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RESEARCH PAPER

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Acute Sensitivity Comparison among *Daphnia magna* Straus, 1820 *Daphnia pulex* Leydig, 1860 and *Simocephalus vetulus* Müller, 1776, Exposed to Nine Toxicants

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Abstract

A comparison of acute toxicity (LC_{50} values) among nine toxicants: a) six metals: Cd, Cr, Cu, Hg, Pb, Ti, and b) three organics: benzene, ethyl acetate, and toluene, between the exotic cladoceran species *Daphnia magna* Straus 1820 and two native strains of freshwater cladoceran species: *Daphnia pulex* Leydig, 1860 and *Simocephalus vetulus* (O. F. Müller, 1776) was performed. We hypothesized that the exotic species would be less sensitive than native species. Our hypothesis was fulfilled. *Daphnia magna* was less sensitive than native species to eight of the nine compounds we analyzed. Results suggest that native species are better adjusted to local environmental conditions, and are more reliable as bioindicators of potential effects of toxicants on aquatic biota. Although the use of *D. magna* is recommended because of its ample toxicity database, some researchers propose the use of native species for toxicity tests. Therefore, to propose a native species to be considered as a test organism for official toxicity test for tropical countries, there is a need to increase the database of toxicants and to compare the sensitivities of several classes of toxicants with a model organism whose sensitivity to a broad variety of toxicants is well known, like *D. magna*.

Introduction

Cladocerans (also known as water fleas) are a group of microcrustaceans commonly distributed in freshwater bodies and occurring in a variety of habitats; they play an important role on food webs, both as consumers of phytoplankton and fish prey (Dettmers & Stein, 1992). Within this group, daphnids have been broadly used in aquatic toxicology, they have demonstrated to be sensitive to a large variety of chemicals (Adema, Kuiper, Hanstveit & Canton, 1982; Baudouin & Scoppa, 1974). *Daphnia magna* has been recommended for toxicity evaluation of many chemicals in water (ASTM, 2014; ISO

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International Standard 6341, 1996; OECD 202, 2004; U.S. EPA, 2002), and has been extensively used in toxicity testing. However, it is not a native species in many countries, is a Holarctic organism with geographic distribution of cold and temperate environments of North America, Europe and Asia (Weber, 1993). Several countries: Austria, Australia, Belgium, Canada, Denmark, France, Germany, Italy, New Zealand, Netherlands, Norway, Portugal, Spain, Sweden, United States and United Kingdom contemplate *D. magna* in their legislature as model organism to quantify toxicity (Wolska et al. 2010). Toxicological information for tropical or subtropical species is very scarce, hence, the countries of these regions have to use international water quality criteria developed for *D. magna* (ASTM, 2014; ISO International Standard 6341, 1996; OECD 202, 2004; U.S. EPA, 2002) which leads to a certain grade of uncertainty. This can be seen in the available data (acute and chronic) found in the ECOTOX (U.S.EPA, 2017) and ECETOC (2003) databases. For *D. magna*, there are 403 toxicity values in ECOTOX (U.S.EPA, 2017) and 217 in ECETOC (2003), while for *Daphnia pulex* and *Simocephalus vetulus*, there are 87 (U.S.EPA, 2017) and 25 (ECETOC, 2003) and 16 ECOTOX (U.S.EPA, 2017) and 4 (ECETOC, 2003) references, respectively. In Mexico, *D. magna* is an exotic species, and it is unable to survive naturally in Mexican reservoirs. In contrast, there are records of *D. pulex* and *S. vetulus* in the states of Aguascalientes, Campeche, Mexico, Hidalgo, Guanajuato and Yucatan (Elías-Gutierrez, Gutiérrez, Silva, Granados & Garfias, 2008). At the beginning of the year 2000, the International Development Research Centre (IDCR) started an international research network called WaterTox, with the participation of institutions from Argentina, Canada, Chile, Colombia, Costa Rica, India and Mexico (Forget et al. 2000a). The goal of WaterTox was to develop and validate toxicity tests with different organisms, including *D. magna* in acute toxicity tests. During the development of this research, the countries of Argentina, Colombia, Costa Rica and India, reported having problems for the maintenance of cultures of *D. magna* and the consequent loss of the species in addition to considering the realization of these tests with a high cost (Forget et al. 2000b). Mohamed (2007) compared the sensitivity to seven chemicals between *Ceriodaphnia rigaudii* (a native species of Trinidad) and the exotic *Daphnia magna* and found that for six toxicants *C. rigaudii* was more sensitive than *D. magna* and concluded that due to this differences in sensitivity perhaps *D. magna* might not be an adequate sentinel species for Trinidad.

The aim of this work was to determine the acute toxicity of nine toxicants (six metals and three organic compounds) and compared the reference values from *D. magna* with those from *D. pulex* and *S. vetulus* to test the hypothesis that native species are more sensitive to toxicants than *D. magna*.

