Lethal concentration (LC_{50}) (120h) of neutral household detergent Limpol in guppy *Poecilia reticulata*.

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Abstract

Aquatic environments have been destroyed because of increase of pollutants dumped into waters. In some poor countries or in developing ones, like Brazil, detergents are one of the main responsible to impact these environments. Guppy (*Poecilia reticulata*) is a common fish in Central and South America, being very used in vitro experiments, since it is an easy specimen to keep in laboratories. This work aimed to determine the LC_{50} (120h) of neutral household detergent for guppy. We tested seven different concentrations (0, 10, 20, 30, 40, 70 and 100 mg/L), and Probit analysis showed that approximately 33.4 mg/L was the lethal dose that killed 50% of guppies in 120h, with doses below 30 mg/L did not killing any fish, while doses above 30 mg/L killed all individuals in few hours. We concluded that even small doses of detergent can be lethal to aquatic organisms, especially if the exposition time is prolonged.

Keywords: Aquatic pollution, Detergent, $LC_{50,}$ Guppy

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Introduction

Water is the main resource for survival of most part of organisms (including human beings) (Spirita *et al.*, 2015). Water pollution has intensified in recent decades, causing damage often irreversible to aquatic environments (Tiburtius *et al.*, 2004, Tavares, 2014). This type of pollution can be caused by various agents, such as heavy metals (Fiori *et al.*, 2013), aromatic hydrocarbons (Heleno *et al.*, 2010), pesticides (Nogueira *et al.*, 2012) and detergents (Barbieri, 2008).

The use of synthetic detergents has caused several impacts to the environment (ex: accumulation in organisms tissues, foam in water bodies, reduced oxygen for aquatic animals) and also to public health (ex: it can cause asthma in children, skin irritation, liver damages, etc.) (Barbieri *et al.*, 2000, Morgado *et al.*, 2000, McWilliams and Payne, 2002, Chandanshive, 2013, Yuan *et al.*, 2014). They are rich in polyphosphates, which when emitted into lake ecosystems by domestic sewage, cause artificial eutrophication (Esteves, 2011). Between the other organisms, organic synthetics can be taken in a food chain, thus contaminating many aquatic species (vertebrates and invertebrates) and also human being, and also can alter the pH and salinity, affecting oxygen consumption by aquatic organisms (as fishes) (Ezemonye *et al.*, 2009, Chandanshive, 2014).

Although some developed countries have an efficient sewage treatment (Scott and Jones, 2000), in some poor countries or in developing like Brazil (Penteado *et al.*, 2006), Nigeria (Ogundiran *et al.*, 2010, Osuala *et al.*, 2017), Turkey (Minareci *et al.*, 2009) and India (Mathew *et al.*, 2013), the sewage treatment is not efficient and cause several damages to aquatic environments. In Brazil, the basic sanitation is poorer in countryside and peripheral regions, where most part of population is poor; and the problems are aggravated with the misuse of financial resources and bad management (Junior and Paganini, 2009).

Currently the main surfactant present in the detergent formula is LAS (linear alkylbenzene sulfonate), which replaces ABS (alkylbenzene sulfonate) by being biodegradable and staying less time in nature (Barbieri, 2005, Penteado *et al.*, 2006). Detergents that contain LAS is widely used around the world, and once a time thrown in aquatic environments, it causes several damages to many organisms that compound these ecosystems (Hansen *et al.*, 1997).

Detergents can cause physiological (like the growth reduction in blue mussels) (Hansen *et al.*, 1997) and behaviours changes (like becoming *Macrobrachium olfersii* shrimps more aggressive and affecting the swimming behaviour in zebra fish, red carp and Japanese medakas) (Martins, 2007, Zhang *et al.*, 2015). One of the ways to measure the effects of pollutants on exposed organisms is through the lethal concentration test (LC_{50}). This test is used to measure the lethal dose that kills 50% of organisms exposed to a certain pollutant (Chandanshive, 2013).

