth, 1.8 μL volume). An ultrasonic bath was used to detach algal cells from the culture flasks' walls and to disperse cell clumps in the solutions. The average specific growth rates of the exponentially growing cultures in the different test media were calculated at 96 h as follows:

$$Growth\ Rate = \frac{\ln N_4 - \ln N_0}{d_4 - d_0}$$

Whereby, N_0 is the nominal number of cells/mL at day 0, N_4 is the measured number of cells/mL at day 4, d_0 is the time of measurement before beginning of test, and d_4 is the time of measurement at 96 h.

2.5. Statistical analysis

Copper toxicity in the standard and modified EPA media was expressed as 96 h-EC_{50} (the concentration of copper that is required to inhibit algal growth by 50% after 96-h compared to the controls). In each treatment, algae count data from triplicate bioassays were pooled and a combined concentration-response curve was plotted using weighted linear regression analysis on Probit transformed data. 96 h-EC_{10} (the concentration of copper that is required to inhibit algal growth by 10% after 96-h compared to the controls) was generated using the same method. NOEC and LOEC were obtained based on the method proposed by Isnard et al. (2001). The effect of copper dosage and the potential changes in growth rates at 96 h were assessed for each of the three different treatments. In each case, growth rates were found to follow a normal distribution using the Shapiro-Wilks' test. Student's *t*-test was then used to evaluate statistically significant differences. The R software (R Core team, 2015) was used to conduct the statistical analysis. Significant difference between 96 h-EC_{50} values in the different treatments was determined depending on confidence interval (CI) overlap. EC_{50}s were considered significantly different when their respective CIs were not overlapping. However, EC_{50}s with overlapping confidence intervals were considered not significantly different only when the following condition was satisfied$(EC50_1 - EC50_2) - (1.96\sqrt{SE_1^2 + SE_2^2}) < 0$, where 1.96 is the critical t-value (Knezevic, 2008).

3. Results and discussion

3.1. Algae growth inhibition tests

For all algae inhibition tests, the canal indigenous species grew well over 96 h in control cultures showing their suitability for toxicity testing. Algae count in all controls exceeded 10^6 cells/mL complying with the EPA toxicity test acceptability criteria (EPA, 2002). Fig. S1 in SI shows algae growth curves of the three conducted bioassays. Also, pH and temperature variation at the end of the inhibition tests are reported in Table S7 in SI, and were within the acceptable limit of pH increase (maximum increase by one unit) and temperature deviation (3°). Only in the tests conducted with the EPA medium at the highest copper concentration (2000 μg/L) and the modified EPA medium with EDTA at copper concentration of 1500 μg/L, a pH increase of 1.37 and 1.29 units were measured, respectively, and were considered tolerable. Algal growth rates measured at 96 h are presented in Table 3.

Adequate algal growth was measured in the controls of the standard non-modified EPA test medium and in test flasks with a copper concentration of 50 μg L^{-1} with growth rates attaining respectively 1.15 ± 0.002 and 1.12 ± 0.001 cells/mL/d after 96 h of exposure. Algae growth rate significantly decreased at copper concentrations of 100 μg L^{-1} (0.36 ± 0.11 cells/mL/d) and was negligible at the highest copper dosages (Table 2). In the bioassays conducted in the reconstituted canal water in the presence of EDTA, substantial algae growth was still measured at copper concentrations of 200 μg L^{-1} (1.16 ± 0.01 cells/mL/d) and was statistically not different from the control ($p > 0.05$). Algae growth significantly decreased with increasing Cu concentrations beyond 200 μg L^{-1} and reached 0.04 ± 0.02 cells/mL/d at 2000 μg Cu L^{-1}. In the tests performed in

Table 3
Average growth rate of algae species at 96 h in the different test media.

Nominal copper concentrations in test solutions ($\mu g\,L^{-1}$)	Growth rates after 96 h (cells/mL/d)		
	Standard EPA medium with EDTA	*Reconstituted canal water with EDTA*	*Reconstituted canal water without EDTA*
0	1.15 ± 0.002 a*	1.29 ± 0.03 e	1.26 ± 0.03 i
50	1.12 ± 0.001 a	1.26 ± 0.04 e	1.26 ± 0.04 i
100	0.36 ± 0.11 b	1.26 ± 0.03 e	1.23 ± 0.02 i
200	0.32 ± 0.01 b	1.16 ± 0.01f	0.20 ± 0.12 j
350	0.18 ± 0.05 c	0.24 ± 0.09 g	0.08 ± 0.07 k
500	0.1 ± 0.07 d	0.08 ± 0.04 h	0.04 ± 0.002 k
1000	0.03 ± 0 d	0.06 ± 0.01 h	0.01 ± 0.01 k
2000	0.01 ± 0.01 d	0.04 ± 0.02 h	0 k

