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Evaluation of effectiveness of EDTA and sodium thiosulfate in removing metal toxicity toward sea urchin embryo-larval applying the TIE

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HIGHLIGHTS

- TIE (Toxicity Identification and Evaluation) is used to work with complex mixtures.
- EDTA and sodium thiosulfate complex some metals in freshwater and seawater tests.
- Metal complexation by EDTA and thiosulfate were applied in embryo-larval tests with the sea urchin.
- The complexing agents, in seawater, have lower capacity to complex with the metals.
- Metals with lower EC_{50} values may present a reduction in toxicity after complexation.

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ABSTRACT

Since the development of the TIE (Toxicity Identification and Evaluation) in 1988 it has been assumed that the capacity of EDTA and sodium thiosulfate to complex some metals, and thus remove their toxicity, can be applied to both freshwater and seawater ecotoxicological tests and the results subsequently interpreted. However, it is now known that there is a wide variability in the extent of this complexation. In this context, the removal of toxicity caused by the presence of Hg^{2+}, Cd^{2+}, Cu^{2+}, Cr^{6+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Ag^{1+} and Se^{2+}, through metal complexation by EDTA and sodium thiosulfate, in relation to the performance of embryo-larval tests with the sea urchin *Arbacia lixula* was investigated. It was observed that EDTA was capable of removing the toxicity of Pb^{2+}, Zn^{2+} and Cu^{2+} while sodium thiosulfate only reduced the toxicity of Ag^{1+}. Compared to the complexation observed in freshwater ecotoxicological tests, the complexing agents used in this study (EDTA and sodium thiosulfate) have a lower capacity to complex metals in the marine ecotoxicological test with *A. lixula*.

1. Introduction

The TIE (Toxicity Identification and Evaluation) procedures, published by the US Environmental Protection Agency (USEPA, 1988, 1991), have been successfully used in complex mixtures observed in the aqueous fraction of sediments to identify toxic agents responsible for environmental impacts. To evaluate whether sample toxicity is due to metals, the initial USEPA TIE protocol for freshwaters suggests the complexation of metals with two chelant agents: EDTA and sodium thiosulfate. The following protocols presented by the USEPA (1996, 2007) were based on the work of Hockett and Mount (1996), who investigated the reduction in the toxicity of various metals toward the Cladocera *Ceriodaphnia dubia* in freshwater. These authors highlighted that cationic metals are chelated by EDTA and sodium thiosulfate, the latter also being an oxidizing agent. In this regard, it is important to note that the current TIE protocol warns that "there is no empirical evidence that EDTA and thiosulfate chelants have the same complexing affinity for metals in freshwater and seawater, and testing needs to be performed to confirm the efficacy of this approach". It has long been known that the degree of metal toxicity is dependent on: (i) factors related to the organism (e.g., animal species, stage of life, mode and duration of exposure, and metabolic pathway of the organism); (ii) environmental conditions (e.g., ionic strength, pH, temperature, salinity or alkalinity, photoperiod); and (iii) chemical speciation and bioavailability of metals. In this respect, chemical speciation, bioavailability and biological effects are examined by means of models and the free ion activity (FIA) model is the conceptual model most used in ecotoxicological studies (Morel and Hering, 1993; Campbell, 1995; Wenger et al., 2005). According to the FIA model, the role of chelants agents is limited to participating in complexation reactions with trace metals, having these chelants no direct physiological effects, despite some apparent exceptions (Morel and Hering, 1993; Campbell, 1995; Wenger et al., 2005). In the case of higher aquatic organisms, it appears that only the free metal ion

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can across the biological membrane and that chelant agents reduce the toxicity and the tissue concentrations of metal (Campbell, 1995).

Notwithstanding, the ability of complexing agents to reduce metal toxicity can vary depending on the degree of complexation with the chloride ions of seawater, as reported by Engel and Fowler (1979) and Mantoura et al. (1978) who demonstrated variability in cadmium toxicity. Various studies focusing on seawater toxicity have discarded metals as the probable cause of observed toxic effects, probably because of a lack of knowledge regarding the efficiency of EDTA and thiosulfate chelation under marine conditions (Carr et al., 2001; Macken et al., 2008, 2009).

