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Short Communication

Assessment of median lethal concentration (CL50) of pollutants on *Macrobrachium tenellum* juveniles

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ABSTRACT. Due to the presence of pollutants in aquatic systems, this research aims to analyze toxicity levels of Faena Clásico®, Asuntol®, sodium hypochlorite and diesel on *Macrobrachium tenellum* juveniles. For the purpose of this study, specimens from 2 to 4 cm in length were used. For glyphosate, hypochlorite and coumaphos bio-assays, a total of 8 individuals were placed per experimental unit, however, only 5 individuals were placed for diesel. Dead individuals were counted at the end of 24 h of exposure. All bioassays were performed in quintuplicate plus a negative control. The mean lethal concentration (LC50) was calculated by a *Probit* analysis and standard error. The mean lethal concentrations obtained are given at 6 h of exposure for 16 mL L^{-1} of diesel aqueous extract; and 24 h of exposure, solutions of Asuntol® 6 mg L^{-1}, Classic Faena® 32 mg L^{-1} and sodium hypochlorite 59 mg L^{-1}. Results show that the different chemicals assessed generate harmful damage to *M. tenellum*, since agrochemical compounds can cause alterations in the composition of ecosystems, reducing biodiversity in a temporarily or permanently manner.

Keywords: pesticides, organophosphates compounds, coumaphos, glyphosate, sodium hypochlorite, prawn.

Aquaculture production has become a growing activity on a global scale, which includes the culture of animal and vegetable species that fulfill all or part of their life cycle in the water. *Macrobrachium tenellum* (Smith, 1871) has a high potential for fishing, aquaculture and as experimental organism, due to its morphological and chromatic plasticity in diverse environments (Guzmán, 1987). The species is distributed on the Pacific coast shore, from the Baja California Peninsula to the Chira River in northern Peru. They can be observed in fresh and brackish water. Despite being a commercial species, there is little research on the effects that pollutants could have on the population of these prawns (Espinoza-Chaurand *et al.*, 2011). However, nowadays there is dependence on the use of pesticides and ectoparasiticides in agribusiness in order to improve production (Benítez-Díaz & Miranda-Contreras, 2013). The toxicity of these compounds depends on their chemical structure, concentration, mode of entry into the organism and the organism metabolism, among others (Jaramillo *et al.*, 2013). These chemical compounds are dispersed in ground and surface water by means of trawling and leaching, so they can reach water bodies (*e.g.*, rivers, lakes, wetlands) distant from the area where pesticides were applied (Benítez-Díaz & Miranda-Contreras, 2013).

For this research, four compounds of easy access and widespread use were chosen to be analyzed based on their toxic potential: coumaphos, glyphosate, sodium hypochlorite and diesel fuel. Coumaphos is an organophosphate that acts by inhibiting enzymes with ester activity, specifically the enzyme acetyl-cholinesterase (ACE) in nerve endings, an enzyme essential for the regular control of nerve impulse transmission and affects nerve fibers, muscles and glands (Fernández *et al.*, 2010). Glyphosate is a broad-spectrum herbicide that acts by inhibiting biosynthesis of aromatic aminoacids in plants (tryptophan, phenylalanine and tyrosine) by inhibiting the enzyme 5-enolpiruvilshikimato-3-phosphates synthetize (EPSPS), which reduces the production of protein and its development, reduces biosynthesis of other compounds such as tetrahydrofolate, ubiquinone and vitamin K (Salazar-López & Aldana-Madrid, 2011). The types of alterations caused depend on the species or organism, type of compound, concentration of glyphosate in water and time of exposure (Salazar-López & Aldana-Madrid, 2011). In Mexico, Ruiz-Toledo *et al.* (2014) found concentrations of glyphosate as high as 36.51 μg L^{-1} in the Cahoacan River, State of Chiapas, which is associated with fields of transgenic soybean crops resistant to glyphosate. Sodium hypochlorite has a wide use as a disinfectant and bleach at a domestic and industrial level; hydrocarbons such as diesel are formed as a mixture of compounds which differ in solubility (Anderson *et al.*, 1974). Its toxicity is associated to its aqueous phase, which corresponds to aromatic compounds (Bellas *et al.*, 2008). The bio-availability of these compounds to the organisms present both in the water column and in sediments shall depend on the type of hydrocarbon and its physical, chemical and biological degradation capacity (Vijayavel & Balasubramanian, 2006).

The aim of this research is to provide knowledge on the impact generated by the presence of coumaphos and glyphosate pesticides, sodium hypochlorite disinfectant and diesel fuel in rivers that could potentially affect the populations of prawn *M. tenellum*. This was done through a series of bioassays to obtain the mean lethal concentration (LC50) of such compounds in juveniles of *M. tenellum*.

Studies were carried out within the facilities of the Laboratorio de Calidad de Agua y Acuicultura Experimental en Puerto Vallarta, Jalisco, Mexico. Juveniles of *M. tenellum* was used in a size range of 2-4 cm in length, measured from the tip of the rostrum at the rear end of the telson. Individuals were collected from the laboratory stock. The compounds used were: Asuntol Bayer® powder presentation containing 50% of coumaphos; Faena Clásico® that according to the manufacturer contains no less than 35.6% glyphosate potassium salt; Cloralex® with 5% sodium hypochlorite. Diesel hydrocarbon was also evaluated.