The guppy *Poecilia reticulata* (Peters, 1859) is a species native to northern South America and Central America, being one of the most widespread ornamental fish species in the world (Magurran and Seghers, 1994, Alves *et al.*, 2000, Andrade *et al.*, 2005). Guppy is a widely used for laboratory model, since it is easy to handle, has low cost and does not require large spaces for maintenance and reproduction (Maya and Marañón, 1998). Fish are widely used in toxicological experiments because they are important in the ecosystems in which they live, and also as an important resource in human nutrition (Barbieri *et al.*, 2000), and this work aimed to determine the LC_{50} (120h) of neutral household detergent for guppy.

Materials and methods

Ethical Note

This work was approved by the Animal Ethics Committee of the Federal University of Ouro Preto (protocol number 2016/18).

Detergent utilized

We utilized neutral household detergent of brand Limpol. The main tensoative presented on its composition is sodium dodecylbenzenesulfonate ($C_{18}H_{29}NaO_3S$).

LC_{50} (120h) of the neutral household detergent test

Forty two adult guppies (21 ♀ and 21 ♂) with lengths between 3 and 4 cm were used during the LC_{50} (120h) test. These guppies were transported in plastic sachets with oxygen to the site of the experiment; the sachets were placed in the water used for the acclimation experiment for 5 minutes. The pH value was between 7 and 8 during the experiment. A control group (without detergent) was used and, with the aid of a syringe, six dilutions of the neutral household detergent (10, 20, 30, 40, 70 and 100 mg/L) were added in plastic containers aerated with Super Air-Pump Kare's air compressors model Kar-3 (Beijing, China); guppies were fed once a day, with 0.5 gram of Nutriflakes flaked ration (Araçoiaba da Serra Municipality, São Paulo, Brazil). Each container had 2 litters of water and housed a couple of guppies, and three replicates were made for each concentration (Dogan *et al.*, 2012). The animals were not fed on the eve of the experiment, and were observed for a period of 120 hours, with the dead being immediately removed (Roy, 1988, Dogan *et al.*, 2012).

Statistical analysis

To determine the LC_{50} (120h), we used the Probit statistical model and the Chi-Square test with 5% of significance.

Results

No death was recorded in control group and at concentrations of 10 and 20 mg/L in the experimental group, but half guppies died at concentration of 30 mg/L after 120h ($\chi2$ = 40.15, DF = 1, p<0.001). The 40, 70 and 100 mg/L concentrations were shown to be too high, with all animals dying at concentrations of 70 and 100 mg/L, and four deaths at the concentration of 40 mg/L approximately 2 hours after the detergent insertion. The Probit analysis showed that approximately 33.4 mg/L is the LC_{50} (120h) of neutral household detergent for guppy (Table 1 and Fig. 1).

Table 1: Detergent concentrations and guppies mortality rate. Value found for the LC_{50} (120h) is in bold font.

Percent	Dose	Lower 95.0% Conf. Limit	Upper 95.0% Conf. Limit
0.1	6.49839	-69.7372	18.2009
0.5	11.0654	-51.6764	21.0146
1.0	13.2803	-42.952	22.414
2.0	15.7004	-33.459	23.9827
3.0	17.2359	-27.4658	25.0078
4.0	18.391	-22.9778	25.7993
5.0	19.3306	-19.3434	26.4594
6.0	20.1303	-16.2638	27.0351
7.0	20.8315	-13.576	27.5524
8.0	21.4593	-11.181	28.0271
9.0	22.0303	-9.01376	28.4697
10.0	22.5559	-7.02936	28.8877
15.0	24.7321	1.04403	30.7609
20.0	26.4616	7.21548	32.4947
25.0	27.9454	12.2269	34.2652
30.0	29.2779	16.391	36.1916
35.0	30.5127	19.859	38.3673
40.0	31.6843	22.7256	40.856
45.0	32.8179	25.0807	43.6823
50.0	33.9335	27.0248	46.8371
55.0	35.049	28.6604	50.3004
60.0	36.1826	30.0791	54.063
65.0	37.3543	31.3557	58.1418
70.0	38.589	32.5511	62.5901
75.0	39.9215	33.719	67.5126
80.0	41.4053	34.915	73.0986
85.0	43.1348	36.2131	79.7058
90.0	45.311	37.748	88.1174
91.0	45.8366	38.1067	90.1611
92.0	46.4076	38.4921	92.3856
93.0	47.0354	38.9113	94.8362
94.0	47.7366	39.3743	97.5782
95.0	48.5364	39.8964	100.711
96.0	49.4759	40.5029	104.399
97.0	50.631	41.2397	108.942
98.0	52.1665	42.2064	114.994
99.0	54.5866	43.7069	124.555
99.5	56.8015	45.061	133.325
99.9	61.3685	47.8127	151.447

