DOI: 10.5327/Z2176-9478201611614

FORMULATION OF SEDIMENTS WITH CHARACTERISTICS OF TROPICAL LOTIC SYSTEMS FOR USE IN ECOTOXICOLOGICAL ASSAYS

FORMULAÇÃO DE SEDIMENTOS COM CARACTERÍSTICAS DE SISTEMAS LÓTICOS TROPICAIS PARA APLICAÇÃO EM ENSAIOS ECOTOXICOLÓGICOS

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ABSTRACT

Investigation of the dynamics and toxicity of pollutants in natural sediments is hindered by the great complexity of their matrix. Given this difficulty, the objective of this paper was to recommend the formulation of sediments representative of tropical lotic environments, considering their use in ecotoxicological assays. Organic and inorganic fractions were used in different percentages, resulting in five sediment formulations with different grain size proportions (fine, medium and coarse sand, and clay). Measured dissolved oxygen and hardness values were suitable for ecotoxicological assays; however, regarding pH values, only formulations with lower levels of organic matter provided suitable water quality for this purpose (between 6.3 and 7.3), for up to 10 days. These formulations were tested using *Hyalella azteca*, which confirmed their suitability for use in toxicological tests, since no lethal effects were observed and there were no significant changes in terms of growth and reproduction of the organisms.

Keywords: formulated sediments; ecotoxicological assays; *Hyalella azteca*; tropical water bodies.

RESUMO

Os estudos relacionados à dinâmica de poluentes e toxicidade em sedimentos naturais são de difícil compreensão por se tratar de uma matriz muito complexa. Diante dessa problemática, o objetivo deste trabalho foi propor a formulação de sedimentos com características representativas de ambientes lóticos tropicais, considerando-se a sua aplicação em ensaios ecotoxicológicos. As frações orgânica e inorgânica foram utilizadas em porcentagens variadas, totalizando cinco formulações de sedimento com diferentes proporções granulométricas (areia fina, média, grossa e argila). Os resultados de oxigênio dissolvido e dureza permaneceram adequados para ensaios ecotoxicológicos, porém, considerando-se o pH, apenas as formulações contendo menores taxas de matéria orgânica apresentaram-se adequadas para este fim (entre 6,3 e 7,3), num período de estabilização de até 10 dias. A espécie *Hyalella azteca* confirmou a aplicabilidade desses sedimentos em testes toxicológicos, não tendo sido observadas taxas de letalidade, tampouco variações significativas em relação aos parâmetros de crescimento e reprodução dos organismos.

Palavras-chave: sedimentos formulados; ensaios ecotoxicológicos; *Hyalella azteca*; corpos de água tropicais.

INTRODUCTION

One of the most important uses of ecotoxicological testing concerns the establishment of safe concentrations of pollutants in order to protect the aquatic life in water and sediment environments (ZAGATTO, 2008). This can be achieved by exposing test organisms to different concentrations of a chemical agent under controlled conditions. Relations between concentration, effect, and exposure time enable the identification of a concentration range, above which the majority of species could suffer injury, and which should not be exceeded (ZAGATTO; BERTOLETTI, 2008).

In Brazil, the resolution 357 (BRASIL, 2005) of the *Conselho Nacional do Meio Ambiente* (CONAMA) sets out water quality standards according to the intended use, considering the protection of aquatic life in Classes 1 and 2 water bodies. However, as to sediments, there is no Brazilian legislation concerning quality standards or toxicological potential of this aquatic compartment. This happens due to the difficulties involved in studying sediments, especially the interpretation of experimental results obtained using natural sediments. These materials represent a highly complex matrix, and many factors can influence both pollutant bioavailability and reaction mechanisms, as well as can hinder the evaluation of toxic effects of a pollutant (MOZETO; ZAGATTO, 2008). The Brazilian environmental agency presents a single legislation concerning the evaluation and disposal of dredged materials in Brazilian jurisdictional waters (BRASIL, 2004), whose reference values for freshwater sediment are based on the official Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 1995).

