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Specification of lethal concentration (LC_{50}) of boron effect on *Daphnia pulex* (Leydig, 1860) using probit model

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ABSTRACT

Statistical models used in toxicity experiments have been quite useful tools in interpreting the organism's susceptibility, exposure response, amount of tolerable concentration, and function of tolerance time. In order to determine and evaluate the toxic effects of boron on *Daphnia pulex* (Leydig, 1860), different boron concentrations considered to be tolerable in aquatic ecosystems were tested for *D. pulex*. Percentage mortality rates at different boron concentrations and probit regression estimates at these concentrations were investigated through the static method. Probit analysis in this study revealed that rising boron concentrations led to mortality, and that finding was statistically significant with P value. These results indicate that the use of episodic boron, which enters the aquatic ecosystem through natural or unnatural means, should be planned due to its potential for stress or toxicity situations on organisms.

1. Introduction

Boron has many important industrial uses such as fiberglass insulation, borosilicate glass, cleaning products, fertilizer, metallurgy and nuclear protective material production [1,2]. However, it is also predicted that boron may become a drinking water contaminant in the next few years [3]. Despite such a risk, it is thought that the use of boron should be done with a planned and technical arrangement [3]. Boron is an important essential micronutrient, but has also been reported to create toxic conditions [4]. Various studies showing an increase its genotoxic effect on organisms have focused on boron as not being biodegradable in the aquatic ecosystem [5,6]. Boron levels can exceed toxic levels in the aquatic ecosystem through natural and unnatural metal corrosion and contamination processes [7]. Although surface waters are generally below the toxic level (0.01-1.5 mg/l) [8], it has been stated that the boron level for aquatic organisms should be below 1.2 mg/l [9]. The toxic properties of boric acid and borates (sodium borate, sodium tetraborate, or disodium tetraborate), especially against arthropods, are known; however [10-12], there is no specific information about their toxicity against water fleas, which have an important place in the food chain in the aquatic ecosystem. The use of invertebrates in monitoring the aquatic ecosystem health provides significant economic benefits [13,14]. As they are very sensitive creatures, invertebrates have become models for protecting public health by providing immediate warnings about unpredictable changes in the ecosystem in advance [13,14]. Water fleas, especially with their many practical features such as short life cycles, parthenogenetic reproductive features, and reduction of traditional animal tests, have provided significant benefit in determining the deviations in ecotoxicological, behavioral, ecophysiological and genetic profiles caused by environmental xenobiotics [15-18].

The invertebrate *Daphnia pulex* with its widespread presence in aquatic ecosystems has been an important animal model in both ecological and laboratory studies [19]. However, little is known about the ecotoxicological probit modeling of boron toxicity on *D. pulex*. Therefore, it is essential to perform different tests to understand the sensitivity variability between organisms along with regression models, which help to evaluate different protection levels of organisms in wildlife [20]. The first aim of our study is to estimate the mortality status and the corresponding lethal concentration of *D. pulex* at the ecotoxicological endpoints of different boron concentrations. It is also aimed to provide a perspective on the sensitivity and applicability of the secondarily applied probit estimation in toxicological tests.

2. Materials and Methods

2.1. Experiment Animals and Chemicals

Water fleas used as the experimental material, were transferred from shallow habitats in northern Anatolia to a culture medium with a stainless steel support

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frame along with freshwater samples of its own habitat. Light microscopy was used to identify water fleas and determine their vital characteristics such as mortality, color change and brightness (Omax Microscope, USA). The guide for the identification of micro-invertebrates in the world's continental waters was used for the species identification of water fleas [19], followed by their adaptation to the culture environment for 1 month. The culture tank was renewed with 20°C spring water and aerated. A 16-hour light/8-dark photoperiodism was maintained for the culture tank, and baker's yeast suspension was fed at 1 ml per tank once a day for one week before the experiments. $Na_2B_4O_7 \cdot 10H_2O$ (Eti Maden®, Turkey) was used in the experiments to determine the effect of boron on mortality in water fleas.