Materials and Methods

The *D. magna* strain is from the EPA and was donated by Dr. Fernando Martinez Jerónimo of the National School of Biological Sciences of IPN, Mexico. The species of *D. pulex* and *S. vetulus* were collected in a pond of the botanic garden of the Autonomous University of Aguascalientes, with geographic coordinates N 21.912, W -102.313. Cladocerans were cultured with EPA medium (96 mg $NaHCO_3$, 60 mg $CaSO_4{\bullet}2H_2O$, 60 mg $MgSO_4$ and 4 mg KCl per liter of deionized water, U.S.EPA, 2002),which is a synthetic moderately hard water (80-100 $CaCO_3$ L^{-1}), pH 7.5, temperature 20ºC ± 2, and photoperiod of 16: 8, (light: darkness) in a bioclimatic chamber (Revco Scientific), fed with the algae *Pseudokirchneriella subcapitata* (Korshikov) F.Hindák 1990 (UTEX 1648) grown in Bold`s Basal Medium (Nichols, 1973). Metals Cd, Cr, Cu, Pb and Ti were standards of atomic absorption diluted in 3% nitric acid (Sigma). Mercuric chloride was diluted in EPA

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medium. Benzene, ethyl acetate and toluene were analytical chemistry standards of Sigma Aldrich and was diluted in acetone. Toxicity tests were performed according to EPA methods (U.S.EPA, 2002). Acute test consisted in exposing ten neonates less 24h for each treatment, one negative control and five nominal concentrations. We performed a solvent control for the organic compounds with the highest acetone concentration used in tests to monitor the toxicity due to solvent.

Ten neonates were selected randomly and placed in one of the five replicates 250 ml glass beakers containing 100 ml of test solution per toxicant concentration. Organisms were incubated for 48h at 20ºC, photoperiod 16:8 (light: darkness) in absence of food. At the end of incubation dead organisms were counted.

Lethal media concentration (LC_{50}) values were calculated using regression with probit units and the logarithm of each toxicant concentration by the software Statistica 10.0 (Stat Soft Inc., 2001). The NOEC (No observed effect concentration) and LOEC (Lowest observed effect concentration) values were calculated by one-way analysis of variance (ANOVA) and Duncan test. We performed a Spearman correlation by transforming the LC_{50} values of each species into logarithm, to determine if there is an association between the values of toxicity of *D. magna* compared to *D. pulex* and *S. vetulus*.

Relative sensitivity were calculated by the followed formula (Von der Ohe & Liess, 2004):

$RS = \log (LC_{50\ D.\ magna} / LC_{50i})$

Where: RS= relative sensitivity; $LC_{50\ D.\ magna}$ = LC_{50} value for *D. magna* and LC_{50} = value for species *i*. A value of zero indicates a sensitivity equal to that of *D. magna*, positive value *D. magna* is less sensitive, negative value *D. magna* is most sensitive.

Results

Table 1 shows the toxicity values achieved for three species of cladocerans used in this work. In terms of LC_{50} values, *D. magna* was more sensitive to Pb; the native species: a) *D. pulex* was the most sensitive to benzene, Cr, Cu, and toluene; b) *S. vetulus* was the most sensitive to Cd, ethyl acetate, Hg and Ti (Table 1). The order of toxicity of the metals in descending order was, Cu = Hg> Cd> Pb> Cr> Ti to *D. magna*, for *D. pulex*: Hg = Cu> Cd> Pb> Cr> Ti and Hg = Cd = Cu> Pb> Ti> Cr to *S. vetulus*. For the organic compounds the order of toxicity was Ethyl acetate> Toluene> Benzene for the three species. In the case of *D. magna* and *D. pulex* the most toxic metals were Cu and Hg and the least toxic Ti, however for *S. vetulus* the Hg, Cd and Cu presented the highest toxicity and Cr least toxic.

The EPA (U.S.EPA, 2002) establishes a range of coefficient of variation of 21 to 58%, results obtained in our tests are below those values (Table 1).

Metal toxicity for cladoceran group according to the geometric mean of the LC_{50} (Table 2) was established as follows Hg> Cu> Cd> Pb> Cr> Ti and organic compounds was ethyl acetate > toluene> benzene. In accordance to LC_{50} values, the difference between the most toxic and less toxic metal was set in this way, *D. magna* 859 fold (Cu-Ti), 2,808 (Hg-Ti) for *D. pulex* and 3,280 (Hg -Cr) for *S. vetulus*. In the case of organic compounds, the differences were 134, 65 and 720 fold (ethyl acetate-benzene) for *D. magna*, *D. pulex* and *S. vetulus* respectively. We compared the mean values of LC_{50} (Figure 1a) to determine if they show significant differences. There are no differences in LC_{50} values for Cu and Hg among the three cladoceran species (Figure 1a). The LC_{50} values for Cr were different among the three species. Metals that did not show differences between *D. magna* and *D. pulex* were: Cd, Pb, and Ti, and between *D. magna* and *S. vetulus* Pb and Ti were different. Ethyl acetate revealed no significant difference between the three species, only toluene was statistically similar to *D. magna* and *S. vetulus* (Figure

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1b). Figure 2a shows that *S. vetulus* was more sensitive than *D. magna* for Cd, Hg, Ti, for organic compounds sensitivity was higher to ethyl acetate and toluene (Figure 2b), *D. pulex* was more sensitive to Cd, Cr, Cu, Hg and the three organic compounds (Figure 2a and 2b) compared to *D. magna*. Titanium was the only toxic without difference in the relative sensitivity among *D. magna* and *D. pulex*.

Table 3 presents different LC_{50} values of the toxics used in this work, mainly found in ECOTOX (U.S.EPA, 2017) and ECETOC (2003) databases, where it is stated that *D. magna* is the species with the highest number of toxicity tests (287) followed of *D. pulex* (137) and finally *S. vetulus* (5). A Spearman correlation of LC_{50} values between *D. magna-D pulex* and *D. magna-S. vetulus* was performed. The correlation coefficient between *D. magna-D. pulex* was 0.88 for metals (figure 3a) and 1.0 for the organic compounds (figure 4a). The correlation coefficient for *D. magna-S. vetulus* was 0.59 and 1.0 for metals and organic compounds respectively (Figure 4a and 4b).