A possible way of minimizing these difficulties is the use of laboratory-manufactured artificial sediments that are spiked with contaminants in order to determine toxic effects under controlled conditions (MURDOCH *et al*., 1997). This can assist in understanding the behavior of different classes of pollutants, including their mobility between water and sediment compartments ("top down" and "bottom up"), degradation time, and formation of secondary compounds, as well as bioavailability and toxicity to biota.

Despite the importance of studies using artificial sediments, new methodologies that take into account physical and chemical factors are needed in order to avoid inaccurate predictions (BURTON *et al*., 2003). The organic content of artificial sediment can influence the nutritional status of the test organism, therefore preliminary studies are essential to formulate the sediment (WÄSTLUND, 1999). Furthermore, most of the sediment formulations that have been described do not represent real environments (GONZALEZ, 2012). In a country like Brazil, with a vast geographical area and a diversity of geological processes that produce sediments, formulations should therefore consider different regional characteristics.

Formulations of sediments containing known amounts of contaminants, based on real North American sediments, have been recommended by the Organization for Economic Co-Operation and Development (OECD) since 1994 to determine observed effect concentrations (OEC), non-observed effect concentrations (NOEC), and lethal concentrations (LC), and to establish standards for sediment quality (SUTER, 2008). However, due to differences in geographical characteristics, individual countries should develop protocols suitable for ecotoxicological and chemical studies designed to determine environmentally safe concentrations and to understand the dynamics of organic and inorganic pollutants. This study would assist in enabling formulations to be adjusted for ensuring their suitability in tests using representative organisms from this type of environment.

Hyalella azteca is an epibenthic species from Northern freshwater. This amphipod was chosen as a matching parameter for the sediment quality, as recommended by international and Brazilian standards for sediment toxicity evaluation (USEPA, 2000; ABNT, 2013). It presents short life cycle, dimorphism, sexual reproduction, and females produce eggs. Such characteristics allow the survival and reproduction to be evaluated by different generations of *Hyalella azteca*, which are recommended criteria for ecotoxicological analysis of many aquatic pollutants. Amphipods play an important role in aquatic environments due to their detritivorous and herbivorous habits and consequent transfer of energy produced by algae and vegetables to consumers of higher trophic levels (USEPA, 2000).

OBJECTIVES

The purpose of this work was to develop suitable formulated sediments for the survival, growth, and reproduction of *Hyalella azteca* with formations that represent water bodies in the State of São Paulo, Brazil. Tietê River system is located in the highest economic and population growth area of the State of São Paulo. The effects of granulometry variation and organic matter content on test organisms were evaluated.

MATERIAL AND METHODS

Formulations

The tested formulations were based on previous studies regarding the formation of natural sediments from 14 locations in the Tietê River system in four different periods (RODGHER *et al.*, 2005). Tietê River is an important aquatic system impacted by a range of anthropogenic influences, and it is used for different purposes such as power generation, public water supply, and navigation. It was chosen as a basis for the formulations due to its varied geology and extensive area, stretching from the East of São Paulo State to its point of discharge into the Paraná River system.

