# ACUTE AND CHRONIC TOXICITY OF WASTE TIRE ASH IN *DAPHNIA MAGNA*

LINARES CAMPOS, A.[1] – MORALES LARA, L.[2] – CEDILLO RAMÍREZ, M. L.[1] – LINARES GONZÁLEZ, Y. A.[1] – RIVERA RODRÍGUEZ, L. B.[3] – PEÑA MORENO, R. D.[4] – FLORES, SOTELO, M. T.[5] – LINARES FLEITES, G.[6] – PANIAGUA SOLAR, L. A.[7] – HERNÁNDEZ ZEPEDA, J. S.[5*]

[1]*Posgrado en Ciencias Ambientales, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Edificio IC 6, Ciudad Universitaria, C. P. 72570 Puebla, México*

[2]*Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Av. 4 Sur 104, C. P. 72000 Puebla, México*

[3]*Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Paseo Claussen S/N, Centro, 82000 Sinaloa, México*

[4]*Centro de Química, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Av. 4 Sur 104, C. P. 72000 Puebla, México*

[5]*Departamento Universitario para el Desarrollo Sustentable, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Av. 14 Sur 6301, Edificio IC2, Ciudad Universitaria, C. P. 72570 Puebla, México*

[6]*Departamento de Investigación en Ciencias Agrícolas, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Av. 4 Sur 104, C. P. 72000 Puebla, México*

[7]*Facultad de Ciencias de la Electrónica, Benemérita Universidad Autónoma de Puebla, Av. 4 Sur 104, C. P. 72000 Puebla, México*

[*]*Corresponding author*
*e-mail: santos.hernandez@correo.buap.mx*

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**Abstract.** Waste tire is a residue that accumulates over time due to the lack of processes for its recycling or transformation; one of the most recurrent anthropogenic activities, whether accidental or provoked, is open burning, whose contamination by ashes affects the environment and its ecosystems. The discharges from these fires could cause an imbalance in freshwater ecosystems, thus deteriorating the conditions necessary for the survival of aquatic species. This work analyzes waste tire ash's acute and chronic toxicity on the organism *Daphnia magna*. For the acute test, bioassays were carried out with three replicates for each of the eight elutriates, and for the chronic test, four groups of studies were formed with three replicates each. The acute test results showed an $EC_{50}$ calculated for a 48-hour exposure of 5.83 g/L, and chronic test results revealed that the total reproduction of the population decreased over the 19 days in all treatments.
**Keywords:** *ash, waste tire, acute toxicity, chronic toxicity, Daphnia magna*

## Introduction

The rapid growth of populations and economies has increased waste generation (Ortiz et al., 2017). One of them is waste tires, which constitute a serious environmental problem worldwide; about 1,000 million are generated annually due to the massive manufacture of new tires, which is approximately 1,500 million units per year (Lopez et al., 2012; Yadav and Tiwari, 2019).

This difficulty arises because they are a complex mixture of different materials, including rubbers, carbon blacks, steel cord and other minor organic and inorganic components (Laresgoiti et al., 2000; Henkelmann et al., 2001). In addition, during the manufacturing process there is a vulcanization stage, in which the tire is treated with sulfur at high temperatures to harden it, thus preventing bacterial attack and allowing it to remain in the environment permanently (Murray, 2003; Sanchez, 2012). Therefore, tires are not biodegradable.

This waste is deposited in backyards, clandestine dumps, public roads and in public and private collection centers. If they are not handled properly, they constitute a risk to the environment and human health.

They are the ideal shelter for rodents and mosquito breeding sites such as *Aedes aegypti* and *A. ablopictus* that transmit dengue and yellow fever, creating visual pollution problems and usurping nature's vital space (BECC, 2008; United Nations Environment Program, 2011).

The scrap tire is difficult to ignite, but once ignited, it is difficult to control and even more difficult to extinguish. A fire can last for months, thanks to its open structure and the properties of the materials that make it up, which give it the characteristic of retaining heat (Health Protection Agency, 2010).