Disscusion

Our original hypothesis is fulfill by the experimental data. *Daphnia magna* was less sensitive than the native species (either *D. pulex* or *S. vetulus*) to eight of the nine compounds we analyzed (See Table 1 and Figure 2). Only in the case of lead *D. magna* LC_{50} absolute value (1.08 mg/L) was lower than that of *D. pulex* (1.09 mg/L) (Table 1), but when we compared the relative sensitivity between both species this value is zero (Figure 2a), which means there is no difference in sensitivity between these two species exposed to lead. These results suggest that native species collected in Mexican reservoirs are better adjusted to the environmental conditions of Mexico and more reliable as bioindicators of the potential effects of toxicants on Mexican aquatic biota. Although the use of *Daphnia magna* is recommended because of its ample toxicity database, our results showed that is less sensitive to toxicant exposure than two native species. Some researchers propose the use of native species in toxicity tests: a) Martínez-Jerónimo, Rodríguez-Estrada & Martínez-Jerónimo (2008) proposed *Daphnia exilis* Herrick, 1895 for Mexico, b) Melnikov & de Freitas (2011) proposed *Daphnia similis* Claus, 1876 for Brazil, c) Aparecida, da Silva, Cristina, & Rocha (2014) proposed *Ceriodaphnia silvestrii Daday* 1902 and *Macrothrix flabelligera* Smirnov 1992, for Brazil, and d) Teklu & Van den Brink (2016) proposed *Diaphanosoma brachyurum* (Liévin, 1848) for Ethiopia. With the results obtained in this work we confirmed the difference between the relative sensitivity of *D. magna*/*D. pulex* and *D. magna*/*S. vetulus*, as well as providing toxicity data for *S. vetulus* for different metals and organic compounds that were not found in the databases. Based on this we can suggest the use of native species for toxicity tests due to their biological and ecological importance, and that are representative of each region.

The analysis of sensitivity of the three cladoceran species we tested exposed to nine toxicants (six metals and three organics), resulted in *D. pulex* being the most sensitive to two metals (Cr and Cu), and two organics (benzene and toluene), and with similar sensitivity to Pb than *D. magna*. *S. vetulus* was the most sensitive to three metals (Cd, Hg and Ti), and one organic (ethyl acetate).

Comparing the LC_{50} results of our work with the ECOTOX (U.S.EPA, 2017) and ECETOC (2003) databases (Table 3), *D. magna* remains within the range of benzene, Cd, Cu, Pb and Hg, for *D. pulex* benzene, Cd, Cr, Cu, Pb and Hg. Moreover, *D. magna* and *S. vetulus* were less sensitive to Cr; otherwise, *S. vetulus* was more sensitive for Cd, Cu and Pb.

In general, *D. magna* is less sensitive to Cu when comparing these ten values to our results (Table 4). On

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the other hand, the values between *S. vetulus* and *S. expinosus* are very similar to those obtained in our strain. For *D. pulex*, the values were intermediate between those reported by these authors (Table 4). Our *D. magna* Cd value in three of the four values was less sensitive. The *D. pulex* strain of Elnabarawy et al. 1986, show greater resistance than our *D. pulex* strain. Badudouin & Scoppa (1974) determined a lethal median concentration value for Cr of 0.023 mg/L to *D. hyaline*, which differs 138, 65 and 149-fold compared to *D. magna*, *D. pulex* and *S. vetulus* respectively (Table 4). The most sensitive organisms to Pb was *D. hyalina* Leydig 1860 in contrast with *D. pulex* strain of Elnabarawy et al. (1986), it was more resistant when compared to the values obtained in this research. The LC_{50} values of Hg to *D. pulex* and *S. vetulus* of this work are very similar to those found by Badudouin & Scoppa (1974) for *D. hyalina* of 0.005 mg/L and Elnabarawy et al.(1986) for *D. pulex* (0.003 mg/L) and *Ceriodaphnia reticulata* (Jurine, 1820) (0.002 mg/L) in contrast to our *D. magna* strain resulted less sensible.

We found differences in the relative sensitivity of *D. magna* compared to *D. pulex* and *S. vetulus* in accordance with Wogram & Liess (2001) and Von der Ohe & Liess (2004), where they assessed the cladocera group against *D. magna*, ensuing the group being more sensitive to metals and organic compounds. The highest differences in relative sensitivity were found between *D. magna* and *S. vetulus* for Cd, Hg and Ti. *D. magna* was more sensitive solely 27% of total test performed.

Results of the spearman correlation evidence that all three species of cladocerans were responding similar to organic compounds (Figure 4). Moreover, we found differences with action mode of metals (Figure 3) mainly with correlation *D. magna-S. vetulus*.

The LC50 values were evaluated by the Spearman rank correlation coefficient. This comparison provided values of 1.0 between *D. magna-D. pulex* (Fig. 4a) and *D. magna-S. vetulus* (Fig. 4b) for organic compounds. We assume the acute toxic responses of three cladoceran are strongly correlated and the mode of action of chemicals is the same. However, the correlation between *D. magna-D. pulex* (Fig. 3a) was strongest (0.80) than *D. magna-S. vetulus* (0.59) Fig. 3b, we presume, metals have a different mode of intoxicated according to the organism.