Five sediment compositions were tested using different proportions of coarse, medium, and fine sand (Jundu®, Analândia, São Paulo), and kaolin clay (Synth®) for the inorganic fraction, totaling 100 g in each formulation. The granulometry followed the classification scheme described in the regulation from the *Associação Brasileira de Normas Técnicas* (ABNT) NBR 6502 (ABNT, 1995), as previously used (RODGHER *et al.*, 2005). These materials were calcined in a muffle furnace at 550 °C for one hour before the experiments. Different proportions of two types of organic matter (*Elodea sp.* and TetraMin®) were added to the formulations. The macrophyte *Elodea sp.* was dried at 40 °C for 24 hours, crushed and sieved to particles < 1 mm in order to ensure good interaction with the sediments (OECD, 2010). In addition, a common commercial fish food (TetraMin®, with a 47% crude protein content) was likely crushed and sieved. There are many types of organic matter sources for artificial sediment composition. Easy acquisition, cost and constant composition for the selection of sources of organic matter were considered important, despite the low representativeness of very complex natural organic matter (GONZALEZ, 2012). US Environmental Protection Agency (USEPA, 2000) does not recommend a specific organic carbon source, but they believe it is important to observe the ratios of carbon to nitrogen to phosphorus when considering the materials to be used in formulated sediments. Some authors (PÉRY *et al.*, 2005; VERRHIEST *et al.*, 2002) have used TetraMin® as an organic carbon to formulated sediments in their studies, and this material is listed as a possible source of organic matter for formulated sediment composition (USEPA, 2000). *Elodea sp*. was chosen as an alternative to α-cellulose, which use is also very common in studies with formulated sediments. The organic matter contents of different formulations varied between 1 and 20% (Table 1).

After mixing manually the dry organic and inorganic components, reconstituted water was added at a ratio of 1 g: 4 mL (sediment/reconstituted water). The water used followed the recommendations for cultivation of *Hyalella azteza* (a representative test organism for sediments). Tap water was filtered and dechlorinated, followed by adjustment of pH, hardness, and dissolved oxygen (DO) to values of 7.2 – 7.6, 40 – 48 mg $CaCO_3$ L^{-1}, and > 4 mg L^{-1} respectively (USEPA, 2000; ABNT, 2013). These parameters were monitored every three days for 15 days, under controlled conditions of temperature and photoperiod. Mild aeration of the surface was maintained throughout the experiment, and three replicates were used for each formulation.

The values of chemical parameters obtained for the formulations were compared with those obtained for the controls using Kruskal-Wallis analysis of variance test, performed with BioEstat 5.0 software (AYRES *et al.*, 2007).

Table 1 – Proportions of organic and inorganic fractions used in sediment formulations (based on reservoirs in middle and lower Tietê River, and the Piracicaba River).

Reference environment (Rodgher *et al.*, 2005)	Formulation	Inorganic fraction				Organic fraction	
		CS (%)	MS (%)	FS (%)	CL (%)	Organic matter (%)	
						Elodea sp.	Fish food
Piracicaba River	Control 1	-	-	25	75	-	-
	1A	-	-	25	75	20	-
	1B	-	-	25	75	-	20
Barra Bonita reservoir	Control 2	-	40	50	10	-	-
	2A	-	40	50	10	15	-
	2B	-	40	50	10	-	15
Nova Avanhandava reservoir	Control 3	90	-	-	10	-	-
	3A	90	-	-	10	5	-
	3B	90	-	-	10	-	5
Três Irmãos reservoir (1)	Control 4	40	40	10	10	-	-
	4A	40	40	10	10	-	1
	4B	40	40	10	10	-	3
	4C	40	40	10	10	1	-
	4D	40	40	10	10	3	-
Três Irmãos reservoir (2)	Control 5	10	10	70	10	-	-
	5A	10	10	70	10	-	1
	5B	10	10	70	10	-	3
	5C	10	10	70	10	1	-
	5D	10	10	70	10	3	-

CS: coarse sand; MS: medium sand; FS: fine sand; CL: clay.

Suitability of sediments for use in ecotoxicological studies: analysis of survival, growth, and reproduction of the test organism *Hyalella azteca*

Formulations 4 and 5 (Table 1) were selected in order to evaluate their suitability for use in ecotoxicological tests due to their satisfactory physical and chemical characteristics for use with test organisms, as required by Brazilian and international protocols. The ecotoxicological studies were performed using the *Hyalella azteca* species cultivated in incubators (Tecnal EX80) on controlled conditions of temperature (24 ± 1 °C) and photoperiod (12 hours – light, 12 hours – dark), as recommended by ABNT NBR 15470 (ABNT, 2013) and USEPA (2000) for evaluation of growth and reproduction, respectively. Organisms' feeding was in accordance with Fracácio *et al*. (2011). The test organisms were fed every day in the experimental period with a mix of 10 mg/mL fish food solution (TetraMin®), 5 mg/mL yeast and 0.1 mL/100 mL primrose oil (0,025 mL/organism/day) and macerated flakes of fish food containing Spirulina (TetraVeggie® Spirulina – enhanced flakes, 0.2 mg/organism/every other day).