These fires generated a large number of emissions (chlorinated methanes, dioxins and furans, polychlorinated biphenyls formed by carbon, hydrogen, zinc, and chlorine) (Health Protection Agency, 2012; Downard et al., 2015) as well as oil, ash and leached toxic pollutants that affect the soil, water bodies and the atmosphere (Health Protection Agency, 2010; United Nations Environment Programme, 2011).

For example, dioxins and furans affect essential organs such as the heart, immune system, liver, skin and thyroid gland, even causing reproductive cancer. In the environment, they degrade very slowly and persist for many years, accumulating in organisms and entering the food chain (Health Protection Agency, 2012; PRTR Spain, 2022).

At present, no studies of the ecotoxicological impact of the acute and chronic effects of waste tire ash on a model organism have been reported. There are studies on the ecotoxicological impact of the tire; most have focused on leachates formed with tread grating and elutriates formed with wear particles, have been subjected to different temperatures and have been evaluated under different conditions, with or without the presence of ultraviolet radiation to identify possible short-term effects; the aquatic reference organisms have been of different taxonomic orders, e.g., fish, crustaceans and algae (Nelson et al., 1994; Wik and Dave, 2005, 2006; Gualtieri et al., 2005; Gualtieri et al., 2005; Wik et al., 2009; Marwood et al., 2011; Panko et al., 2012). One of the most widely used model crustaceans in these ecotoxicological studies has been the bioindicator *Daphnia magna*; since it is an essential representative of freshwater communities due to its wide geographical distribution, it is susceptible to a wide range of toxic compounds, which is one of the main characteristics for its international use in toxicity tests (ISO, 2012; OECD, 2012; Linares et al., 2020). In addition, it is relatively easy to cultivate in the laboratory (NMX-AA-087-SCFI-2010 Análisis de Agua - Evaluación de Toxicidad Aguda Con *Daphnia Magna*, Straus (Crustacea - Cladocera) - Método de Prueba, 2010). It also has a vital role in the food chain as a primary consumer in the aquatic ecosystem (Cui et al., 2018).

In 1995, the Pasteur Institute in Lille (France) conducted two studies to determine the toxicity of rubber dust extracted from tire carcasses on organisms such as


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*S. capricornutum algae*, *Daphnia magna* crustaceans and *Brachydanio rerio* fish (United Nations Environment Programme, 2011).

In 2005, Wik and Dave conducted a study investigating whether toxicity testing with *Daphnia magna* could be used as a screening test for environmental labeling of automobile tires. This study, 12 car tires were measured for toxicity to this organism (Wik and Dave, 2005). In 2006, they investigated the toxic effects of 25 different tires (Wik and Dave, 2006); In 2009, they investigated the toxicity of three different tires using a battery of test organisms which were green algae *Pseudokirchneriella subcapitata* (growth inhibition 72 h), crustaceans *Daphnia magna* (immobility 24 and 48 h) and *Ceriodaphnia Dubia* (48 h survival and nine-days reproduction and survival) and zebrafish eggs *Danio rerio* (48 h lethality) (Wik et al., 2009).

In 2011, Marwood et al. conducted a study with tread wear particulate sediment elutriates; the sediments were obtained from a road simulator laboratory of the German Federal Highway Research Institute, three different tires were rolled, and the sediments were combined to form a single compound. Acute toxicity evaluations were performed on three test organisms, fish *Pimephales promelas*, crustacean *Daphnia magna* and the alga *Pseudokirchneriella subcapitata* (Marwood et al., 2011). In 2012 they conducted a chronic toxicity assessment on freshwater aquatic organisms, including *Ceriodaphnia dubia*, *Pimephales promelas*, *Chironomus dilutus* and *Hyalella azteca*, exposed them to sediment elutriate enriched with 10 g/kg of tire and road wear particles (Panko et al., 2012).

### *Target*

The objective of this study was to evaluate the acute and chronic toxicity of waste tire ash on the organism *D. magna* under NMX-AA-087-SCFI-2010.