Information obtained from the Web of Science (2018) registered 238 publications of *D. magna* on metal acute toxicity during 1984 to 2017. The contribution by country was: United States 62, China 59, Canada 22, Mexico 15, France 14, Portugal 14, Brazil 12, India 12 Netherlands 12, United Kingdom 10, Australia 9, Spain 9, England 6, Germany 6, Japan 6, Turkey 5, Belgium 4, Chile 4, Finland 4, Taiwan 4, Italy 3, Scotland 3, Slovakia 3, South Korea 3, Argentina 2, Egypt 2, Malaysia 2, Nigeria 2, Poland 2 and Vietnam 2. With respect to *D. pulex* the total of publications are 31, compiled in the years 2006 to 2017 and they are distributed in the following way: United States 12, France 5, United Kingdom 4, Mexico 3, Belgium, Canada, England, Germany and China with two works for each one. The Web of Science database does not contain any publications related to *S. vetulus*.

In this work the acute toxicity tests were developed under the same conditions of temperature, pH, hardness of the test solution, photoperiod and chemical purity, these parameters being indispensable for the quality control of the results obtained from acute toxicity test. According to the USEPA (2002), an important factor for the accuracy of the results is the coefficient of variation (CV). In this research, the CV ranges were 0.48-17.14% and 4.65-21.93 for metals and organic compounds respectively, which indicates the reliability of the results. We report the first acute toxicity values of ethyl acetate and toluene for the three species of cladoceran tested and benzene for *S.*

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vetulus. This is the first report in which relative sensitivity and the correlation between species for acute toxicity tests with six metals and three organic compounds are included. Some authors (Wogram and Liess, 2001; Sanchez-Bayo, 2006) have reported comparisons of the sensitivity of *D. magna* among other species of cladocerans. However, they did not perform the toxicity tests, they used values of the USEPA database; in these cases, feed data (species and concentration) of the different species of cladocerans was not taken into account, which can reduce the accuracy when comparing the sensitivity between species. On the other hand, Cowgill et al. (1984) performed toxicity tests to compare the sensitivity between *D. magna-Cedriodaphnia dubia/affinis*, although the feeding of the cultures was with different species of algae, *S. capricornotum* and *A. convolutus* respectively, and this may alter the sensitivity of the organism.

We have a large database for *D. magna*, which includes 2475 toxicants with 48-h exposure tests, and 3184 total toxicants. In contrast, the database for *D. pulex* includes 316 for 48-h tests and 524 total toxicants. In the case of *S. vetulus* includes only 34 for 48-h tests and 49 total toxicants (U.S.EPA, 2017). ECETOC (2003) reports 113 total toxicants for *D. magna*, but only 16 for *D. pulex* and 15 for *S. vetulus*. In the case of ECOTOX (U.S.EPA, 2017) for 48-h tests for the toxicants used in this work, the database include 157 toxicants for *D. magna*, 97 for *D. pulex*, but only 13 for *S. vetulus*. Therefore, to propose a native species as a model organism for official toxicity test for the Mexican legislation (or for any tropical country), there is an urgent need to increase the database of toxicants. It is necessary to compare the sensitivities of several classes of toxicants with a native model organism whose ecology, genetics, sensitivity to a broad variety of toxicants is well known as is the case of *D. magna*.

Conclusions

The exotic species *D. magna* was the least sensitive species to nine toxicants (six metals and three organics), when compared with the native species *D. pulex* and *S. vetulus*. Our results suggest that native species collected in Mexican reservoirs are better adjusted to the environmental conditions of Mexico and more reliable as bioindicators of the effects of toxicants on Mexican aquatic biota. There is a need to increase the knowledge on native species (like *D. pulex* and *S. vetulus* used in this contribution), to match information on *Daphnia magna* (especially the ample toxicity database), to propose a native species as a sentinel organism for Mexican reservoirs. This contribution includes the first: a) acute toxicity values of ethyl acetate and toluene for the three species of cladocerans, b) acute toxicity value of benzene for *S. vetulus*, and c) relative sensitivity and correlation between three cladoceran species for acute toxicity tests with six metals and three organic compounds.

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Table 1. Endpoints of the acute toxicity test performed with three species of cladoceran. The most sensitive LC_{50} values are highlighted with bold numbers. N = 5.

Species	Parameter	Benzene	Cadmium	Chromium	Copper	Ethyl acetate	Lead	Mercuric chloride	Titanium	Toluene
D. magna	LC10	62.01	0.10	2.53	0.006	1.04	0.75	0.017	9.75	33.13
	LC50	207.53	0.20	3.04	0.013	1.54	**1.08**	0.028	11.17	44.16
	NOEC	50	<0.125	2.0	<0.005	1.0	0.25	<0.015	<10.0	20.0
	LOEC	100	0.125	2.5	0.005	1.5	1.0	0.015	10.0	30.0
	CL	145.64 295.80	0.1998 0.2174	2.9020 3.2033	0.0120 0.0140	0.4585 1.6166	0.9474 1.2445	0.0258 0.0315	11.0077 11.3448	39.6551 49.1926
	CV%	17.72	3.09	1.96	8.52	4.75	14.26	6.71	0.48	8.27
	R^2	0.81	0.98	0.76	0.94	0.93	0.83	0.76	0.93	0.74
D. pulex	LC10	31.11	0.11	1.16	0.003	1.07	0.56	0.0019	10.71	9.74
	LC50	**86.49**	0.17	**1.43**	**0.007**	1.32	1.09	0.004	11.22	**15.45**
	NOEC	25	<0.10	0.5	0.001	<1.0	0.1	0.0005	10.0	5
	LOEC	50	0.10	1.0	0.005	1.0	0.5	0.001	11.0	10
	CL	72.2680 96.8500	0.1670 0.1857	1.2755 1.6062	0.0059 0.0087	1.2855 1.3930	0.9516 1.2676	0.0030 0.0053	11.0509 11.3972	13.0797 18.2767
	CV%	9.06	0.73	3.70	17.14	6.15	3.61	13.17	2.10	21.93
	R^2	0.93	0.95	0.80	0.81	0.91	0.93	0.76	0.78	0.75
S. vetulus	LC10	288.60	0.004	2.21	0.013	0.42	0.75	0.0003	0.51	22.54
	LC50	475.66	**0.015**	3.28	0.017	**0.66**	1.34	**0.001**	**2.34**	42.89
	NOEC	<200.0	0.001	<2.0	0.010	0.25	0.5	0.0001	<0.2	<20.0
	LOEC	200.0	0.005	2.0	0.015	0.50	1.0	0.0005	0.2	20.0
	CL	422.2795 535.9200	0.0113 0.0208	3.0746 3.5067	0.0160 0.0182	0.5834 0.7603	1.1738 1.5441	0.0008 0.0014	2.2222 3.5620	38.7257 47.5006
	CV	4.65	5.22	2.39	7.42	4.65	7.12	6.55	5.25	8.37
	R^2	0.83	0.85	0.85	0.81	0.83	0.87	0.85	0.76	0.87