In Brazil, following the publication of CONAMA Resolution 344 (CONAMA, 2004), which establishes guidelines for the evaluation of degraded material and its disposal, concern has been focused on evaluating the quality of sediments, whereas chemical and toxicological analyses have been limited because there are no recommendations for evaluating the probable factors responsible for the toxicity observed. According to the USEPA (2007), current information available on sediment toxicity is not sufficient to provide a basis for decision-making in the area of environmental management. However, it can be useful when the exact origin of the toxicity is known, which can be revealed through applying the TIE method. However, there has been little application of this method in Brazil, to sediments or effluents (Badaró-Pedroso and Rachid, 2002).

Thus, the objective of this study was to evaluate the chelation capacity of EDTA and sodium thiosulfate, and their consequent capacity to reduce the availability/toxicity of the metals Hg^{2+}, Cd^{2+}, Cu^{2+}, Cr^{6+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Ag^{1+} and Se^{2+} in the embryo-larval tests with the sea urchin *Arbacia lixula* carried out in seawater. The results were compared with those reported by Hockett and Mount (1996), seeking to identify if the two studied chelants have the same complexing affinity for metals in seawater under optimal conditions of salinity, hardness and pH for the development of embryos of *A. lixula*. The results of this study will be of interest in the evaluation of the marine sediment quality which is assessed very often by the embryo-larval tests with sea urchins.

2. Materials and methods

Chronic short-term embryo-larval trials were carried out with the sea urchin *A. lixula* using the methodology described in the standard NBR/ABNT (2006) and recommendations presented by Máximo et al. (2008). The basic procedure involved collecting ovules and sperm of the sea urchin *A. lixula* in the municipal district of Penha, on the north coast of the State of Santa Catarina (Brazil). KCl at 0.5 M was injected into the oral region, and the sperm were collected dry with a Pasteur pipette, and kept on ice until use. The ovules were obtained in seawater, filtered through a 0.5 μm filter at a salinity of 32, and fertilized with sperm solution prepared with 0.5 mL of sperm and 24.5 mL of filtered seawater.

The toxicity tests carried out on the sea urchin embryos and larvae with EDTA, sodium thiosulfate and the different metals were performed in 15 mL plastic flasks, using 10 mL of test solution. Six concentrations and a control (seawater) were prepared for each test substance, with four replicates. For the metals Hg^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Ag^{1+} and Se^{2+} standard solutions for atomic absorption produced by Titrisol® and Merck® were used. For the metals Cu^{2+}, Cr^{6+} and Zn^{2+} and the complexing agents EDTA and sodium thiosulfate, the salts $CuSO_4.5H_2O$, $K_2Cr_2O_7$, $ZnSO_4{\cdot}7H_2O$, $C_{10}H_{14}Na_2O_8{\cdot}2H_2O$, and $Na_2S_2O_3{\cdot}5H_2O$, were used, respectively, purchased from Merck®.

After determination of Effective Concentration of the 100% (EC_{100}) in the metal sensitivity tests, new tests were carried out with these metal concentrations but now metals were complexed with EDTA or sodium thiosulfate. The maximum concentration of EDTA and sodium thiosulfate used was 100 mg L^{-1} (based on the results obtained in the complexing agent toxicity tests) with a 2-h waiting period between preparing the solutions and adding the eggs to the test flasks for stabilization. In these tests six concentrations of each complexing agent and the control (complexing agent without metals) were prepared.