According to ABNT NBR 15470 (ABNT, 2013) and USEPA (2000), tests can be validated when they present less than 20.0% of mortality in control sediment. Control consisted of inorganic sediment fraction alone for assessment of survival, growth, and reproduction of *H. azteca.*

Growth evaluation

For each formulation and experimental control (Table 1), 10 replicas were prepared for a 10-day test, each with one organism aged between 7 and 14 days that had been allowed a stabilization period of five days. All the experiments used 10 g of sediment and 40 mL of reconstituted water, which were placed in glass vessels with a capacity of 50 mL. The experiment lasted 10 days, and a semi-static regime was adopted, with exchange of water every three days, and measurement of pH, hardness, and DO. The incubators were equipped with controlled temperature (24 ± 1°C), photoperiod (12 hours – light, 12 hours – dark), and illumination (500 – 600 lux).

After the experimental period, the test organisms were evaluated biometrically to obtain the dry weight and length. Dry weight was determined using a five-decimal analytical balance (Ohaus), after drying the organisms for 24 hours at 60 °C, and the length was measured from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface using a Vernier caliper (USEPA, 2000). Kruskal-Wallis analysis of variance test was used to identify significant differences between the data obtained for different experimental treatments and controls. With regard to dry weight, Fisher's exact test was applied because the values were obtained as the average of five organisms for each treatment, rather than individually (AYRES *et al.*, 2007). According to ABNT NBR 15470 (ABNT, 2013), the length or dry weight of organisms in control sediment must be compatible with organisms of the same age previously cultivated in the laboratory.

Reproduction evaluation

Adult organisms aged between 24 and 30 days were selected to prepare couples that were kept for 14 days in vessels containing the formulations and controls, on the environmental conditions that have already been described. Five replicates were used in each experiment. Every seven days, adults were removed to new vessels maintained under the same conditions, and the offspring numbers were counted. Statistical analysis of the data was performed using the Kruskal-Wallis analysis of variance test. Reproduction parameter is highly variable within and among laboratories. More than 50% of laboratories participating in a round-robin testing reported that reproduction from day 28 to 42 was >2 young/female in control sediment (USEPA, 2000).

RESULTS AND DISCUSSION

Formulations: analysis of chemical water parameters

DO and hardness values were within acceptable ranges for all the tests, with values greater than 2.5 mg/L (USEPA, 2000; ABNT, 2013) and between 40 and 48 mg $CaCO_3$ L^{-1} (ABNT, 2013) respectively. Maximum and minimum DO values obtained for formulations 1 to 5 were 7.5 and 6.0 mg/L respectively, and the hardness was in the range of 40.0 – 46.8 mg $CaCO_3$ L^{-1}. As to these two parameters, the formulations were suitable for use in ecotoxicological tests.