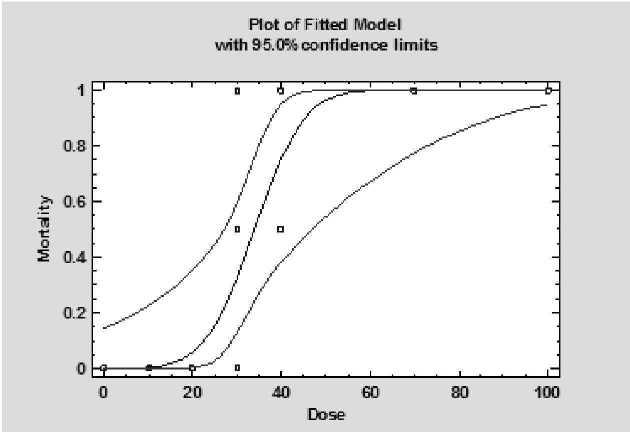

Figure 1: Lethal concentration of neutral household detergent for guppy, found in the LC_{50} (120h) test.

Discussion

The LC_{50} (120h) of neutral household detergent found for guppy was 33.4 mg/L. Lower doses such as 10 and 20 mg/L did not kill any of the guppies at the end of the 120 hours of exposure, 30 mg/L killed half of guppies while doses above 40 mg/L were extremely lethal in just a few hours.

Concentrations less than 30 mg/L did not kill any fish during the LC_{50} (120h), but a study tested two different types of detergent (Surf excel and Nirma) in individuals of *Mystus montanus*, and the LC_{50} values founded by him were 20 mg/L (Surf excel) and 23.5 mg/L (Nirma) during 96h of exhibition (Chandanshive, 2013). The values found can suggest that these detergents are more toxic than the neutral household detergent used in this work, because the species tested were larger (length between 12.3 and 14.5 cm) than the guppies used in our experiment (length between 3 and 4 cm) and 50% of the fishes died before 120h.

In an experiment with individuals of *Clarias gariepinus* exposed to commercial detergent effluent with LAS (the main compound presented in detergents currently) during 56 days (Ogundiran *et al.*, 2010), authors founded that LC_{50} for 1344h was 0.0166 mg/L of detergent effluent. The long-time exposed to the pollutant can suggest why a low quantity of detergent was lethal to 50% of those individuals, and surely it will be less than 33.4 mg/L for guppies if we had prolonged the time of exposition to detergent.

In an experiment analyzing the effects of alkylbenzene sulphonate (one of the compounds of detergents) in individuals of Zebra fish (*Danio rerio*), researchers observed that LC_{50} (12h) value (the shortest observation time) was 36.427 mg/L, while LC_{50} (96h) value (the longest observation time) was 27.310 mg/L[1] (Spirita *et al.*, 2015). One more time, we could suggest that the detergent used in the present study was less toxic than the mentioned earlier, since none of our guppies died in quantities less than 30 mg/L (during 120h).

Almost 50% of guppies (46.66%) died after 24h exposed of 0.00000004 mg/L of an herbal detergent present in a shampoo (Najan and Bhowate, 2010). This result suggests that also detergents presented in shampoo can be much more toxic than the neutral household detergent used, since the LC_{50} (24h) value was too short. LC_{50} values for guppies after 96h exposed of two different laundry detergents were 0.773 mg/L (in a middle contained detergent of brand Persil) and 28.841 mg/L (in a middle contained detergent of brand Klin) (Osuala *et al.*, 2017). The found values showed that both of detergents are very toxic, killing 50% in 96h, comparing with our highest value that killed 50%: 33.4 mg/L in 120h.

Our results showed that even a simpler and biodegradable detergent frequently used by populations around the globe can have dramatic effects on fish's life, being lethal in low concentrations. The prolonged effects of detergent in the fish's physiology and behaviour still have to be investigated.

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