In order to establish the concentrations to be evaluated, the following considerations were made: In the case of glyphosate, from the recommended concentration of use 108.9 mg m^{-2} and assuming 1 mm of rainfall, the expected concentration in a runoff would be 108.9 mg L^{-1} and a higher concentration was taken from 180 mg L^{-1} dilutions were made successive to 1/2 and the concentrations tested were 180, 90, 45, 22.5 and 11.2 mg L^{-1}. For sodium hypochlorite, which despite having recommended doses is commonly used indiscriminately, successive dilutions were made to 1/10 of the commercial solution, then the concentrations evaluated were 5500, 550, 55 and 5.5 mg L^{-1}. The coumaphos have a recommended usage concentration of 500 mg L^{-1}, dilutions were made at 1/10 and the concentrations evaluated were 50, 5, 0.5 and 0.05 mg L^{-1}. In the case of diesel, the volume that formed the thinnest but most uniform layer on water in experimental units was used as a minimum addition, and from this volume it was doubled in each addition, hence the proportions used for diesel/water were 7.5, 15, 22.5, 30 and 37.5 mL L^{-1}, these mixtures were emulsified by shaking them for 15 min and allowing them to stand for 24 h. Subsequently, the aqueous fraction was collected with a siphon to be used in the bioassays. The oily fraction was removed because it could show false positives due to the possibility of generating anoxia in the aqueous phase by preventing the exchange of oxygen with the atmosphere.

For the glyphosate, hypochlorite and coumaphos bioassays, 8 individuals were placed per experimental unit, which consisted of PET containers with a capacity of 2 L, which were covered with a mesh to prevent escape. The record of dead individuals was registered every 6 h for 24 h of exposure. They were considered dead when no response to stimuli, or movement of their appendix and in most cases, individuals showed an opaque white color. In the case of diesel, 5 individuals were placed per experimental unit, with the aforementioned characteristics. The data record was registered every 2 h, for 24 h of exposure. All bioassays were performed in quintuplicate plus a control without treatment. In addition, they were carried out at room temperature of 28°C and water conditions prior the addition of compounds was pH 7.5 and dissolved oxygen 5 mg L^{-1}. The mean lethal concentration (LC50) was calculated in the IBM® SSPS® Statics Software (IBM Corp, 2012), as described by Finney (1952). In order to determine the standard error, Randhawa (2009) instructions were followed.

Table 1, shows the LC50 values of the different compounds acting on *M. tenellum* juveniles, as well as standard errors and exposure time. It is also observed that the aqueous fraction of the diesel emulsion, showed a LC50 = 16 mL L^{-1}. As for sodium hypochlorite, glypho-

Table 1. Average lethal concentration (CL50) of different compounds on *Macrobrachium tenellum* juveniles.

Compound	Time (h)	CL50 ± SE (mg L^{-1})
Coumaphos	24	6 ± 3
Glyphosate	24	32 ± 6
Sodium hypochlorite	24	59 ± 31
Diesel	6	16 ± 2

sate and coumaphos, the first one showed the lowest toxicity when presenting the highest LC50, followed by glyphosate. In contrast, coumaphos showed the highest toxicity when a LC50 of 6 mg L^{-1}.

Results show that the different chemicals products evaluated generate harmful damage to *M. tenellum* in various concentrations. The compound that caused the greatest toxic effect on *M. tenellum* prawn juveniles was coumaphos, with an LC50 = 6 mg L^{-1}, this mean that among the evaluated compounds was the one that needs less quantity to cause the mortality of half of the population studied. Glyphosate showed less toxicity to *M. tenellum*, finding an LC50 of 32 mg L^{-1}, followed by sodium hypochlorite, with an LC50 of 59 mg L^{-1}. In the case of glyphosate, this herbicide is labeled as a non-harmful product for crustaceans, however it was found that continuous exposure to this chemical can be harmful to this particular organism. These results validate the theory presented by Nimmo (1985), who states that agrochemical compounds can cause alterations in the composition of ecosystems by reducing biodiversity temporarily or permanently. Diesel presented a response at 6 h of exposure with an LC50 of 16 mL L^{-1}, to cause 50% mortality in *M. tenellum*. These results are highly alarming because hydrocarbons are the most found pollutants on river beds. Likewise, other research studies mention that hydrocarbons spillage such as diesel and crude oil in currents and water bodies causes an increase in mortality and abnormalities in offspring and juveniles of different freshwater species (Harmon & Wiley, 2010).

The first impact of oil spills and their by-products in surface water occurs with phytoplankton, forming an impermeable layer that obstructs sunlight transmission and inhibits the photosynthetic process, decreasing oxygen, the amount of solids contribution and organic/inorganic substances (Vera *et al.*, 2009). Delays in larval development and growth and molt inhibition have also been reported for larvae of numerous species of crustaceans (Weiss, 1992; Amin & Comoglio, 2002), associating these affectations to the interference of this type of compounds (diesel) on the normal metabolic pathways of fatty acids and to energy disruptions in general (Capuzzo & Lancaster, 1981). In addition, Vera *et al.* (2009) reported inhibition effects on the population growth of the *Chaetoceros gracilis* diatom, affecting the intrinsic growth rate and division rate per day, when exposed to solutions with kerosene.

It is widely known that the use of chemical compounds has increased as a consequence of agriculture development (Ecobichon, 2001) and the global industrial boom resulting in accidental dumping of waste during handling, transport or treatment processes or directly as residual effluents (Ramírez, 1989). The indiscriminate release of these compounds in land environments and indirectly into aquatic systems can cause chronic changes in the behavior and fundamental physiological functions of aquatic organisms such as growth, reproduction and even affect survival (Newman & Unger, 2003). However, research works addressing the issue of toxicity on freshwater crustaceans is limited and even more difficult to find when related to studies on *M. tenellum*, where the information is virtually non-existent. Therefore, this research work is a great contribution towards the knowledge on the effect of the main chemical compounds found in aquatic ecosystems.

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