However, the first three formulations with organic matter contents of 20, 15, and 5% showed substantial acidity throughout the experiment. The environment was therefore unfavorable for the survival of *H. azteca* during ecotoxicological tests, even with the exchange of water in every three days. When a 20.0% proportion of organic matter was used (based on sediments from the Piracicaba River), the maximum and minimum pH values were 6.8 and 4.7. In the case of formulation 2, containing 15.0% of organic matter in the form of triturated *Elodea sp.*, the maximum and minimum pH values were 7.1 and 5.5 respectively, and when the fish food was used, the pH ranged between 6.0 and 7.3. Slightly higher pH values were observed for formulation 3, which contained 5.0% of organic matter, with pH ranges of 6.8 to 7.2 (macrophyte) and 6.8 to 7.4 (fish food). In this case, pH showed signs of reaching equilibrium between days 10 and 15, and the average value was 6.8. These data are in agreement with the findings of Verrhiest *et al*. (2002), who reported that the physicochemical and biological parameters of sediment formulations showed important changes during the first 10 to 15 days. These suggest that toxicity tests should only be performed after 10 days at minimum. The used formulations consisted of 65.0% sand, 30.0% kaolin clay, 4.85% α-cellulose, 0.15% TetraMin® fish food, and 0.1% calcium carbonate. It was shown that, during the conditioning period, parameters such as pH became stabilized and there was the initiation of organic matter degradation, which is a fundamental process in natural ecosystems. The pH values obtained for formulations 1 to 3 are shown in Figure 1 (A, B and C).

Results of pH analyses indicated that formulations 1 and 2 were not suitable for use in toxicological tests during the 15-day period. The systems showed instability and the pH values were below the pH 7.0 – 7.6 range, which is recommended for the *Hyalella azteca* cultivation (ABNT, 2013). Formulation 3 presented better stability for pH values than formulations 1 and 2, therefore the lower amount of organic matter, the more stable will be the system regarding this parameter.

Values of pH obtained for formulations 4 and 5 were similar to those of the controls and remained closer to neutral (Table 2), which could be explained by the low organic matter content. These findings are in agreement with the work of Campagna (2010), in which a simple sediment formulation (90.0% coarse sand and 10.0% clay) was used with 1.0, 3.0, and 5.0% organic matter content (derived from an aquatic macrophyte). It was concluded that the use of organic matter content exceeding 1.0% resulted in unsuitable water quality for the survival of test organisms during periods shorter than 10 days.

For both formulations, those with 1.0% organic matter derived from fish food (formulations 4A and 5A) showed the best characteristics in terms of water quality suitable for the survival of *H. azteca*, as the pH values were slightly closer to the range recommended for this organism (Table 2, Figure 1D and 1E). Excluding the controls, the maximum and minimum pH values were 7.3 and 6.3 for formulation 4 and 7.3 and 6.8 for formulation 5.

According to Kemble *et al*. (1999), changes in water quality using formulated sediments are related to the bioavailability of byproducts produced during the decomposition of organic matter, which in turn depends on the microbial community and physical and chemical characteristics of the sediment and water. Herein, the water pH was closer to neutrality for formulation 5, which consisted mainly of fine sand. Between days 7 and 15, it was slightly closer to those of the controls, for both types of organic matter, compared to formulation 4 (which had a higher content of coarse sand). In the latter case, the pH was slightly more acidic for formulation 4D, in which 3.0% *Elodea sp.* were used. As discussed by Kemble *et al*. (1999), sediments with finer particle size may have a greater capacity to adsorb the byproducts of organic matter decomposition, making the byproducts less available to the water column.

Analysis of variance using the Kruskal-Wallis test revealed $p \leq 0.05$ for comparison of the pH values obtained for formulations 4B and 4D (3.0% organic matter) with those for the control. However, the first of these formulations showed stability after day 10, with values close to neutrality, while for the second formulation the pH remained acidic up to the end of the experiment. For formulation 5, comparisons between all treatments (including the control) resulted in $p \geq 0.05$, which indicate good pH stability. In this case, there was a pH fluctuation of only 0.5 between the minimum and maximum pH values for the four formulations (with 1.0 and 3.0% organic matter).

Overall, considering the results of the statistical tests and the fact that the pH values were closer to those recommended for cultivation, survival, and reproduction of *H. azteca*, together with the time taken for pH stabilization, formulation 5A was found to be the most satisfactory. Nonetheless, all the variations of formulation 5 could be considered suitable, but they only showed slight differences regarding the time taken for pH stabilization. All variations of formulation 4 also presented conformity in terms of measured parameters, with the exception of formulation 4D (using 3.0% *Elodea sp.*).