* a–k denote whether growth rates in the different test media are significantly different ($p < 0.05$) across copper concentrations. Same letter means no significant difference ($p > 0.05$).

reconstituted canal water in the absence of EDTA, copper concentrations of 200 $\mu g\,L^{-1}$ and above showed to be inhibiting for algae growth, while adequate algae growth was observed at the lower copper concentrations and was not significantly different from the control. These results indicate lower copper toxicity in the bioassays carried out in the reconstituted canal water in the presence and absence of EDTA as compared to the bioassays conducted in the non-modified EPA medium. Measured 96 h-EC_{50} values in the different treatments are presented in Table 4.

3.2. Effect of water chemistry on copper toxicity

Water characteristics that influence speciation and bioavailability can significantly alter exposures of copper-containing algaecides and subsequent responses of target algae. To demonstrate the effect of water chemistry, namely pH, hardness and alkalinity on copper toxicity, two types of laboratory bioassays were conducted using standard and modified EPA test media. The standard EPA medium exhibited moderate hardness and low alkalinity. The modified EPA medium presented hard water characteristics as in the case of the canal (Table 1). Both media contained EDTA ($Na_2EDTA{\cdot}2H_2O$ = 300 µg/L) as part of the standard EPA nutritive medium used to prevent ions precipitation in the solution, namely iron. Lower copper toxicity was observed in the modified EPA medium. Measured 96 h-EC_{50} value in this case was 386.67 $\mu g\,Cu\,L^{-1}$ and was significantly ($p < 0.05$) higher than the EC_{50} value measured in the standard EPA medium (96 h-EC_{50} = 65.93 $\mu g\,Cu\,L^{-1}$). These results are expected since cations that are involved in water hardness (i.e., Ca^{2+} and Mg^{2+}) would have competed with Cu^{2+} for algae binding sites, hindering thus the metal uptake and toxicity for sensitive biotic receptors (Heijerick et al., 2002). Also, the high water alkalinity in the simulated canal water would have affected aqueous metal speciation through complexation with bicarbonate ions (carbonate alkalinity = 0 in the canal water), which decreases the free copper ion activity in water and thereby reduces metal bioavailability and toxicity (De Schamphelaere and Janssen, 2002). Durborow (2014) reported that copper sulfate in waters with high total alkalinity levels will settle before algae are completely controlled. Snoeyink and Jenkins (2011) showed that at pH 7, an increase in alkalinity from 50 to 250 $mg\,L^{-1}$ (as $CaCO_3$) decreases the Cu^{2+} levels from 25% to 9% of the total copper present.

These results emphasize the importance of performing laboratory algal toxicity tests using site water characteristics to predict responses of target algae in field-scale applications.

3.3. Effect of EDTA on copper toxicity

EDTA, a synthetic chelating agent, is commonly added to algae nutritive culturing media to enhance iron solubility and increase the availability of essential trace metals. In field applications, chelated copper is used because it remains in the water column longer than the non-chelated form, which increases the duration of exposure (Masuda and Boyd, 1993). However, algae may respond differently to different forms of copper. Closson and Paul (2014) found that copper becomes less toxic when it is chelated, providing an additional margin of safety to non-target organisms compared to copper sulfate. In their study on the growth inhibition of copper to the freshwater algae *Scenedesmus subspicatus,* Ma et al. (2003) reported that both EDTA and fulvic acid could reduce toxicity of copper by the way of preventing it from being adsorbed by the algae cell wall. Likewise, the results from this study showed significantly lower copper toxicity to algae in the bioassays conducted in the reconstituted canal water with added EDTA (386.67 $\mu g\ Cu\,L^{-1}$) as compared to their analogous test waters excluding the chelating agent (217.17 $\mu g\ Cu\,L^{-1}$). These results demonstrate that caution should be taken when extrapolating laboratory results to field situation. The use of EDTA in laboratory nutritive culture media could underestimate copper toxicity in field waters. This could be more relevant when impacted aquatic environments are deficient in natural organic chelators such as DOC as in the case of the treated Canal water. The use of metal chelators in toxicity bioassays should thus be dependent on the characteristics of the water to be treated.