The tests were carried out in an incubator at 25 ± 2 °C, with a 12 h light-dark cycle, for 24 h and ended with the addition of 1 mL of 4% formaldehyde. The eggs, embryos and larvae were counted in a Sedgewick Rafter counting chamber under a microscope. The percent effect was determined by the number of eggs, embryos and deformed larvae observed out of a total of 100 quantified in each case. The test was validated by the percent effect control with less than 20%. The salinity and pH were measured at the start and end of the experiments using a ThermoOrion 162A conductivity meter and a ThermoOrion 370 pH meter, respectively. The salinity and pH of the tests presented averages of 32.9 (29.5–36.1) and 8.08 (7.12–8.47), respectively. In the test solution, changes in pH and salinity were not observed after the addition of EDTA and sodium thiosulfate.

The median Effective Concentration values (EC_{50}) for the metals, EDTA and sodium thiosulfate were estimated by the Trimmed Spearman-Karber method, using the TSK program, version 1.5 (EPA, Cincinnati, Ohio), on the basis of the percent effect observed in the tests.

For the graphic representation of the tests performed, the original data were analyzed applying the Abbott equation (USEPA, 2002) in order to eliminate the variability between the batches of organisms used for the different assays, based on the effects observed in the control flasks.

3. Results

The sensitivity of the *A. lixula* embryos to the metals tested is shown in Table 1 and Fig. 1. According to the EC_{50} values generated in this study, the species presented greater sensitivity to Hg^{2+} and Ag^{1+} and higher resistance to Cr^{6+} and Cd^{2+}.

For the sodium thiosulfate, the EC_{50} was above the highest concentration tested (600 mg L^{-1}) while EDTA presented an EC_{50} of 232.74 mg L^{-1} (±14.52). The highest concentration of complexing agent used in the metal chelation experiments (100 mg L^{-1}) was not found to be associated with any adverse effects on the development of the sea urchin eggs and larvae (Fig. 2).

When the tests were performed with effective concentrations of EC_{100}, representing a 100% effect of the metals on the organisms, complexed with the addition of different concentrations of EDTA, it was observed that this complexing agent has the capacity to remove the toxicity of Pb^{2+}, Zn^{2+} and Cu^{2+} even at low concentrations (Fig. 3). For the sodium thiosulfate, only the Ag^{1+} presented a reduction in toxicity, although this chelating agent may be able to remove the toxicity of Hg at higher concentrations than that used in this study (>100 mg L^{-1}) (Fig. 4). Even so, it was observed that the complexation and toxicity removal capacity of sodium thiosulfate in the sea urchin embryo-larval was lower than that observed for EDTA.

Table 2 shows a summary of a comparison between the results obtained in this study and those published by Hockett and Mount (1996) for the complexation of metals in tests with the Cladocera *C. dubia*.

4. Discussion

The results obtained in this study showed that the sensitivity of *A. lixula* to different metals varies, with 24-h EC_{50} values being up to 239 higher for the more toxic (Ag^{1+} and Hg^{2+}) compared with the less toxic (Cr^{6+}) metals. The ranking of increasing metal toxicity

Table 1
Values of 24-h Effective Concentration (EC_{50} and EC_{100}) and Confidence Interval (CI – 95%) for metal toxicity toward the sea urchin *Arbacia lixula* embryo-larval test.

Metal (mg L^{-1})	Hg	Cd	Cu	Cr	Zn	Ni	Pb	Ag	Se
EC_{50}, 24 h(CI)	0.01 (±0.002)	1.58 (±0.05)	0.046 (±0.002)	2.39 (±0.09)	0.075 (±0.006)	1.27 (±0.05)	0.26 (±0.01)	0.01 (±0.001)	0.54 (±0.02)
EC_{100}, 24 h	0.025	4.0	0.13	3.54	0.11	4.0	0.5	0.03	1.5