It has been widely reported that, amongst the abiotic factors, the content of organic matter in the sediment is the main factor controlling the bioavailability of organic pollutants in toxicological studies (DI TORO *et al*., 1991; FLEMING; HOLMES; NIXON, 1998). In addition to the amount, the type of organic matter used in sediment formulations is also important because it influences the sorption of contaminants as well as their bioavailability to micro- and macroinvertebrates, and determines how well the formulations represent natural environments (GONZALEZ, 2012). Various sources of organic matter (such as peat, dung, humus, plants, and fish food) can be used in sediment formulations. The selection of organic matter for this work was based

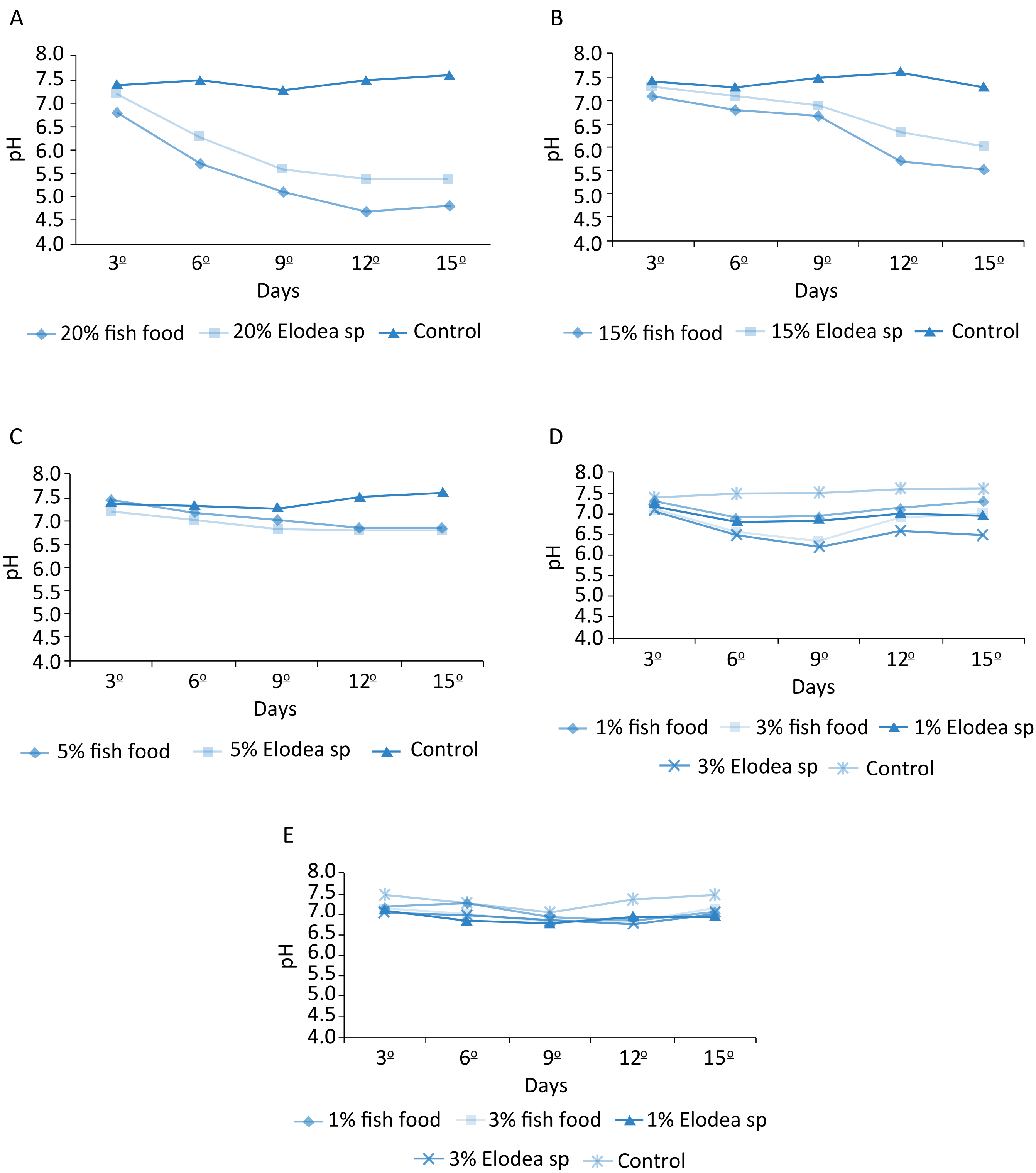

Figure 1 – Values of pH obtained every three days during 15 days of experiment: (A) Formulation 1, containing 20.0% organic matter; (B) Formulation 2, containing 15.0% organic matter; (C) Formulation 3, containing 5.0% organic matter; (D) Formulation 4 and (E) Formulation 5, containing 1.0 and 3.0% organic matter.

Table 2 – Results of chemical analyses of sediment formulations consisting mainly of coarse and medium sand (formulation 4) and fine sand (formulation 5), using two sources of organic matter in proportions of 1.0 and 3.0%.

Formulation	Days	pH	Hardness (mg $CaCO_3$ L^{-1})	Dissolved oxygen (mg L^{-1})
Control 4	3	7.4	45.8	7.4
	6	7.5	44.6	7.5
	9	7.5	44.2	7.3
	12	7.6	44.0	7.4
	15	7.6	43.2	7.3
4A	3	7.2	44.2	7.3
	6	6.8	43.2	7.0
	9	6.9	41.9	7.0
	12	7.1	43.9	7.2
	15	7.3	44.2	7.0
4B	3	7.1	44.6	7.0
	6	6.5	42.5	6.8
	9	6.3	42.4	6.3