LC_{50}=Lethal concentration where 50% of animals. LC_{10}= Lethal concentration where 10% of animals die. NOEC = No Observed Effect Concentration. LOEC = Lowest Observed Effect Concentration. All values in mg/L. R^2 = *C*oefficient of determination of the LC_{50}. CV = Coefficient of variation of LC_{50}. CL = 95% Confidence limits for the LC_{50} values.

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Table 2. Geometric mean of the CL_{50} toxicity values of three cladoceran species. All values in mg/L

Endpoint	Benzene	Cadmium	Chromium	Copper	Ethyl acetate	Lead	Mercuric chloride	Titanium	Toluene
Geometric mean LC_{50}	204.384	0.079	2.424	0.011	1.102	1.164	0.004	6.643	30.815

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Table 3. Acute toxicity values for *D. magna*, *D. pulex* and *S. vetulus*, 48h, LC_{50}. All values in mg/L

Toxicant	Species	Range	Mean	n	Reference
Benzene	*D. magna*	56.65-1130	275.72	18	U.S. EPA (2017) , ECETOC (2003)
	D. pulex	15-345	208.33	3	
Cd	*D. magna*	0.0009-22.6	0.21	171	U.S. EPA (2017) , ECETOC (2003)
	D. pulex	0.0004-1.04	0.15	82	U.S. EPA (2017) , ECETOC (2003)
	S. vetulus	0.024-0.089	0.056	2	U.S. EPA (2017)
Cr	*D. magna*	0.02-2.4	0.60	21	U.S. EPA (2017) , ECETOC (2003)
	D. pulex	0.57-2.0	0.57	18	U.S. EPA (2017) , ECETOC (2003)
	S. vetulus	-	0.50	1	U.S. EPA (2017)
Cu	*D. magna*	0.008-0.12	0.03	57	U.S. EPA (2017) , ECETOC (2003)
	D. pulex	0.006-0.37	0.07	23	U.S. EPA (2017) , ECETOC (2003)
	S. vetulus	-	0.057	1	U.S. EPA (2017)
Pb	*D. magna*	0.13-4.4	1.0	5	U.S. EPA (2017)
	D. pulex	0.37-9.9	3.54	6	U.S. EPA (2017) , ECETOC (2003)
	S. vetulus	-	4.5	1	U.S. EPA (2017)
Hg	*D. magna*	0.001-0.02	0.005	13	U.S. EPA (2017)
	D. pulex	0.002-0.024	0.008	5	U.S. EPA (2017)

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Table 4. Acute toxicity values for *D. magna*, *D. pulex* and *S. vetulus*, 48h, LC_{50}. All values in mg/L.

Toxicant	Species	LC_{50} values	References
Cd	*D. hyalina*	0.055	Baudouin & Scoppa, 1974
	C. reticulata	0.18	Elnabarawy et al.1986
	D. pulex	0.31	
	D. pulex	0.13	Santos-Medrano & Rico-Martínez, 2015
Cr	*D. hyalina*	0.023	Baudouin & Scoppa, (1974)
Cu	*D. hyalina*	0.005	Baudouin & Scoppa, (1974)
	D. pulex	0.03	Elnabarawy et al.1986
	Ceriodaphnia reticulata	0.02	
	D. galeata G. O. Sars, 1864	0.02	
	D. pulex	0.003	Bossuyt & Janssen, 2005
	Ceriodaphnia pulchella G. O. Sars, 1862	0.016	
	D. galeata	0.009/0.018	
	D. longispina (O. F. Mueller, 1785)	0.022	
	Simocephalus expinosus (O. F. Müller, 1776)	0.018	
	S. vetulus	0.021	
Pb	*D. hyalina*	0.60	Baudouin & Scoppa, 1974
	C. reticulata	1.80	Elnabarawy et al. 1986
	D. pulex	2.02	
	D. pulex	1.62	Santos-Medrano & Rico-Martínez, 2015
Hg	*D. hyalina*	0.005	Baudouin & Scoppa, 1974
	C. reticulata	0.002	Elnabarawy et al. 1986
	D. pulex	0.003	