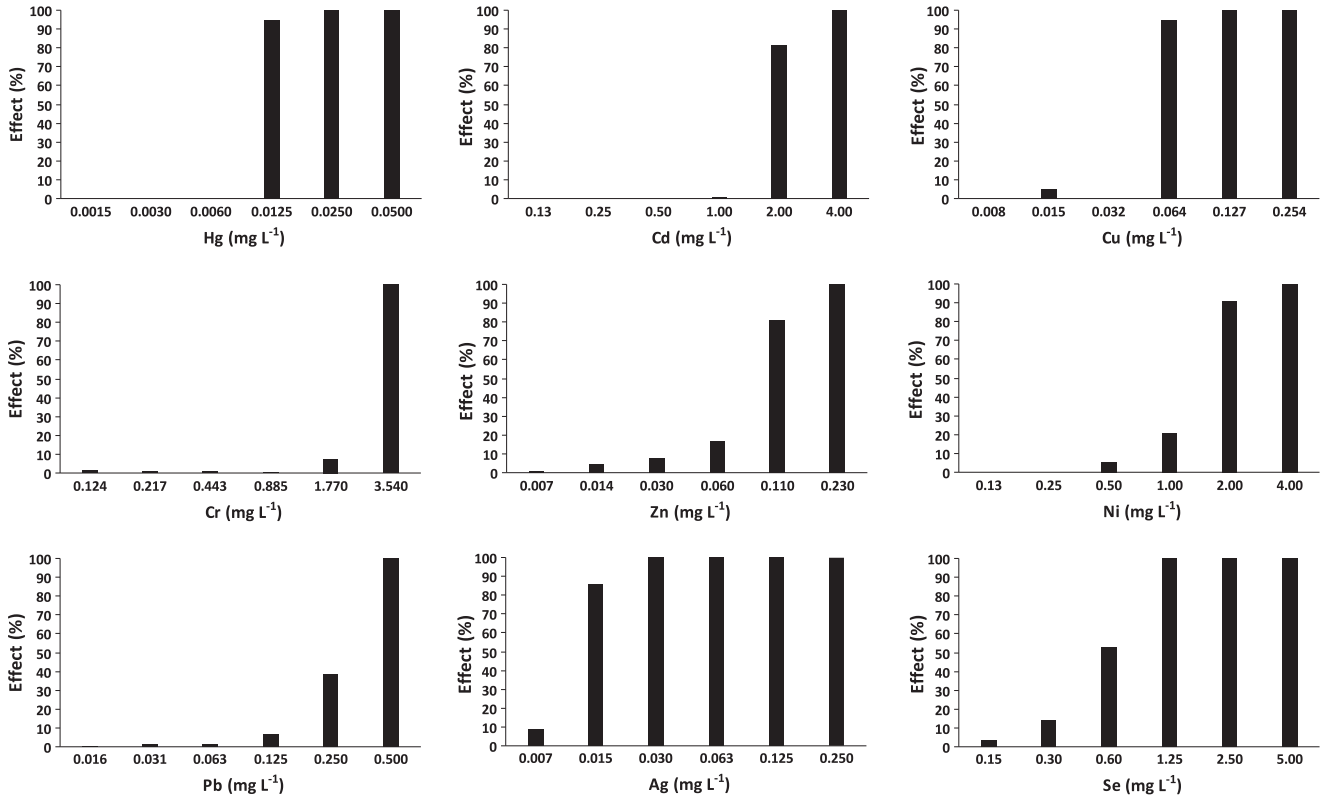

Fig. 1. Dose-response ratio observed in the embryo-larval tests with the sea urchin *A. lixula* in relation to the metals Hg^{2+}, Cd^{2+}, Cu^{2+}, Cr^{6+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Ag^{1+} and Se^{2+}.

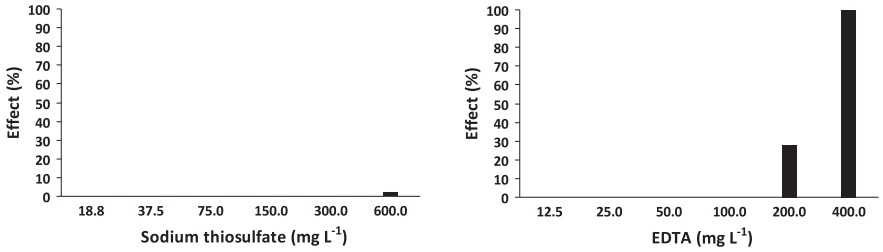

Fig. 2. Dose-response ratio observed in the embryo-larval tests with the sea urchin *A. lixula* in relation to the complexing agents sodium thiosulfate and EDTA.