3.4. Comparison between laboratory and field testing

In order to confirm the accuracy of predictions from laboratory studies of responses of indigenous algal species to copper algaecide, results from laboratory bioassays were compared to copper dosages used in a small scale field testing simultaneously conducted in the impacted irrigation canal, by the corresponding water authority (BAMAS, 2005). The locations of the application sites are provided in Table S1 of SI and Fig. 1. Copper sulfate was introduced into the canal in the non-chelated form. At each testing location, fine grain copper sulfate was applied evenly downstream of the pump intakes to maximize contact time of copper sulfate to algae. A concentration of between 500 $\mu g\,L^{-1}$ and 1000 $\mu g\,L^{-1}$ as copper sulfate (corresponding to 198.9 and

Table 4
Bioassays toxicity endpoints.

	Test medium		
	Standard EPA medium with EDTA	*Reconstituted canal water with EDTA*	*Reconstituted canal water without EDTA*
96 h-EC_{50} ($\mu g\,Cu\,L^{-1}$)[a]	65.93 a*	386.67 b	217.17 c
95% Confidence Interval at 96 h-EC_{50}	49.01–89.99	226.83–559.15	172.23–73.84
96 h-EC_{10} ($\mu g\,Cu\,L^{-1}$)	31.28 a	52.60 a	116.19 b
95% Confidence Interval at 96 h-EC_{10}	23.26–42.08	30.857–76.067	92.15–146.52
NOEC ($\mu g\,Cu\,L^{-1}$)	25.32 a	29.88 a	97.32 b
LOEC ($\mu g\,Cu\,L^{-1}$)	53.52 a	221.32 b	182.32 b

[a] Measured copper concentrations were used to determine toxicity endpoints values.
* a–c denote whether endpoints values are significantly ($p < 0.05$) different. Same letter means no significant ($p > 0.05$) difference.

397.9 μg L^{-1} as copper) was initially targeted in May to evaluate the degree of algae control. Adequate control of algae was noted in 3 days with chlorophyll-a concentrations decreasing to 1.7 ± 0.8 μg L^{-1}. Consequently, the amount of copper sulfate added to the canal in June and July was tapered to a dose of 100 μg L^{-1} (corresponding to 40 μg L^{-1} as copper) and exhibited acceptable control until middle July. Increased algae presence was noted in late July with chlorophyll-a concentrations reaching 40.2 μg L^{-1}. As a result, the dosing copper sulfate target was increased to 1000 μg L^{-1} for the month of August and was later decreased to 500 and 200 μg L^{-1} during September.

The results from the field testing demonstrate that copper sulfate dosages of 500 and 1000 μg L^{-1} (198.9 and 397.9 μg L^{-1} as Cu) were necessary to control massive algae growth in the irrigation canal. These dosages correlate with the predicted copper treatment concentrations determined in laboratory toxicity bioassays conducted in test media with simulated canal water chemistry of high hardness and alkalinity. In these treatments, algae growth rates were significantly reduced at copper concentrations of 350 and 200 μg L^{-1} (879.7 and 502.7 μg/L as $CuSO_4$), with corresponding 96 h-EC_{50} values of 386.67 and 217.17 μg Cu L^{-1} in the presence and absence of EDTA, respectively. Copper toxicity was significantly overestimated (96 h-EC_{50} = 65.93 μg Cu L^{-1}) in toxicity testing performed in the standard EPA medium with relatively lower alkalinity and hardness. Algae growth in this medium was substantially decreased (0.36 ± 0.11 cell/mL/d) at copper concentrations of 100 μg L^{-1}, while in field applications relatively higher copper dosages (198.9 and 397.9 μg Cu L^{-1}) were required to achieve algae control. These results demonstrate the effect of test water chemistry on toxicity results in laboratory bioassays.