The second objective was to analyze under Mexican Standards NMX-AA-051-SCFI-2001 and NMX-AA-103-SCFI-2006, what heavy metals and volatile organic compounds the ashes contain.

## Materials and Methods

### *Reference agency*

Initially, the culture of *D. magna* was developed according to NMX-AA-087-SCFI-2010, which indicates the environmental conditions for the maintenance and optimal culture of organisms, which were previously obtained and characterized in the Laboratory of Ecology and Restoration of Aquatic Systems of the Faculty of Biological Sciences of the Benemérita Universidad Autónoma de Puebla. Groups of 20 daphnids were maintained in 1 L of reconstituted hard water (total hardness 250 mg/L $CaCO_3$, pH=7.5), and the medium was renewed three times a week. The cultures were maintained at a temperature of 21 ± 2 °C, with a photoperiod of 16:8 (light: dark) and a Spirulina based feeding and a yeast mixture, TetraVeggieTM at a concentration of 250,000 cells/mL, every third day. Subsequently, 20 of these units were implemented and monitored. As a result, normal development in the population dynamics was obtained during 21-day monitoring of 20 initial organisms, where the number of neonates exceeded the number of adults and juveniles; at the end of this time, there was a population of 342 individuals; this guaranteed the quality of the population for the evaluation of chronic acute toxicity.


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### *Scrap tire ash collection*

Five scrap tires were selected to obtain the ashes from a vacant lot located in the La Pedrera neighborhood, Puebla, Pue; their characteristics are shown in *Table 1*; three fragments of different parts of the tread were taken from each tire and weighed on a gram scale (Pocket model M-HSeries Scale for a maximum weight of 500 g ± 0.01 g.), the weights were between 19.10-20.47 g.

***Table 1.*** *Registration of scrap tires*

| Tire name | Year of manufacture | Rhine | Certified maximum speed |
|---|---|---|---|
| V Steel Bridgestone | 2015 | 16 | Z (240 km/h) |
| Michelin | 2019 | 16 | W (270 km/h) |
| Premiere | 2014 | 14 | H (210 km/h) |
| Multi Hawk Firestone | / | 15 | T (190 km/h) |
| Scorpion ATR Pirelli | 2018 | 15 | H (210 km/h) |

The incineration was carried out in an open-air patio and was performed separately for each fragment; in a 10*10 cm steel plate, they were exposed to a direct flame, this was maintained until the presence of black smoke and a sustainable flame, when the combustion was finished they were combined to form a single compound, At the end of the combustion, they were combined to form a single compound, to which only the metallic pieces that remained from the steel rings were removed and mixed in a mortar to homogenize the ashes, obtaining a particle size of 344 µm and stored in an amber container with a capacity of 500 mL.

### *Elutriate preparation*

Elutriates were performed with eight different concentrations of scrap tire ash in reconstituted hard water (dilutions were selected based on the literature cited (Wik and Dave, 2005, 2006; Wik et al., 2009; Marwood et al., 2011).); they were 1.5 g/L, 5 g/L, 7 g/L, 8 g/L, 11 g/L, 12 g/L, 14 g/L and 16 g/L, were shaken for 10 continuous minutes and rested for 24 hours, after which the supernatant was decanted to obtain the liquid phase. The acute toxicity evaluation was carried out.

### *Acute toxicity test*

The methodology followed is the one described in (NMX-AA-087-SCFI-2010 Análisis de Agua - Evaluación de Toxicidad Aguda Con *Daphnia Magna*, Straus (Crustacea - Cladocera) - Método de Prueba, 2010).

For the acute toxicity test the characteristics of the organism used were neonates less than 24 hours old, obtained from the 3$^{rd}$ generation of a single young mother with adequate growth and reproduction.

The bioassays were performed in three replicates for each of the eight elutriates, each container contained 30 mL of the corresponding dilution and 10 neonates, two controls (positive control and negative control), at a temperature of 21 ± 2 °C, with a photoperiod of 16:8 (light: dark), the immobility or mortality count for the calculation of the $EC_{50}$ was carried out at 48 hours.