toward *A. lixula* embryo-larval observed in this test was: chromium < cadmium < nickel < selenium < lead < zinc < copper < silver ≈ mercury, which does not correspond exactly with the ranking published by Abel (1989), which established the following approximate order of decreasing toxicity for heavy metals in aquatic organisms: mercury > cadmium > copper > zinc > nickel > lead > chromium > aluminum > cobalt. However, the toxicity of a given metal can vary greatly from one species to another, depending on various physiological/biochemical factors related to the absorption, storage, removal or detoxification capacities of the organisms through the use of proteins (e.g. metallothioneins, phytochelatins) or cell structures (e.g., cell wall, lysosomes).

Studies of the sensitivity of *A. lixula* under the metals are not very common, but the results obtained in this work are comparable with those obtained by Cesar et al. (2004) which found EC_{50} values of 1.26 mg L^{-1} for Cd and 0.016 mg L^{-1} for Zn. Castagna (1976) obtained an EC_{50} of 0.06 mg L^{-1} for Hg, while Soyer (1963) obtained an EC_{50} value of 0.001 mg L^{-1} for Ag.

Concerning the toxicity of EDTA and sodium thiosulfate toward *A. lixula*, this organism showed weak sensitivity to these chelating agents, since the EC_{50} values were as high as 232.74 mg L^{-1} (±14.52) for EDTA and >600 mg L^{-1} for sodium thiosulfate. This low toxicity of the complexing agents has been described by the USEPA (1996) for other test organisms, and is of fundamental