	12	6.9	41.3	6.1
	15	7.0	44.3	6.9
4C	3	7.2	45.2	6.9
	6	6.9	43.1	6.8
	9	6.9	44.1	7.0
	12	7.0	44.3	7.1
	15	7.0	46.3	6.9
4D	3	7.1	44.6	7.0
	6	6.5	42.5	6.8
	9	6.2	42.4	6.3
	12	6.6	41.3	6.1
	15	6.5	44.3	6.2
Control 5	3	7.5	44.3	7.2
	6	7.3	44.1	7.0
	9	7.1	43.2	7.3
	12	7.4	44.8	7.4
	15	7.5	46.2	7.3
5A	3	7.2	43.0	7.0
	6	7.3	43.2	6.9
	9	7.0	44.1	6.9
	12	6.9	43.1	7.0
	15	7.1	42.8	7.0
5B	3	7.1	45.4	7.0
	6	7.0	41.8	6.6
	9	6.8	46.8	6.9
	12	6.9	45.6	6.0
	15	7.1	44.0	7.0
5C	3	7.2	43.2	7.0
	6	6.9	44.0	6.9
	9	6.9	45.6	6.7
	12	7.0	46.2	7.0
	15	7.0	46.3	6.9
5D	3	7.1	42.8	7.0
	6	7.0	42.1	6.5
	9	6.8	44.7	6.7
	12	6.8	40.3	6.3
	15	7.1	41.8	6.8

on the natural sediment characteristics, as well as the commercial availability and ease of use in the laboratory. Further studies will be necessary to evaluate the interaction of different types of organic matter with contaminants, together with their influence on the bioavailability of toxic pollutants to benthic organisms.

Tests of survival, growth, and reproduction of *Hyalella azteca* using sediment formulations

Results of the ecotoxicological analyses provided support for the results obtained in chemical analyses of water for formulations 4 and 5. All the exposed organisms survived until the end of the experiments, and the values obtained for growth parameters were very similar, even in the comparison of the two formulations. The average weight and length values (using 10 replicates for each formulation) are shown in Table 3. The minimum and maximum values obtained for the lengths of organisms were 2.07 and 2.67 mm respectively for formulation 4, and 2.09 and 3.41 mm respectively for 5.