Furthermore, while natural organic ligands were deficient in the canal water (DOC < 0.5 mg/L), copper field applications were well correlated to the laboratory bioassays conducted in simulated canal water in the presence of the synthetic ligand EDTA. Indeed, copper concentrations of 397.9 μg L^{-1} were necessary for adequate control of algae growth in the irrigation canal in periods of intensive algal proliferation (May and August), and better relate to laboratory results from the tests conducted in reconstituted canal water with added EDTA (96 h-EC_{50} = 386.67 μg Cu L^{-1}). This is relevant when considering that the high alkalinity and hardness of the treated water decrease the bioavailability of copper. Higher initial dosages of the non-chelated copper sulfate were thus necessary in field applications to account for the processes capable of reducing free copper concentrations and maintain adequate copper levels in the treated waters to control algae growth. In laboratory bioassays, the addition of EDTA had prevented copper precipitation out of the test solution keeping it biologically available. EDTA allows a slow and gradual release of the chelated copper maintaining it for longer period in treated waters (Masuda and Boyd, 1993). This indicates that the original concentration of the free toxic metal ion in test solutions is lower than the ultimate total biologically available copper concentration. This makes both field and laboratory treatments using the non-chelated and chelated form of copper, respectively, comparable in terms of inhibitory copper dosages. This also demonstrates the preferable use of chelated copper forms in hard and alkaline waters to avoid excessive algaecide applications.

Determined algaecide inhibitory concentrations measured in laboratory assays under simulated water chemistry in the absence of EDTA (96 h-EC_{50} = 217.17 μg L^{-1} as Cu) also proved to be efficient in field situation. The wider copper range used in the field to achieve algae growth control (198.9 and 397.9 μg Cu L^{-1}) is probably due to the higher algae proliferation in the field as compared to the biomass growth under the test conditions. In the latter case, the original inoculum of 10,000 cell/mL in test solutions might not have well represented the field case during the different treatment periods. The role of EDTA in simulating naturally occurring DOC was not demonstrated in this study in the absence of a field reference for comparison and further studies are still needed to clarify this point.

4. Conclusion

While natural systems represent the actual set of conditions, in space and time, under which algae are present, reproduction of these systems in the laboratory is very difficult constituting the principal limitation of laboratory cultures. This has caused some workers to question the use of cultures in ecological research. This study has proposed a strategy to obtain laboratory information that can improve the prediction of effective algaecide applications in the field. Representative samples of algae in site water were shipped to the laboratory and responses of the indigenous algae to copper exposures were measured in the standard EPA culture medium and in simulated site water with high hardness and alkalinity. A better correlation of the laboratory results with copper dosages used in the field was obtained in the latter case indicating the major effect of water chemistry in toxicity tests. Furthermore, while the role of EDTA in reducing copper toxicity in laboratory bioassays was demonstrated, its role in mimicking naturally occurring ligands in treated waters was not confirmed in this study. Although, laboratory toxicity bioassays conducted under simulated field conditions could improve predictions of algaecide treatment dosages in natural waters, care must be taken in extrapolating laboratory data to field situation. Small scale field testing to determine efficient algaecide concentrations to control algae growth in impacted water bodies remains necessary.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2018.02.054.

References

Araújo, C.V.M., Diz, F.R., Lubián, L.M., Blasco, J., Moreno-Garrido, I., 2010. Sensitivity of Cylindrotheca closterium to copper: influence of three test endpoints and two test methods. Sci. Total Environ. 408, 3696–3703.

Bellinger, E.G., Sigee, D.C., 2015. Freshwater Algae: Identification, Enumeration, and Use as Bioindicators. John Wiley & Sons Ltd., Chichester, UK.

Bossuyt, B., Janssen, C., 2004. Long-term acclimation of Pseudokirchneriella subcapitata (Korshikov) Hindak to different copper concentrations: changes in tolerance and physiology. Aquat. Toxicol. 61–74.

Button, K.S., Hostetter, H.P., 1977. Copper sorption and release by Cyelotella meneghiniana (Bacillariophyceae) and Chlamydomonas reinhardtii (Chlorophyceae). J. Phycol. 13, 198–202.

Closson, K.R., Paul, E.A., 2014. Comparison of the toxicity of two chelated copper algaecides and copper sulfate to non-target fish. Bull. Environ. Contam. Toxicol. 93 (6), 660–665.

Contreras, L., Moenne, A., Gaillard, F., Potind, P., Correaa, J.A., 2010. Proteomic analysis and identification of copper stress-regulated proteins in the marine alga Scytosiphon gracilis (Phaeophyceae). Aquat. Toxicol. 96, 85–89.

Cook, C.M., Kostidou, A., Vardaka, E., Lanaras, T., 1997. Effects of copper on the growth, photosynthesis and nutrient concentrations of Phaseolus vulgaris plants. Photosynthetica 34, 179–193.

Cooke, G.D., Welch, E.B., Martin, A.B., Fulmer, D.G., Hyde, J.B., Schrieve, G.D., 1993. Effectiveness of Al, Ca, and Fe salts for control of internal phosphorus loading in shallow and deep lakes. Hydrobiologia 253 (1–3), 323–335.