### *Acute toxicity data analysis*

The data obtained from the count of immobile or dead organisms of each dilution after 48 h of exposure to the toxicant were analyzed using the probit method with the statistical package R commander (The R Foundation for Statistical Computing, 2022) in order to obtain the mean effective concentration ($EC_{50}$) with a 95% confidence interval.

### *Acute toxicity results*

In the negative control group, all individuals survived, in the positive control with the reference toxicant potassium dichromate the $EC_{50}$ was 1.1 mg/L which was consistent with the average value of the historical $EC_{50}$ of the laboratory, therefore, our organisms had the necessary sensitivity in the experiment.

All eight waste tire ash elutriates exhibited toxicity on *Daphnia magna* groups *Table 2*.

***Table 2.*** *Relationship between concentration (g/L) and mortality (%)*

| **Concentration (g/L)** | 0 | 1.5 | 5 | 7 | 8 | 11 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| **Mortality at 48 hours (number of organisms)** | 0 | 0 | 5 | 7 | 7 | 6 | 6 | 6 | 7 |

The acute toxicity test resulted in an $EC_{50}$ calculated for a 48-hour exposure of 5.83 g/L, *Figure 1*.

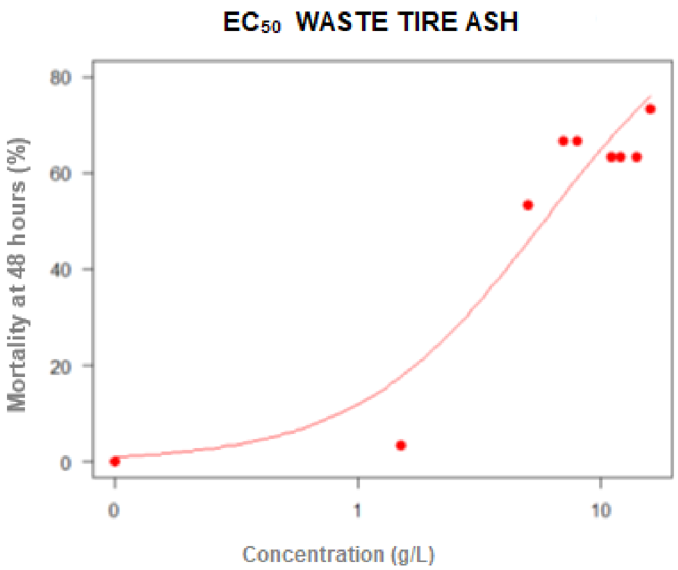


***Figure 1.*** *The average effective concentration of scrap tire ash*

### *Preparation of elutriates for chronic toxicity testing*

For the chronic toxicity test, three sublethal concentrations (1.5 g/L, 3.5 g/L, 4.5 g/L) were selected, and the corresponding elutriates were made, shaken for 10 continuous minutes and rested for 24 hours.


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### *Chronic toxicity test*

Four study groups were formed with three replicates each, 1) Control group in culture medium only, 2) Group with culture medium in the presence of sublethal ash concentration of 1.5 g/L (A), 3) Group with culture medium in the presence of sublethal ash concentration of 3.5 g/L (B), 4) Group with culture medium in the presence of sublethal ash concentration of 4.5 g/L (C). The study groups consisted of 20 hatchlings of the original culture under 24 hours, arranged in cylindrical containers with a capacity of 150 mL containing 120 mL of culture medium, which was changed every third day to ensure that the ash concentration was constant, the temperature of 21 ± 2 °C, with a photoperiod of 16:8 (light: dark).

Each treatment was monitored on days 1, 3, 5, 7, 7, 9, 11, 13, 15, 17 and 19 by manual counting, recording changes in population development to evaluate finite population growth rate, survival percentage, the record of first reproduction (days), total reproduction, hatchlings per female yield (daphnids/days) in order to establish a sketch of conditions in life history characteristics.