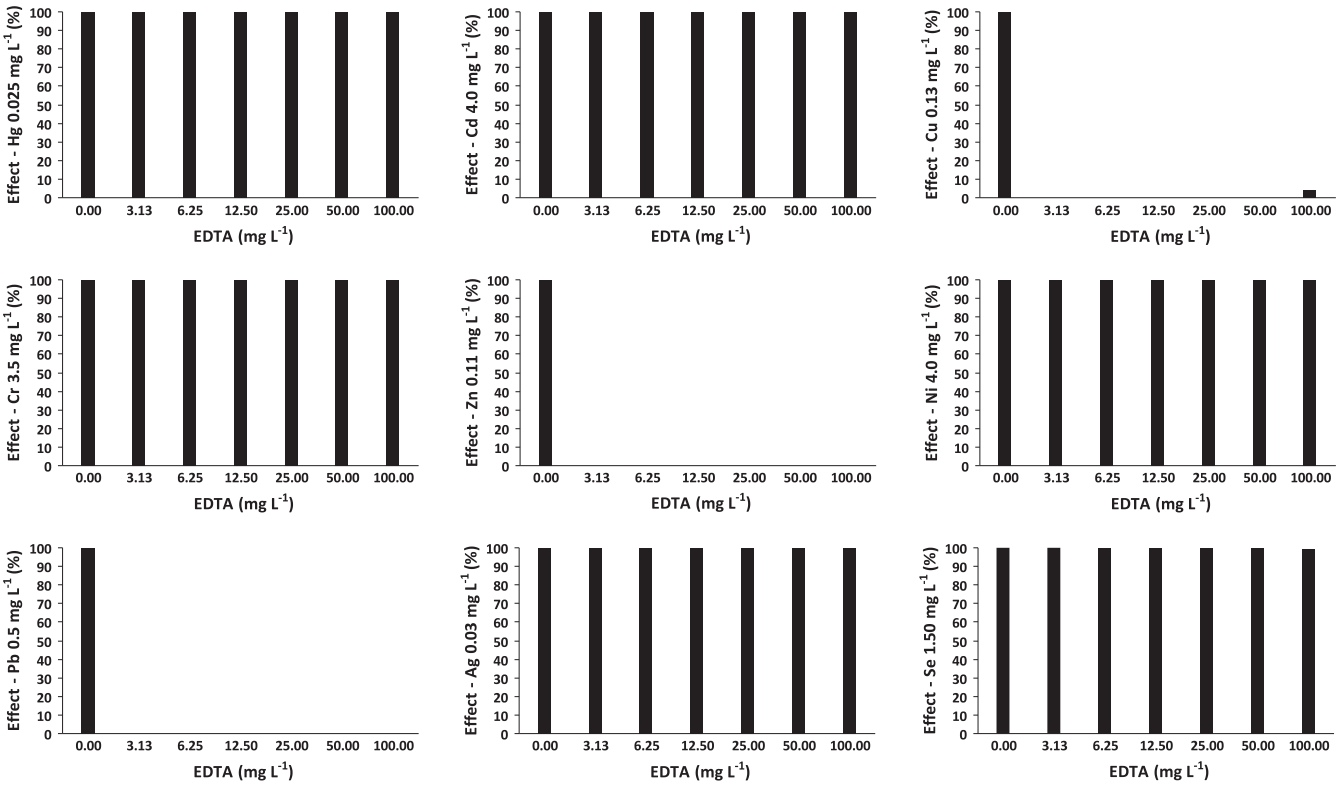

Fig. 3. Dose-response ratio observed in the embryo-larval tests with the sea urchin *A. lixula* in relation to EC_{100} (24 h) of the metals associated with the complexing agent EDTA.

