2. Environmental Consequences

Artemias and microplastics:What are the effects?

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Topic: Natural Sciences and Biology
Description: This scenario is suitable for students aged between 12-18 years. The students have to reflect about the harmfulness of microplastics to organisms
Aims: Evaluate the potential of microplastics as contaminants or transmission vehicles of environmental contamination through a test of toxicity with artemia.
Outcome: Raise awareness to the problem of marine litter and, in particular, of microplastics pollution, and the harmful effects of this type of pollution.

Title	Procedure	Time
Presentation	The topic is introduced to the students 15 min	15 min
Workgroup	In groups students carried out the lab activities. Each student group should have their own sample	60 min
Discussion	After the activities the student fill in the experimental log, registering data obtained and answering the questions	15 min
Presentation	Students present their work to classmates and discuss the differences among samples	30 min

Activities :

Experimental protocol

Artemia and microplastics: what are the effects?

State of the art

Oceans constitute the largest reservoirs of water (97.2%) of the planet Earth, being a vital source of biological, natural and economic resources. However, Man has neglected the importance of this environmental compartment, polluting the oceans through the various anthropogenic activities, both terrestrial and maritime, that it develops. Plastic waste accounts for 60-95% of marine litter and has become a worldwide problem. It is now considered one of the main pollutants responsible for marine pollution. It is estimated that about 8 million of the 300 million tons of plastic produced per year end up in the ocean. About 70% sinks and accumulates on the ocean floor and it is estimated that ca. 270 thousand tons will float. Despite the high durability, the plastic is fractionated into smaller and smaller particles. Small plastic waste (<5mm) is denominated by microplastics. The microplastics may also come from the use and discharge of cleansing creams, body scrubs, tooth whitening agents and from several clothes. As a consequence, microplastics have been accumulating in the oceans and sediments in the last decades.

Due to their small size, microplastics can be ingested by organisms of the various trophic levels and can be bioaccumulated until they affect the major predators. Since microplastics have the capacity to absorb contaminants, such as Persistent Organic Pollutants (POPs), when ingested by marine species, they are also a transported of these POPs into the marine food chain.

The artemia is a small crustacean of 10 to 15 mm in length, quite sensitive to the quality of the water in which it lives and to chemical substances in general. For this reason it is widely used in toxicity assessments.

Aims

The specific objectives of this activity are: to carry out a test of toxicity with artemia in order to evaluate the potential of microplastics as contaminants or transmission vehicles of environmental contamination. This activity will make possible to raise awareness of the problem of marine litter and, in particular, of microplastics pollution and the harmful effects of this type of pollution. This protocol is part of the Curricular Areas of Natural Sciences of the 3rd Cycle of Basic Education and Biology (12th year) of Secondary Education. It is inserted in Essential Principle 6 "The Ocean and

humanity are strongly interconnected" on the scientific culture of the Ocean fomented by the project Knowing the Ocean[8].

Material

- Artemia larvae with about 2 weeks of age (can be obtained in advance through the hatch of cysts).
- Microplastics collected on the beach
- Microplastics obtained from clean plastic, by cutting into small pieces (<5mm)
- liquid soap for hand dishwashing
- Marine salt
- Petri dishes or test tubes (7 per work group)
- A plastic bottle of 1.5L
- Pasteur pipets
- Graduated pipettes of 2, 5 and 10 mL
- Pen
- Dechlorinated tap water (water left in contact with the air for at least 24 hours so that chlorine evaporates)
- Beakers of 100 mL

Procedure

A. <u>Preparation of test solutions</u>

1. Collect about 10g of microplastics on the beach.
2. From several clean plastic residues, from our daily use, cut small pieces (<5mm) in order to produce 20g of microplastics.
3. Using the 1.5 L bottle, prepare seawater for the test by dissolving 25 g of sea salt in 1 L of dechlorinated tap water.
4. Prepare 3 solutions with microplastics:
    a. <u>Solution 1:</u> mix 10g of microplastics collected on the beach with 100 mL of saline solution.
    b. <u>Solution 2:</u> mix 10g of clean microplastics with 100 mL of saline solution.

[8] http://www.cienciaviva.pt/oceano/home/

c. Solution 3: mix 10g of clean microplastics with saline solution containing liquid soap (95 mL of saline solution + 5 mL of liquid soap).

5. Keep the 3 solution in rest for one week.
6. After 1 week, decant all the liquid from solution 3, leaving in the container only the microplastics.
7. Refill the vessel containing the microplastics from solution 3 with 100 ml of saline solution.
8. Leave the new solution (solution 3.1), together with the other two solution, another week at rest.
9. Prepare the positive control solution. To do this, mix 1.5 ml of the liquid soap into 50 ml of the prepared saline solution. The solution should be gently agitated with a Pasteur pipette to obtain a homogeneous mixture without producing foam.