### *Chronic toxicity data analysis*

All treatments were carried out in triplicate, and the results were analyzed using a generalized linear model with Poisson distribution and with logarithm as the link function; then, the Tukey test was performed post hoc with the R commander statistical package (The R Foundation for Statistical Computing, 2022).

### *Chronic toxicity results*

The most critical effects recorded during the trial were in the reproduction variable of treatment C (4.5 g/L) since the day of first reproduction was delayed four days concerning the control and the remaining treatments ($p < 0.05$). Regarding the total reproduction, the total number of hatchlings obtained during 19 days was much lower for all treatments concerning the control ($p < 0.05$).

In agreement with the above, the average number of hatchlings per gravid female was lower for the treatments compared to the control group ($p < 0.05$), and no significant differences were observed between treatments; this has repercussions on the population yield as a parameter that allows knowing the daily number of daphnids per milliliter, which shows the impact of the feeding on survival and reproductive stability. Thus, this indicator is lower for all treatments compared to the control.

Finally, the value of the Finite Growth Rate (PGR) stands out, with positive data for the treatments. It should be noted that positive values of this parameter are indicative of an increasing population; however, although positive values were recorded, it should be noted that the data is well below that of the control group, specifically in treatment C (4.5 g/L). The results are shown in *Table 3*.

In order to find a possible explanation for the behavior of the organism, an analysis of the ashes was carried out under NMX-AA-051-SCFI-2001 (Análisis De Agua - Determinación De Metales Por Absorción Atómica En Aguas Naturales, Potables, Residuales Y Residuales Tratadas - Método De Prueba, 2001) and NMX-AA-103-SCFI-2006 (Residuos – Determinación De Compuestos Orgánicos Volátiles Por Cromatografía De Gases Acoplado A Un Espectrometro De Masas En Productos De Extracción De Constituyentes Tóxicos (Pect) – Método De Prueba, 2006).


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***Table 3.*** *Life history parameters of D. magna were monitored for 19 days*

| | Control | A (1.5 g/L) | B (3.5 g/L) | C (4.5 g/L) |
|---|---|---|---|---|
| **First reproduction (days)** | 11.00 ± 0.00$^{a}$ | 11.00 ± 0.00$^{a}$ | 11.00 ± 0.00$^{a}$ | 15.00 ± 0.00$^{b}$ |
| **Total Reproduction** | 356.00 ± 19.31$^{c}$ | 178.67 ± 9.07$^{b}$ | 75.67 ± 13.58$^{a}$ | 65.00 ± 3.00$^{a}$ |
| **Neonates /Gravid Female** | 4.39 ± 0.17$^{b}$ | 2.68 ± 0.18$^{a}$ | 2.57 ± 0.18$^{a}$ | 2.75 ± 0.34$^{a}$ |
| **Survival (%)** | 95.55 ± 1.47$^{c}$ | 96.36 ± 0.71$^{c}$ | 83.35 ± 3.93$^{b}$ | 74.92 ± 3.42$^{a}$ |
| **Yield (daphnids/day)** | 0.11 ± 0.01$^{c}$ | 0.05 ±0.00$^{b}$ | 0.02 ± 0.00$^{a}$ | 0.02 ± 0.00$^{a}$ |
| **Finite Growth Rate (PGR)** | 2.56 ± 0.09$^{c}$ | 1.78 ± 0.07$^{b}$ | 0.72 ± 0.24$^{a}$ | 0.63 ± 0.11$^{a}$ |

Values are means ± standard deviation. Different letters indicate significant differences ($p < 0.05$)

The results of the former are shown in *Figure 2*, in which lead (Pb) is the heavy metal with the highest presence, however, there is the presence of zinc (Zn) in smaller quantities, also shown are the concentrations obtained for arsenic (As) and other heavy metals such as cadmium (Cd), chromium (Cr), mercury (Hg), copper (Cu) and nickel (Ni).