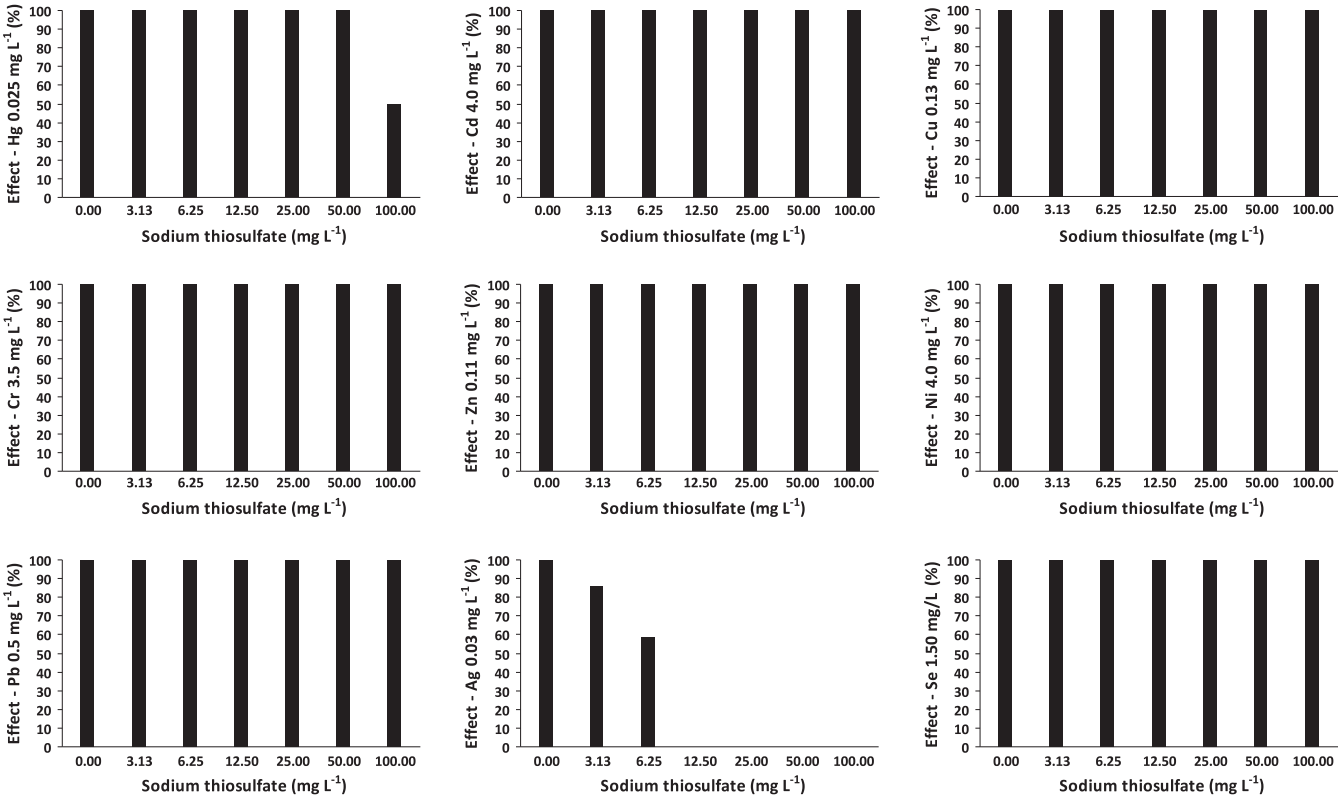

Fig. 4. Dose-response ratio observed in the embryo-larval tests with the sea urchin *A. lixula* in relation to EC_{100} (24 h) of the metals associated with the complexing agent sodium thiosulfate.

Table 2
Comparative results of the toxicity removal capacity of EDTA and sodium thiosulfate for the metals tested in this study and their comparison with the results obtained by Hockett and Mount (1996).

	Toxicity removal by EDTA					
	Adapted from Hockett and Mount (1996) (*C. dubia*, DMW–HRW)[a]			This study (*Arbacia lixula*, seawater)		
	None	Weak	Strong	None	Weak	Strong
Toxicity removal by thiosulfate						
Strong		Ag	Cu Cd	Ag		
Weak		Hg	Zn Ni Pb	Hg		
None	Se Cr			Se Cd Ni Cr		Cu Zn Pb

[a] DMW–HRW – Diluted mineral water–hard reconstituted water.

importance for the establishment of a base line for complexation tests when the TIE methodology is applied.

Comparing our results with those published by Hockett and Mount (1996) (Table 2), the assumption that freshwater and seawater are equivalent for the purposes of TIE methodology is not valid for metal complexation by EDTA or sodium thiosulfate. This assumption (surrogate approach) has been criticized by Burgess et al. (1995) who have reported that for the majority of chemicals tested for toxicity, as part of the Water Quality Criteria of the USEPA, marine species were shown to have greater sensitivity than freshwater species. Albeigt marine Phase I TIE methods have been developed for several marine species (Burgess et al., 1993). However, no toxicity testing of the species *A. lixula* to evaluate the metal chelation potential of EDTA and sodium thiosulfate chelants were found in the literature.

With regard to the results for *A. lixula*, both EDTA and the sodium thiosulfate presented the capacity to complex and reduce toxicity of a smaller range of metals in seawater (Figs. 2 and 3). It should be noted that the concentrations of complexing agents used in the tests (100 mg L^{-1}) were greater than the amounts needed to chelate the metals present in the medium (Figs. 3 and 4). According to SAIC (2003), using 60 mg L^{-1} of EDTA, 26 mg L^{-1} of bivalent metals can potentially be chelated in freshwater, but this ratio will probably be different for seawater.

EDTA is a chelating agent that forms non-toxic complexes with the metals Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Se^{2+} and Zn^{2+} (USEPA, 2007). The chelation is dependent on the pH, the type of metal, the presence of other ligands in the solution, and the affinity of the metal with the EDTA. Primarily, EDTA forms complexes with Ca^{2+} and Mg^{2+}, leading to higher toxicity of the metals as the hardness and salinity of the water increase.