2.2. Experimental Design

The boron concentrations tested in the experiment were selected according to the prerequisite of performing at least five tests for the toxic substance in the limit determination tests recommended by the Environmental Protection Agency (EPA) [21] and the availability limits of boron in surface waters [22]. Depending on these prerequisites, deaths in daphnia were monitored during 48-hour exposure periods in jars containing different concentrations of boron (0.05, 0.10, 0.20, 0.40, 0.80, 1.60, 3.20, 6.40 mg/l) compared to the jar containing no boron in the control group. The median lethal concentration (LC_{50} values) for *D. pulex* were determined by using 48h static bioassay experiment [23-25]. It is stated and recommended that 20 test organisms are suitable for each toxicity application in probit toxicity studies [26]. Since there were a total of 9 test jars with 20 *D. pulex* for each boron concentration in the probit prediction experiment, in total 180 *D. pulex* were observed in the entire experiment. The preparation of the stock solution was made according to the stock preparation procedures of the APHA methods [27], and the chemical at the specified concentrations was diluted into the test aquarium and injected into the experimental environment. The mean and standard error of the carapax length and carapax width of neonatal *D. pulex* (~24h) at the beginning of the experiment were determined as 0.721±0.01 mm, 0.092±0.01 mm. By adding 100 ml of the organisms' culture medium and 100 ml of spring water to the experimental environments, each experimental area was created sequentially in 200 ml of medium. Under the light microscope, the *D. pulex* color change, inability to move, and cessation of heartbeat were noted for each group as indicators of its mortality.

2.3. Instrumentations

Inductively coupled plasma mass spectrometry was used to determine boron concentrations in application aquariums (ICP-MS Agilent 7500ce series, Octopole Reaction Systems, Agilent Technologies, Japan). The recovery percentage of the boron limit determined in the spectrophotometer was determined as 95.7%. In the validation parameters of the analytical method for the analyte, the detection limit (LOD) value was 1.45 $ng.g^{-1}$; limit of quantification (LOQ) value was 5.25 $ng.g^{-1}$; the relative standard deviation (RSD, %) and the coefficient of determination (R^2) values were determined as 1.07% and 0.99, respectively.

The physical-chemical properties of the experimental environments and the methods used to determine these properties are given in Table 1. Analyzes were performed three times to determine the physical and chemical properties of the experimental tank ambiance.

Table 1. Physical and chemical properties of the tank

Parameters, Unit	Analytical method	Mean ± Std. Error
Water temperature, °C	Temperature probe	20.10±0.91
Dissolved oxygen, mg/l	Oxygen meter probe	7.83±0.09
pH	pH probe	7.12±0.13
Total hardness, mg/l	Titrimetric method	98.75±2.23

2.4. Statistical Analysis

Statistical analyses were performed using the SPSS statistical software (V 27.0.1.0, IBM, Corp., USA) to determine the effects of boron on the *D. pulex* population [28]. The relationship between boron concentrations applied to *D. pulex* and mortality and the direction of the relationship were determined by regression-correlation analysis. The level of significance was set to be at least $P \leq 0.01$. Depending on the significance determined, probit analysis estimates were made [29,26]. The sigmoidal mortality chart of the population was drawn, and the lethal concentration (LC_{50}) value was checked with the calculated value [30].

3. Results and Discussion

In this study examining boron toxicity, the direction of the relationship between boron concentrations applied to a total of 180 *D. pulex* exposed to boron and their mortality rates is shown in Figure 1, and Pearson correlation matrices are given in Table 2. As boron concentration increased, the average mortality level similarly increased; thus, a strong positive correlation was observed ($P \leq 0.01$) (Pearson's R=0.802; df=7; p=0.005). In a study examining boron toxicity, it was observed that high concentrations of boron potentially affected the weight of Diptera larvae [31]. In another study where the water quality parameters of water samples taken from different drainage channels were examined seasonally, the Pearson correlation coefficient between boron concentrations in the spring season and the mortality rate of *Daphnia magna* was found to be positively significant with 0.71 (p = 0.003) [32]. The high positive correlation levels indicated that boron derivatives had toxic effects on macroinvertebrate populations.