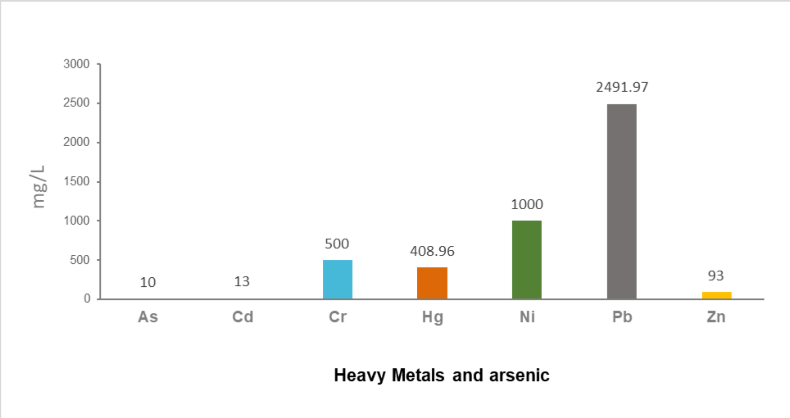


***Figure 2.*** *Analysis under NMX-AA-051-SCFI-2001 of scrap tire ash*

*Table 4* shows the values of As and the seven heavy metals Cd, Cu, Cr, Hg, Ni, Pb and Zn, and compares them with the maximum permissible limits (MPL) for heavy metals contained in water for agricultural irrigation use of the NOM-001-SEMARNAT- 1996, which establishes the maximum permissible limits for pollutants in wastewater discharges into national waters and property, 1996, and the Acuerdo por el que se establecen los Criterios Ecológicos de Calidad del Agua CE-CCA-001/89., 1989 and the Maximum Permissible Limits (MPLs) for natural waters of the EPA 440/5-86-001 / Quality Criteria for Water, 1986. The concentrations obtained exceeded the MPLs of both the Standard, the Agreement and the EPA statute.

The results obtained from the analysis of eleven volatile organic compounds are presented in *Table 5*. NMX-AA-103-SCFI-2006 (RESIDUOS – DETERMINACIÓN DE COMPUESTOS ORGÁNICOS VOLÁTILES POR CROMATOGRAFÍA DE GASES ACOPLADO A UN ESPECTROMETRO DE MASAS EN PRODUCTOS DE EXTRACCIÓN DE CONSTITUYENTES TÓXICOS (PECT) – MÉTODO DE PRUEBA, 2006). Furthermore, the MPLs of the Standard, as mentioned above, is also shown. Therefore, the concentrations obtained exceeded the MPLs of the Mexican Standard.


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***Table 4.*** *Maximum permissible limits for heavy metals and arsenic*

| PARAMETER (mg/L) | Results | NOM-001-SEMARNAT-1996 | Agreement EC CCA-001/89 | EPA 440/5- 86-001 |
|---|---|---|---|---|
| **As** | 10.00 | 0.2 | 0.1 | 0.05 |
| **Cd** | 13.00 | 0.2 | 0.01 | 0.01 |
| **Cu** | 400.00 | 4.0 | 0.2 | 1.5 |
| **Cr** | 500.34 | 1.0 | 1 | 0.1 |
| **Hg** | 408.96 | 0.01 | - | 0.002 |
| **Ni** | 1000.92 | 2.0 | 0.2 | 0.632 |
| **Pb** | 2491.97 | 0.5 | 5 | 0.0015 |
| **Zn** | 93.00 | 10.0 | 2 | 5 |