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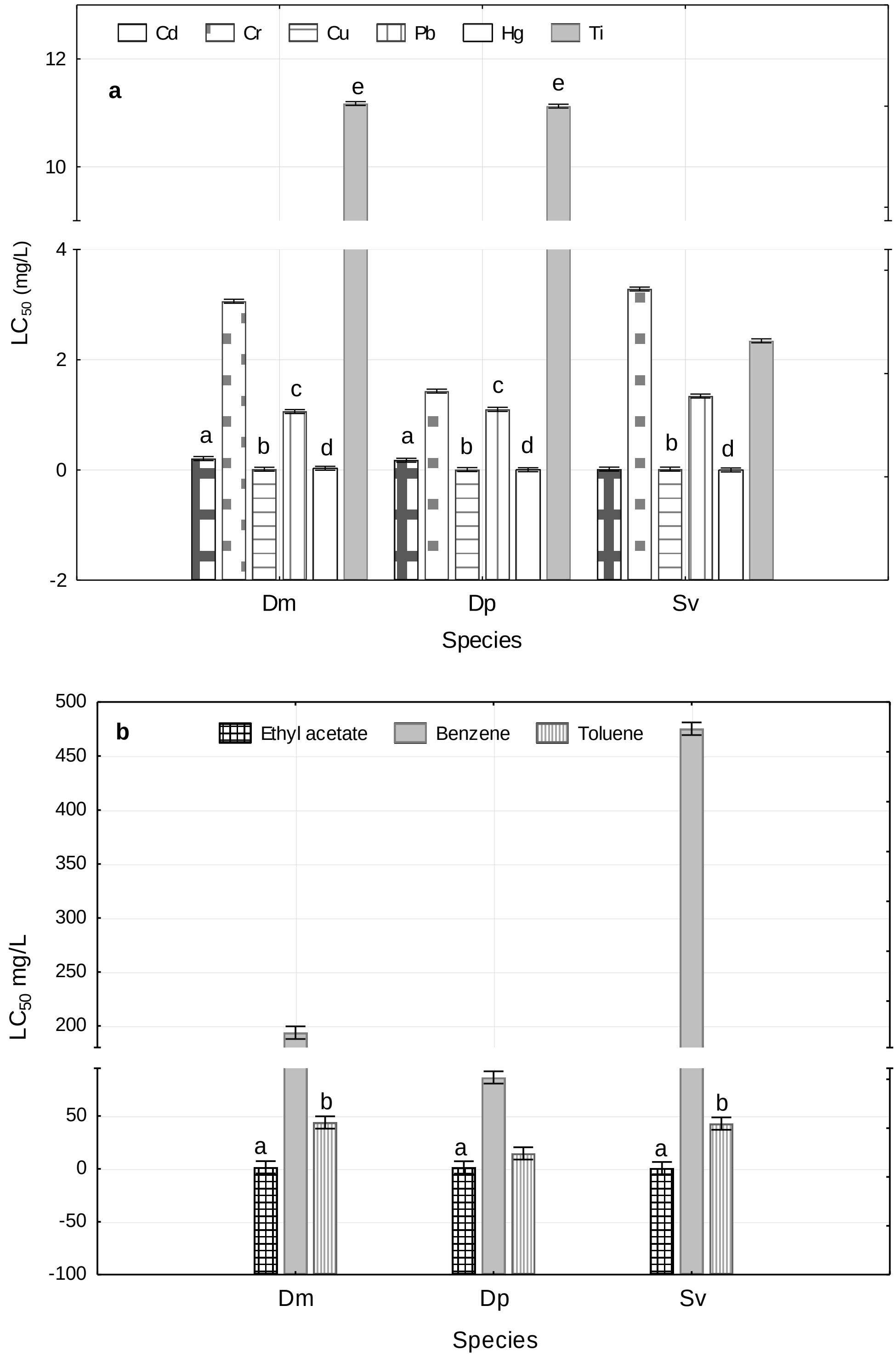

Figure 1. Comparison of LC_{50} values of *D. magna* against *D. pulex* and *S. vetulus* by ANOVA and Duncan test. Vertical bars represented standard error. The same letter means no significative difference. $p<0.05$. a) metals, b) organic compounds. Abbreviations: Dm=*D. magna*, Dp= *D. pulex*, Sv=*S. vetulus*