Kruskal-Wallis analysis of variance test showed that for the length parameter there were no significant differences between formulations 4 and 5 and their control ($p > 0.05$). Additionally, for the average weight of the organisms, application of Fisher's exact test revealed no significant differences between the test formulations and their corresponding controls ($p > 0.05$ in both cases).

The reproduction parameter also showed considerable similarity between the treatments using the different formulations. The average numbers of offspring produced per couple at the end of two weeks (using five replicates for each formulation) are listed in Table 4. The smallest number was three offsprings (formulation 4B), and the greatest number was 11 offsprings (formulations 4C, 5A, and 5D). These results are similar to those of Fracácio *et al*. (2011), who found about 7 to 11 offsprings per couple aging between 30 to 42 days. Kruskal-Wallis analysis of variance test resulted in $p > 0.05$ for formulations 4 and 5 and between both formulations, therefore they suggest that there were no significant differences between the varied treatments in terms of numbers of produced offspring.

Table 3 – Physiological parameters of the organisms after 10 days of contact with sediment formulations.

Formulation	Average weight (µg)	Average length (mm)
Control 4	0.123	2.300 ± 0.081
4A	0.132	2.307 ± 0.061
4B	0.136	2.276 ± 0.104
4C	0.141	2.386 ± 0.146
4D	0.128	2.307 ± 0.147
Control 5	0.138	2.280 ± 0.093
5A	0.155	2.300 ± 0.084
5B	0.143	2.438 ± 0.245
5C	0.139	2.251 ± 0.118
5D	0.132	2.266 ± 0.086

Table 4 – Total numbers of offsprings produced per couple after 14 days of contact with sediment formulations.

Formulation	Control 4	4A	4B	4C	4D	Control 5	5A	5B	5C	5D
Average offspring per couple	8.0 ± 1.0	8.0 ± 1.0	7.6 ± 2.8	7.2 ± 2.3	7.0 ± 1.2	8.0 ± 1.0	8.6 ± 1.5	7.6 ± 1.7	7.6 ± 1.1	8.4 ± 1.5

Toxicological analyses therefore showed that both formulations 4 and 5, containing 1.0 or 3.0% of organic matter (derived from *Elodea sp.* or TetraMin® fish food), could be used in toxicological tests, because no significant differences were observed for the growth and reproduction parameters. Despite showing unfavorable behavior in terms of pH, formulations 4B and 4D (containing 3.0% organic matter) were nonetheless suitable for use in experiments with *H. azteca*.

CONCLUSIONS

With regard to the water quality and period of time taken for the system to stabilize, formulation 4 (40.0% coarse sand, 40.0% medium sand, 10.0% fine sand, and 10.0% kaolin clay) with 1.0% organic matter derived from TetraMin® fish food or *Elodea sp.*, as well as all variations of formulation 5 (10.0% coarse sand, 10.0% medium sand, 70.0% fine sand, and 10.0% kaolin clay), showed satisfactory characteristics with rapid stabilization and pH values close to neutral after day 7 (in most cases). In support of the chemical analyses, the toxicological tests revealed that both formulations 4 and 5 could be used in experiments with *Hyalella azteca*, since no significant differences were observed between the treatments in terms of growth and reproduction parameters.

It is recommended that tests with sediments employing *Hyalella azteca* should be modified to aid detection of the organism, especially due to the difficulty in identifying offspring (because their coloration is very similar to that of sand). The use of fine mesh screens might enable easier handling of the sediment in order to locate the organisms at the end of experiments.

ACKNOWLEDGMENTS

The authors are grateful to *Universidade Estadual Paulista "Júlio de Mesquita Filho"* (UNESP) and *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP – Process 2012/14583-5) for their financial support. Bioagri Company kindly provided the test organisms.

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