***Table 5.*** *The Concentration of volatile organic compounds in scrap tire ashes according to NMX-AA-103-SCFI-2006*

| | Results | Maximum Permissible Limits (NMX-AA-103-SCFI-2006) |
|---|---|---|
| **BENCENO** | 1.58 LPC µg/L | 0.03 LPC µg/L |
| **CHLOROBENZENE** | 2.37 LPC µg/L | 0.03 LPC µg/L |
| **CHLOROPHORM** | 1.98 LPC µg/L | 0.04 LPC µg/L |
| **VINYL CHLORIDE** | 10.41 LPC µg/L | 0.04 LPC µg/L |
| **1,4-DICHLOROBENZENE** | 2.33 LPC µg/L | 0.04 LPC µg/L |
| **1,2-DICHLOROETHANE** | 1.30 LPC µg/L | 0.02 LPC µg/L |
| **1,1-DICHLOROETHYLENE** | 3.23 LPC µg/L | 0.03 LPC µg/L |
| **HEXACHLOROBENZENE** | ND | ND |
| **HEXCHLOROBUTADIENE** | 6.13 LPC µg/L | 0.10 LPC µg/L |
| **METHYL ETHYL KETONE** | 9.34 LPC µg/L | ND |
| **PYRIDINE** | ND | ND |
| **TETRACHLOROETHYLENE** | 6.13 LPC µg/L | 0.05 LPC µg/L |
| **CARBON TETRACHLORIDE** | 11.73 LPC µg/L | 0.02 LPC µg/L |
| **TRICHLOROETHYLENE** | 12.70 LPC µg/L | 0.02 LPC µg/L |

LPC= Practical limit of quantification based on 25 mL of sample, ND= Undetermined

## Discussion

The results of this study show that waste tire ash elutriates pose an acute and chronic toxicity risk to the *D. magna* organism.

We obtained an $EC_{50}$ calculated for a 48-hour exposure of 5.83 g/L; this contrasts with the findings of Marwood et al. (2011), who reported that sediment elutriates enriched with tire and road wear particles showed no toxicity to three common aquatic test species at nominal concentrations of 10g/L or less.

However, our result agrees with Wik and Dave (2006), who reported toxic effects on *P. subcapitata*, *D. magna* and *Ceriodaphnia dubia* exposed to a series of sequential aqueous extracts of tire dust scraped from tire treads. They obtained an $EC_{50}$ range at 48 h that was 0.5 g/L > 10.0 g/L. The differences between the findings can be attributed to the elutriates and the test conditions.


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As for the chronic test, essential data were recorded regarding the effect on the population when exposed to three sublethal treatments. Specific effects were observed on parameters associated with total reproduction: the delay in sexual maturity and the reduction in the average number of hatchlings per gravid female, which disturbed the population yield and the finite growth rate. This reproductive effect is supported by the "allocation principle", which mentions that energy is distributed in demands such as maintenance, growth and reproduction of the organism; if extra energy is spent in one of them, the other two will have less energy available; therefore, the decrease in reproduction allowed the maintenance and survival of the organisms (Heckmann et al., 2007; Linares et al., 2020).

It should be noted that the PGR value has positive data for the treatments, which are indicative of an increasing population; however, despite the fact that positive values were recorded, it should be noted that the data are well below the control group, i.e., the population is reduced as the ash concentration increases; this disagrees with the data obtained by Panko et al. (2012), who mention that the sediment elutriates enriched with tire and road wear particles did not show toxicity, nor did they affect the growth and survival of their test organisms.

Marwood et al. (2011) have shown that zinc is the most abundant heavy metal in tires and is most likely to present toxicity in the test organism; the results obtained are consistent with previous investigations (Gualtieri et al., 2005; Wik et al., 2009); The Zn concentration obtained in the ash study exceeded the acute soluble lethal concentrations in 48 h exposures of *D. magna*, which ranged from approximately 50 µg/L to > 3 mg/L depending on the test conditions (Mount and Norberg, 1984; Oda et al., 2006; Muyssen and Janssen, 2007; Marwood et al., 2011).

It is crucial to evaluate the life history parameters of the *D. magna* organism and its mortality to infer the possible damage to the aquatic communities receiving this pollutant caused by the open burning of waste tires.

## Conclusion

This study has shown that ashes from the burning of waste tires have a negative effect on the organism *D. magna*, with only 5.83 g/L 50% of the organisms die; in addition, when exposed to sublethal concentrations in the long term (19 days) it has as a consequence a decrease in total reproduction, observing that the higher the concentration of the toxicant, the lower the finite growth rate. More research is needed on the effects of ash on reference aquatic organisms of different taxonomic orders.

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