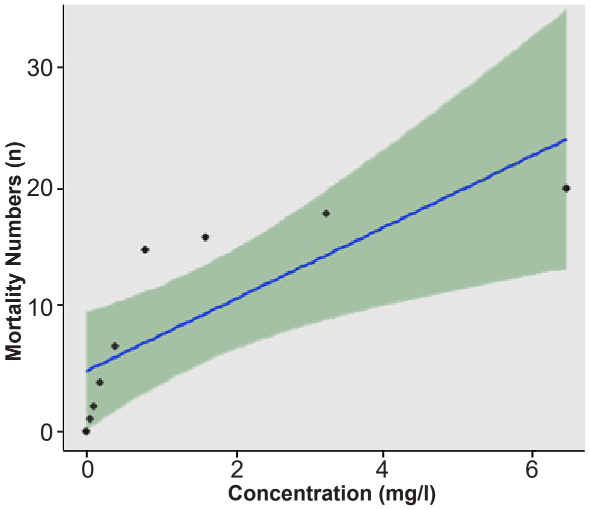

Figure 1. Pearson correlation between boron concentrations and mortality.

Table 2. Pearson correlation matrix between exposed concentrations of boron and mortality of *D. pulex*.

	Pearson's *R* matrix	**Concentration (mg/l)**
Number of Mortality (n)	Pearson's *R*	0.80 (*P*<0.01)*
	df	7
	p-value	0.005
	95% Confidence Interval Upper	0.965
	95% Confidence Interval Lower	0.394

The results of estimating the probit line plots (with confidence limits) and probit analyses of cladocerans are given in Figure 2 and Table 3. The probit regression results of *D. pulex* exposed to boron for 48 hours were found to be R^2=0.73; y= -0.96+0.85x. Accordingly, as a result of probit analysis in this study, the effect of boron application doses on survival was found to be statistically significant with p values. Since the estimated coefficient was a positive value (1.002), it indicated that the effects were positive i.e. the number of deaths occurred more frequently as the dose increased. As seen from the relevant chi-square values and the corresponding p values, the established probit regression model was found to be significant.

The probit analysis is used extensively in ecotoxicology to determine the relative toxicity of toxic substances on organisms and assumes that the relationship between the concentration and response state is normally distributed [33]. In this study, the acute toxic effects of boron on *D. pulex* were determined using the probit analysis with LC_{50} determination method due to the cumulative normal distribution. In the analysis, the dose-response curve was visualized as a straight line, providing maximum probabilities and least squares or regression estimates. The concentration values that caused 50% mortality at the end of the 48-hour period were analyzed, and the results are shown in Table 3. LC_{50} was found only at 0.51 mg/l boron concentrations.

The percentage mortality in the *D. pulex* population determined as a response to boron toxicity yielded an upward sloping sigmoidal curve (Figure 3). Basically, the probit cumulative standard is inverse to the normal distribution [34,25]. It is seen that the values of the fit hypothesis (Pearson Goodness of Fit Test) were significant for regression (Table 3). Moreover, probit regression seems more suitable for this study as it shows the 95% extreme values of the chemical.

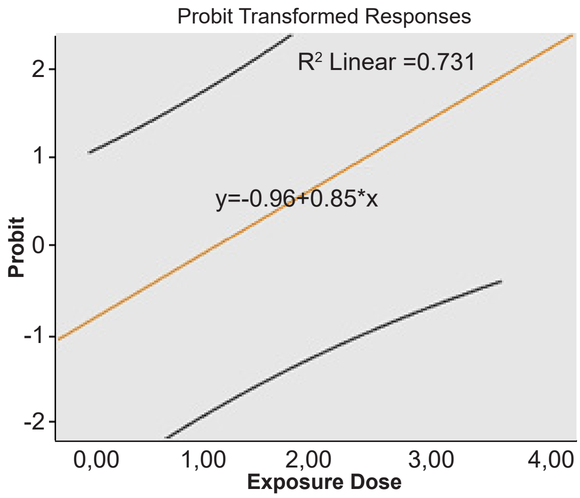

Figure 2. Result of probit regression estimation.