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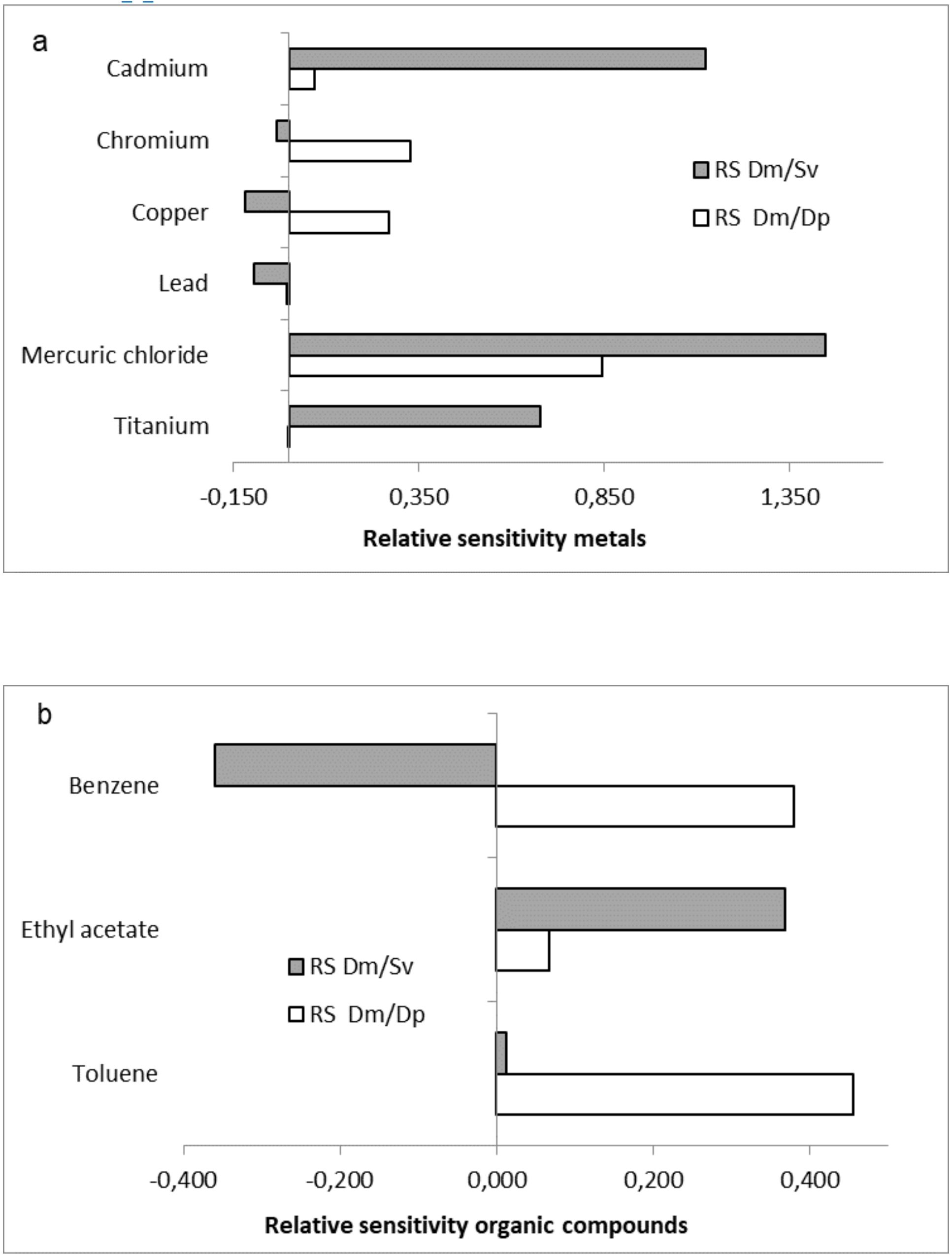

Figure 2. Relative sensitivity expressed as a logarithmic proportion (LC_{50}) relative to the sensitivity of *D. magna*. a) metals and b) organic compounds.

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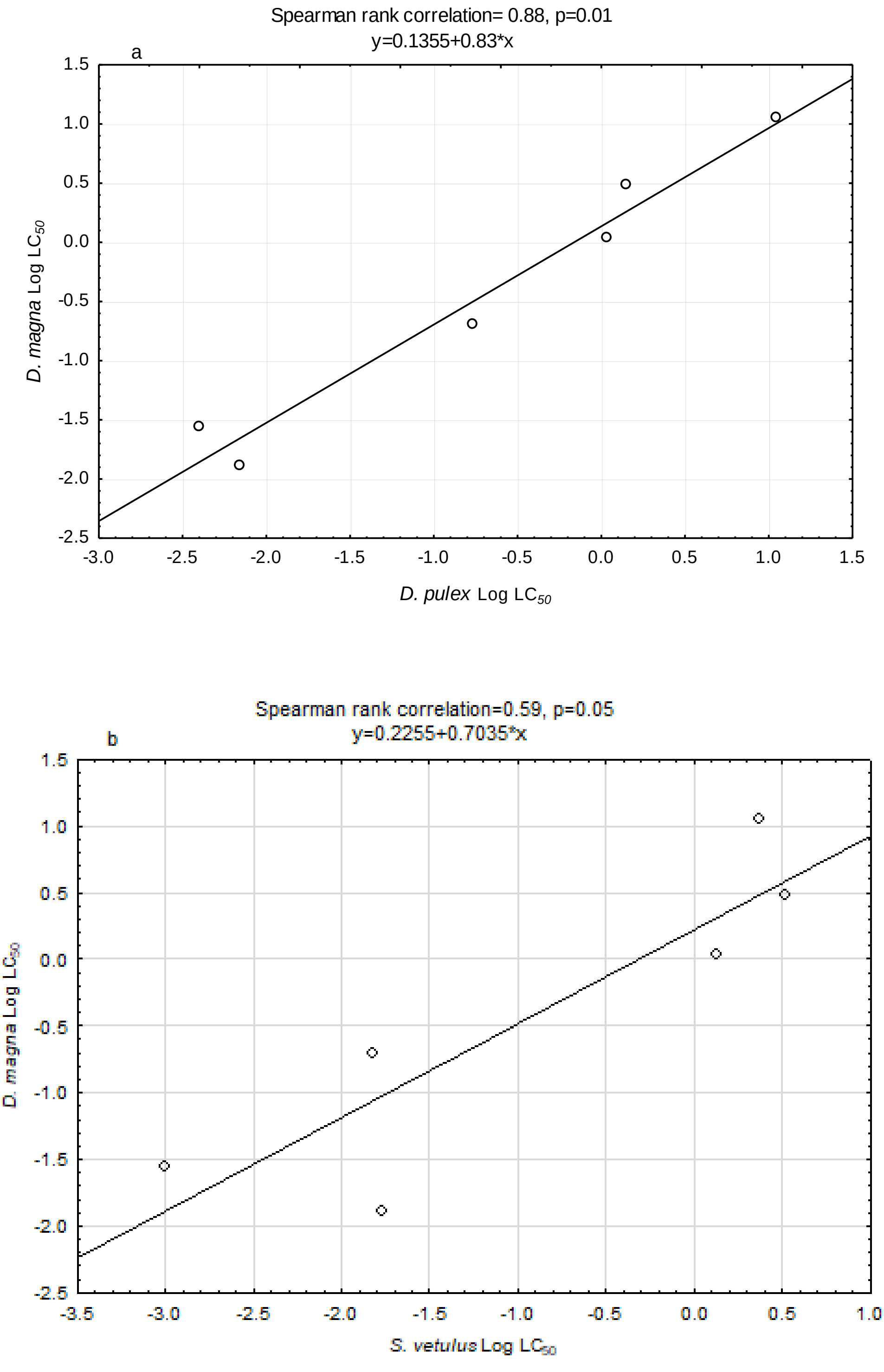