B. Toxicity trial

1. Divide the class into 4 groups, each group testing a different solution (solution 1, 2, 3.1
and positive control solution)

2. Each group should receive 7 petri dishes or test tubes and mark each one with the following conditions to be tested: 0% (negative control), 10%, 17%, 26%, 40%, 64% and 100%.

3. Fill each plate or tube with the solution to be tested by pipetting with the graduated pipettes the volumes indicated in table 1.

Table 1. Volumes of saline solution and of solution with contaminant (solution 1, 2, 3.1 and positive control solution) to be pipetted for each treatment.

Concentration (%)	Solution with liquid soap (mL)	Solution with the contaminant (mL)
0	0.0	10.0
10	1.0	9.0
17	1.7	8.3
26	2.6	7.4
40	4.0	6.0
64	6.4	3.6
100	10.0	0.0

4. Place 10 larvae of artemia in each of the plates, using Pasteur pipettes. During this step, it should be attempted to carry the smallest possible amount of water from the larval culture to the test plates. Placement of the last larva at the highest concentration marks the start of the test (zero hours). Students should make sure that all larvae placed on the plaques are alive. In order for this type of assay to be considered valid, the mortality observed on all negative control plates should not exceed 10%.
5. Evaluate mortality 30 and 45 minutes after the start of the trial, counting how many larvae are dead in each treatment tested and recording these results. In case test tubes are use of, will be easier to count the number of live larvae, obtaining the total of dead larvae by subtracting the number of live ones to the total of exposed larvae.
6. From the results, draw the toxicity curve (Figure 1), determining the concentration at which the 50% mortality is expected to occur in the experimental conditions (CL50).

Results analysis

One of the most commonly used parameters to assess the toxic potential of chemicals for aquatic organisms is the median lethal concentration (LC50), i.e. the concentration of the substance being tested which causes the death of 50% of the test organisms under the experimental conditions chosen. This value is determined on the basis of the toxicity test results, achieved from the toxicity curve or concentration-response curve (Figure 1). This curve relates the concentration of the chemical substance tested to the percentage of animals showing the measured effect.

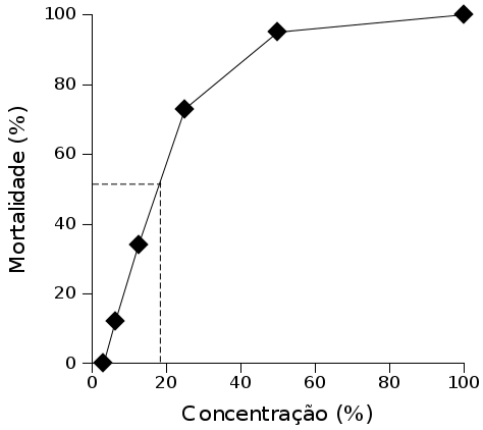

Figure 1. Toxicity curve of a laundry detergent to artemia. Determination of the value of CL50

Experimental Log

Artemia and microplastics: what are the effects?

1. Formulate and indicates the hypotheses to be tested with this experiment.

2. Indicate in the following tables the results obtained in the tests that you performed and calculates the respective percentages of mortality.

Table 1: Results obtained with solution 1 (microplastics collected on the beach in saline solution).

Concentration (%)	Total number of exposed larvae per concentration	Larvae killed by concentration / Percentage of mortality		Larvae killed by concentration / Percentage of mortality	
		30min		45min	
		N°	%	N°	%
0					
10					
17					
26					
40					
64					
100					

Table 2: Results obtained with solution 2 (clean microplastics in saline solution).

Concentration (%)	Total number of exposed larvae per concentration	Larvae killed by concentration / Percentage of mortality		Larvae killed by concentration / Percentage of mortality	
		30min		45min	
		N°	%	N°	%
0					
10					
17					
26					
40					
64					
100					

Table 3: Results obtained with solution 3.1 (clean microplastics in saline solution with liquid soap and then transferred to new saline solution).

Concentration (%)	Total number of exposed larvae per concentration	Larvae killed by concentration / Percentage of mortality		Larvae killed by concentration / Percentage of mortality	
		30min		45min	
		N°	%	N°	%
0					
10					
17					
26					
40					
64					
100					

Table 4: Results obtained with positive control solution.

Concentration (%)	Total number of exposed larvae per concentration	Larvae killed by concentration / Percentage of mortality		Larvae killed by concentration / Percentage of mortality	
		30min		45min	
		N°	%	N°	%
0					
10					
17					
26					
40					
64					
100					

3. What is the percentage of mortality recorded in the four negative controls? What can a control plate mortality of more than 10% mean? Why can this invalidate the test?

Represent the toxicity curves for the four solutions tested, write the caption for X and Y axes and for the figures you have made. Calculates the LC50 value of the four solutions.