De Laender, F., De Schamphelaere, K., Vanrolleghem, P., Janssen, C., 2009. Comparing ecotoxicological effect concentrations of chemicals established in multi-species vs. single-species toxicity test systems. Ecotoxicol. Environ. Saf. 310–315.

De Schamphelaere, K.A.C., Janssen, C.R., 2002. A biotic ligand model predicting acute copper toxicity for Daphnia magna: the effects of calcium, magnesium, sodium, potassium, and pH. Environ. Sci. Technol. 36, 48–54.

Durborow, B.S., Chauhan, G., 2014. Recent Advances in Weed Management. In: Springer Science+Business Media, New York. http://dx.doi.org/10.1007/978-1-4939-1019-9_13.

EPA, E.P., 2002. Method 1003.0: green alga, Selenastrum capricornutum, growth test; chronic toxicity. In: Environmental Protection Agency, Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Washington, DC, pp. 197–230.

Franklin, N., Stauber, J., Lim, R., 2004. Development of multispecies algal bioassays using flow cytometry. Environ. Toxicol. Chem. 1452–1462.

Heijerick, D.G., De Schamphelaere, K.A.C., Janssen, C.R., 2002. Predicting acute zinc toxicity for Daphnia magna as a function of key water chemistry characteristics: development and validation of a biotic ligand model. Environ. Toxicol. Chem. 21, 1309–1315.

Hochmuth, D., Asselman, J.D., J.A.C., De Schamphelaere, K.A.C., 2014. Are interactive effects of harmful algal blooms and copper pollution a concern for water quality

management? Water Res. 60, 41–53.
Huff, F.A., Angel, J.R., 1989. Frequency Distributions and Hydroclimatic Characteristics of Heavy Rainstorms in Illinois. 70 Bulletin/Illinois State Water Survey, Illinois.
Hullebusch, E.V., Deluchat, V., Chazal, P.M., Baudu, M., 2002. Environmental impact of two successive chemical treatments in a small shallow eutrophied lake: Part II. Case of copper sulfate. Environ. Pollut. 120, 627–634.
Isnard, P., Flammarion, P., Roman, G., Babut, M., Bastien, P., Bintein, S., Saroli, M., 2001. Statistical analysis of regulatory ecotoxicity tests. Chemosphere 45 (4), 659–669.
Knezevic, A., 2008. Overlapping confidence intervals and statistical significance. *73* Cornell University Statistical Consulting Unit.
Litani Basin Management Advisory Services (BAMAS), 2005. Canal 900 Algae Control: Testing and Validation.
Litani River Authority Executed projects, hydraulic, Southern Bekaa Irrigation Project 2018.http://www.litani.gov.lb/en/?page_id=121.
Litani River Basin Management Support Program, LRBMS Project Document: Wet Season water quality survey of the Litani River Basin project 2011.
Ma, M., Zhu, W., Wang, Z., Witkamp, G., 2003. Accumulation, assimilation and growth inhibition of copper on freshwater alga (Scenedesmus subspicatus 86.81 SAG) in the presence of EDTA and fulvic acid. Aquat. Toxicol. 63 (3), 221–228.
Masuda, K., Boyd, C.E., 1993. Comparative evaluation of the solubility and algal toxicity of copper sulfate and chelated copper. Aquaculture 117, 287–302.
R Core team., 2015. *R: A Language and Environment for Statistical Computing. Retrieved from Vienna, Austria:.* Retrieved from https://www.r-project.org/.
Snoeyink and Jenkins: D.M. Whitacre (ed.), Reviews of Environmental Contamination and Toxicology, 1 Reviews of Environmental Contamination and Toxicology 213, DOI 10.1007/978-1-4419-9860-6_1, C Springer Science+Business Media, LLC 2011.
Stevenson, R.J., Bothwell, M.L., Lowe, R.L., Thorp, J.H., 1996. Algal Ecology: Freshwater Benthic Ecosystem.
Wang, D., Xia, W., Kumar, K., Gao, K., 2017. Increasing copper alters cellular elemental composition (Mo and P) of marine diatom. Ecol. Evol. 3362–3371.
Yu, Y., Kong, F., Wang, M., Qian, L., Shi, X., 2007. Determination of short-term copper toxicity in a multispecies microalgal population using flow cytometry. Ecotoxicol. Environ. Saf. 49–56.