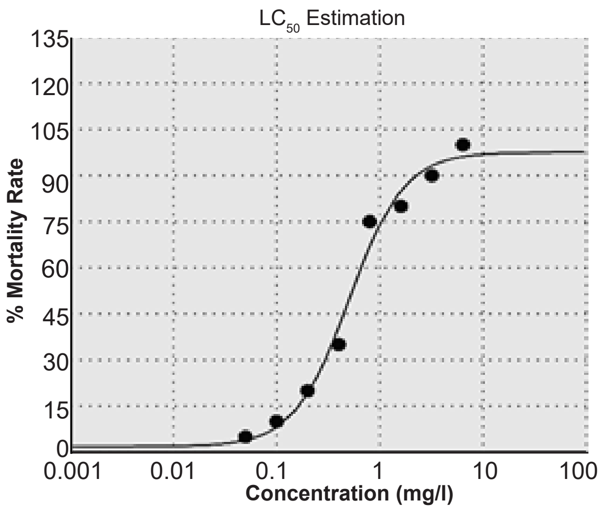

Figure 3. Lethal concentration (LC_{50}) of *D. pulex*.

4. Conclusions

The results revealed that environmental increases in boron may affect *D. pulex* mortality. The increase in the concentration of boron compounds such as boron and borate, especially in domestic and agricultural areas, causes the extinction of key species in the food chain as well as the destruction of harmful species. Planned use should be ensured, taking into account that increased concentrations of boron for various reasons may cause a negative situation in the welfare of aquatic organisms. In addition, it is thought that the sensitivity of *D. pulex* determined in this study to the effect of boron concentrations and the suitability

Table 3. Probit regression parameters of *D. pulex* under boron exposure.

Parameter Estimates							
						95% Confidence Interval	
PROBIT[a]	**Parameter**	**Estimate**	**Std. Error**	**Z**	**Sig.**	**Lower Bound**	**Upper Bound**
	Exposure Dose	1.002	0.15	6.56	0.00	0.70	1.30
	Intercept	-1.034	0.15	-6.76	0.00	-1.19	-0.88

[a.] PROBIT model: PROBIT(p) = Intercept + BX

Chi-Square Tests				
		Chi-Square	**df[b]**	**Sig.**
PROBIT	Pearson Goodness-of-Fit Test	27.20	7	0.00[a]

[a.] Since the significance level is greater than 0.150, no heterogeneity factor is used in the calculation of confidence limits.

[b.]Statistics based on individual cases differ from statistics based on aggregated cases.

95% Confidence Limits for Exposed								
PROBIT	**Probability**	**Estimate**	**Lower Bound**	**Upper Bound**	**Probability**	**Estimate**	**Lower Bound**	**Upper Bound**
	0.01	-1.29	-6.14	-0.38	0.55	1.16	0.61	2.76
	0.05	-0.61	-3.84	0.07	0.60	1.28	0.73	3.15
	0.10	-0.25	-2.65	0.34	0.65	1.41	0.85	3.57
	0.15	-0.00	-1.87	0.55	0.70	1.56	0.96	4.01
	0.20	0.19	-1.28	0.74	0.75	1.71	1.08	4.51
	0.25	0.36	-0.81	0.95	0.80	1.87	1.20	5.06
	0.30	0.51	-0.43	1.17	0.85	2.07	1.33	5.71
	0.35	0.65	-0.12	1.43	0.90	2.31	1.49	6.54
	0.40	0.78	0.12	1.72	0.95	2.67	1.73	7.77
	0.45	0.91	0.31	2.04	0.97	2.91	1.87	8.57
	0.50	1.03	0.47	2.40	0.99	3.35	2.15	10.10

LC_{50} Regression Results	**Value**
LC_{50}	0.51
Equation Form	$Y = \mathrm{Min} + \dfrac{\mathrm{Max} - \mathrm{Min}}{1 + \left(\dfrac{X}{LC_{50}}\right)^{\text{Hill coefficient}}}$

of the applied probit model for the study will provide importance in the use of both, preferably for different ecotoxicological studies.

5. Contribution Statement

The author declares that she has contributed to 100% of the article.

6. Conflict of Interest

The author has no conflicts of interest to declare.

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