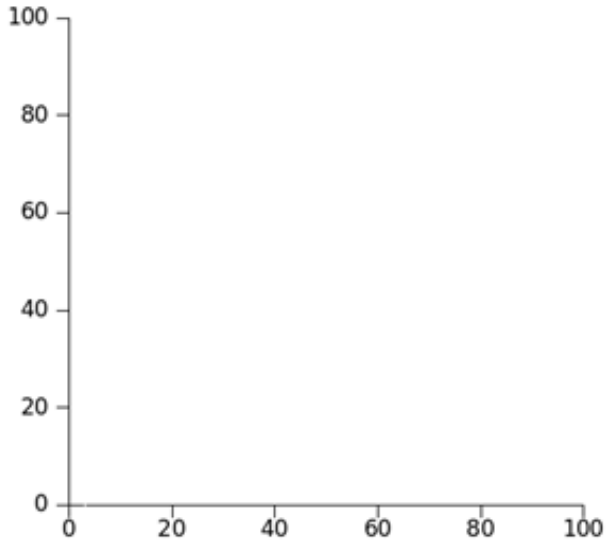

Figure 1.

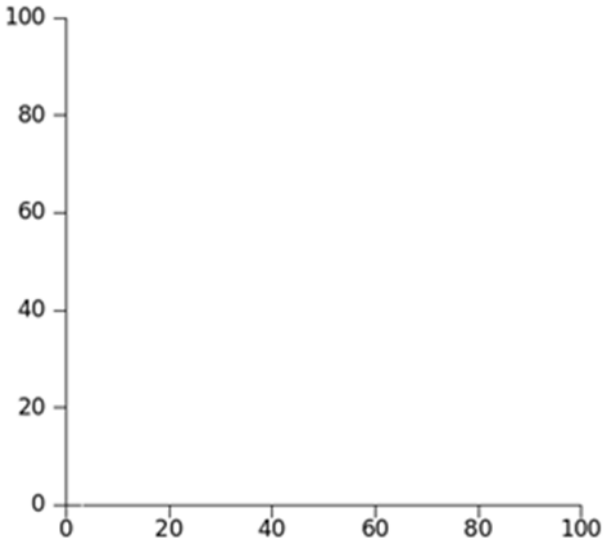

Figure 2.

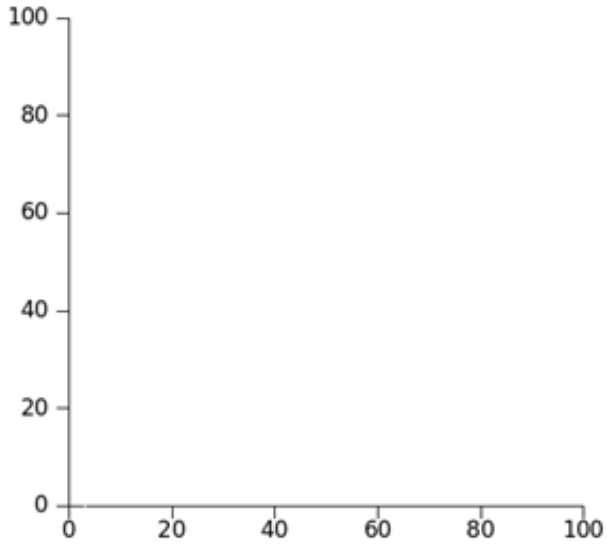

Figure 3.

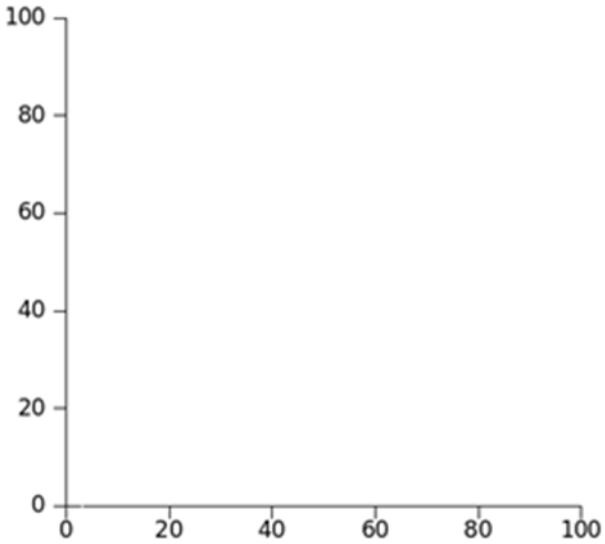

Figure 4.

Which conclusion you take from the experience you have made?

4. Based on your findings, indicate the main harmful effects of microplastics on marine life, taking into account the adsorption capacity of contaminants that microplastics have.

5. Since microplastics can be an entry point for pollutants into food chains, which can affect the entire marine food chain until the top predators, do you think microplastics could affect humans in this context? Justify your answer.