For the metals Zn^{2+}, Pb^{2+} and Cu^{2+}, the pH (average of 8.08) of the tests carried out in this study would explain the EDTA chelation capacity (Holleman and Wiberg, 2001). This result is in agreement with those obtained by Jirik et al. (1998) who carried out fertilization tests on sea urchins, but the capacity of EDTA to remove toxicity in our study is lower than similar results reported in the literature (Ho and Burgess, 2009). Pb as a bi- and tetravalent cation is also in the group of metals capable of being complexed by EDTA at a slightly basic pH. The low toxicity removal of Ag^{1+} by EDTA is consistent with its lower stability constant, but we were unable to find a reasonable explanation for the lack of toxicity removal by EDTA in the case of the metals Ni^{2+}, Cd^{2+} and Hg^{2+}. Nickel and cadmium have similar chemical properties to copper, zinc and lead, while mercury is similar to the silver cation. In this respect, slow equilibration was observed when adding copper to a mixture of natural and synthetic ligands in the presence of calcium in seawater. Thus, the assumption of rapid re-equilibration is less likely to be valid for the reaction of strong, slow-reacting ligands, such as EDTA, with competing transition metals or with transition metals at seawater calcium concentrations (Hering and Morel, 1989). In relation to the weak chromium toxicity or lack of removal of this element by EDTA addition, Hockett and Mount (1996) noted that when the pH of the chrome solution was adjusted up to pH 8 a visible precipitate was formed, and the solution was no longer toxic. No other chemical evidence could be noted in terms of metal complexing properties and therefore the specific sensitivity of *A. lixula* may play an important role in the toxicity of these metals. Independently of the interpretation of the chemical complexation data, it is clear from our results that EDTA is not suitable for removing the toxicity of metals, for the purposes of TIE, when a bioassay with *A. lixula* is used, except in cases where the suspected toxicity is due to the presence of Zn^{2+}, Pb^{2+} or Cu^{2+}.

Sodium thiosulfate ($Na_2S_2O_3$) is used in TIE procedures to remove the toxicity of oxidants such as chlorides, bromides, sulfides and iodides, which can also be removed by aerating the sample. Theoretically, thiosulfate can also complex some cationic metals like cadmium, copper, silver and mercury in freshwater tests, due to its anionic property (SAIC, 2003). However, the complexation capacity is dependent on the contact time of the thiosulfate with the metal, as the rate of the complex formation is slow (>24 h) and is also dependent on the concentration of oxidants in the sample (USEPA, 1991). In the study reported herein, the sea urchin eggs were inoculated into the test solutions 2 h after preparing the mixtures with the complexing agents, which may have limited their complexation capacity. However, the reduction of the Ag^{1+} toxicity is probably related to the monovalent characteristic, the only metal with this characteristic used in this study.

With respect to thiosulfate complexation (Fig. 4), with the exception of Ag^{1+} cation, for all other metals with higher or lower stability constants for complexation, the toxicity is not removed by thiosulfate addition. As for the interpretation of the results for EDTA, it is clear that sodium thiosulfate is not suitable for metal removal for the purpose of TIE when a bioassay with *A. lixula* is used, unless the sample toxicity is due to the Ag^{1+} cation.

In conclusion, a literature survey shows that there are few studies on the chelation capacity of metals by EDTA and sodium thiosulfate in marine ecotoxicological tests, and that the complexation capacity is dependent on several factors, ranging from differences in sensitivity of the species used in the tests (Burgess et al., 1995) to the type of treatment which the environmental sample undergoes before use in the ecotoxicological experiments

(Schubauer-Berigan et al., 1993). Nevertheless, the fact that marine test organisms are more sensitive than freshwater species to metals in the presence of complexing agents suggests that complexants may act in a selective way. In other words, metals with lower EC_{50} values, such as Hg^{2+} and Ag^{1+}, may present a reduction in toxicity even with a low complexation rate, while metals with higher EC_{50} values, like Cr^{6+} and Cd^{2+}, do not respond to the complexing agents when the test is carried out with *A. lixula* in seawater. More detailed studies need to be carried out to understand the chelating potential of different complexants when other species and media/conditions are used.

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