Figure 3. Correlation between acute toxicity values of *D. magna*, *D. pulex* (a) and *S. vetulus* (b) for metals.

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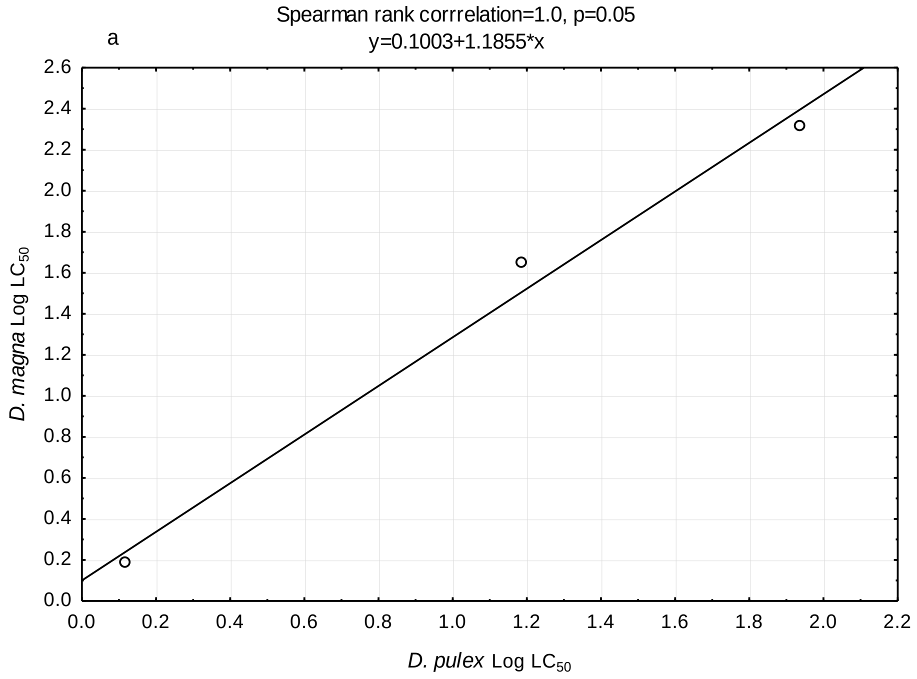

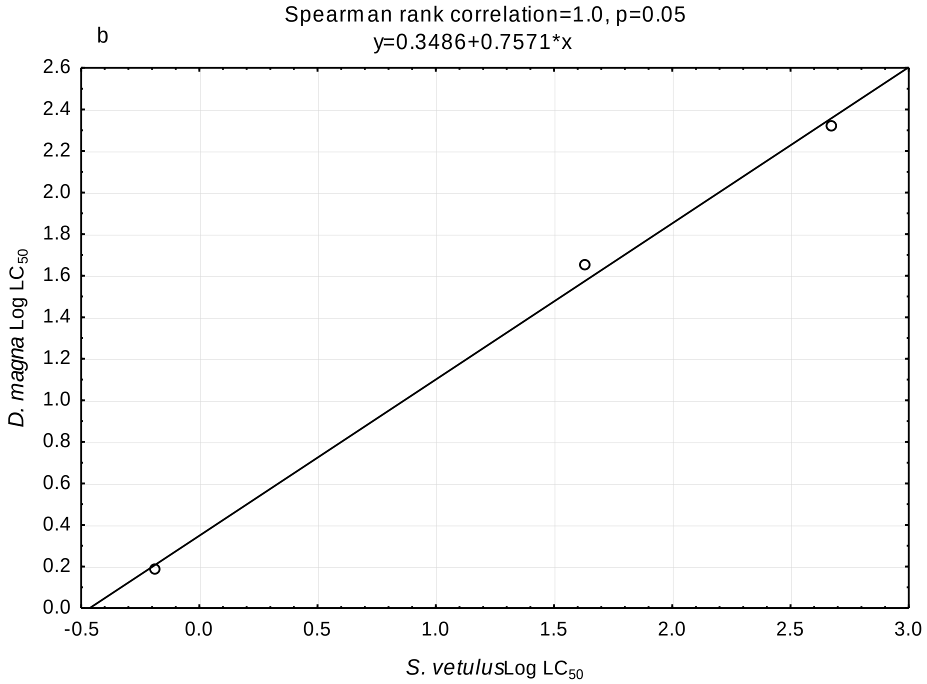

Figure 4. Correlation between acute toxicity values of *D. magna*, *D. pulex* (a) and *S. vetulus* (b) for organic compounds.