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Investigation of diazinon toxicity of water treated with electrochemical process using *Daphnia magna*

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ABSTRACT

Diazinon is an organic phosphorus insecticide with extensive application in agriculture but it contaminates soil and water. The aim of this research was to examine detoxification performance of the electrochemical process to treat water contaminated with diazinon using bioassay tests. This was a batch system experimental study using *Daphnia magna* as the bio-index; divided into two equal groups (with/without electrochemical process application). The electrochemical process was applied for a contact time of 15 min and at the current density 9.55 mA cm^{-2}. Consequently, mortality was counted for *D. magna* in each group during a monitoring period of 168 h. Probit analysis was applied to analyze the experimental data. The results showed that the electrochemical process had a noticeable effect on elimination of diazinon toxicity. LC_{50} values for the exposure times 24, 48, 72, 96, 120, 144, and 168 h were 3.5, 3.2, 2.6, 2.0, 1.2, 0.3, and 0.3 mg L^{-1} for the groups without electrochemical process treatment and 5.2, 4.6, 3.5, 2.5, 2.4, 2.1, and 1.8 mg L^{-1} for the groups that received electrochemical process treatment, respectively. Therefore, electrochemical process caused a decline in toxicity. Although, the electrochemical process could properly decrease the 96-h toxicity and completely eliminate the diazinon pesticide, results of long-term monitoring (168 h) revealed that effectiveness of the electrochemical process on complete elimination of toxicity is not confirmed with certainty.

Keywords: Diazinon; Electrochemical process; Toxicity; *Daphnia magna*

1. Introduction

The organophosphorus group of pesticides has been widely used all over the world. Diazinon (chemical formula; *O*,*O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate and empirical formula; $C_{12}H_{21}N_2O_3PS$) as a cholinesterase pesticide belongs to this organophosphorus group of pesticides. Diazinon is stable at pH 7 and can remain stable in the environment for 6 months [1–3]. A high concentration of diazinon in an aquatic ecosystem can create problems for its aquatic organisms [4] and it is also harmful to aquatic macrofauna such as invertebrates, mammals, and fish [5,6]. Diazinon causes rapid hydrolysis of acetylcholine and inhibition of cholinesterase enzyme, where overstimulation of this enzyme is lethal to living things [2]. According to WHO, diazinon is a middle-risk compound. The WHO has set the permissible limit for aquatic exposure at 350 ng L^{-1} [7,8]. On the other hand, the European Union has banned application of this pesticide for aquaculture [2,9]. It is inevitable that diazinon concentration must be reduced to meet the approved standards for aquatic ecosystems. In recent years, various methods have been applied for removing this pesticide from aqueous solution; these include reverse

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osmosis, biological methods, adsorption by active carbon, oxidation by ozone, and photo-catalytic oxidation [10–15]. Electrochemical treatment has been considered as an environmentally friendly and cost-effective method [16,17]. The electrochemical process works by application of an electric current (alternating and direct current) to a sacrificial anode electrode to produce coagulants in situ. Trivalent irons or aluminum ions are generated by use of aluminum, and/or iron anode electrodes are used as anodes. Electrochemical reactors might be conducted on monopolar and dipolar connections [18,19]. The electrochemical process includes anode scarification and formation of a coagulant, destabilization of the suspended substances and pollutants, and removal of unstable particles using floc production. There are mechanisms that can be applied to destabilize pollutants. The destabilization mechanism that takes place in the electrochemical process includes compression of an electrical double layer, adsorption and charge neutralization, enmeshment in a precipitate and inter-particle bridging [20]. In many developing and developed countries, control of wastewater quality is still performed based on physical and chemical parameters. Consequently, because of economic limitations, only few parameters, such as biochemical oxygen demand and chemical oxygen demand, are considered for evaluations. However, application of distinct parameters cannot be presented as a realistic measurement to judge the level of toxicity in a body of water. Alternatively, use of a chemical-sensitive living organism is valuable in order to test the effectiveness of a removal method [21]. Physical and chemical experiments could not be appropriate for evaluating the potential effects of contaminants on aquatic life. Bioassay tests can provide suitable and direct criterion for toxicity or a lack of toxicity [22–24]. Bioassays have been widely used as a reliable index to evaluate the effects of contamination on an aquatic environment. Simplicity, low cost, effectiveness, and short procedural time are some advantages of bioassay application [5,21,25]. Indeed, the results of bioassay can determine the efficiency of a removal method in relation to the health of biological creatures and eventually to human beings. Various creatures such as species of fish and invertebrates can be employed in the bioassay method. As *Daphnia magna* has a short reproductive duration, it can be easily cultured in a laboratory with few facilities. Furthermore, experimentation time is shorter for *D. magna* compared with other bioassay indicators; hence, it is frequently used as a bio-index for risk analyses of toxins in water [5,21,26]. In recent years, a few studies have been done using the bioassay method to monitor toxicity. Research has determined that *Lebistes reticulatus* fish was a good indicator for bioassay assessment of pharmaceutical wastewater treated by an electrochemical process. The results of that study indicated that the mentioned process did not decrease toxicity to an acceptable level. Another study showed that the electrochemical process had been able to completely remove diazinon [27,28]. Considering the widespread application of diazinon around the world, especially in Iran, along with its damaging effects on the environment, human, and aquatic life, the aim of this study was to determine the effect of the electrochemical process on detoxification of diazinon in water samples. The water samples were collected from the Saadabad river and pesticide was added to the samples.

2. Materials and methods

2.1. Chemical and water samples

This experimental research was conducted on water samples collected from Saadabad river with diazinon added to the samples. Raw water samples were taken from Saadabad river of Barzok, near Kashan. These raw water samples were then transferred to the laboratory, maintained at 4°C. Then, an amount of diazinon (0.5, 1, 2, 2.5, 3, 3.5, 4, 5, and 6 mg L^{-1}) was added to each sample. In addition, one sample of raw water without pesticide was considered as the control in each group. The diazinon (60%) used in these experiments was purchased from Modern Insecticide Ltd. (India).

2.2. Electrochemical process

The electrochemical process was applied to the first group in a separate batch system. The electrochemical processes were not applied to the second group and the other conditions were exactly the same as those of the first group. The reactor used for the electrochemical process included a Plexiglas electrochemical cell with effective volume of 2 L. Stainless steel electrodes were 2.0 mm thick and dimension of 15 × 2.5 × 0.2 cm. The stainless steel electrodes were connected by monopolar mode to DC power and there was a distance of 15 mm between the electrodes. Mixing in the reactor was done with a magnetic stirrer at the rate of 300 rpm. Differences in electric potential and current intensity were measured with voltmeter and ohmmeter (Fig. 1). According to the results of a previous study, the electrochemical process was performed with eight stainless steel electrodes, electrical current density of 9.55 mA cm^{-2}, and reaction time of 15 min [28]. It should be noted that the concentration of diazinon was determined by high pressure liquid chromatography (HPLC; CE 4200, Cecil, UK). Also, diazinon concentration in the blended raw water samples was determined as zero by HPLC.

2.3. Bioassay with D. magna

D. magna was applied as a bio-index to assess the levels of toxicity reduction. *D. magna* population was provided

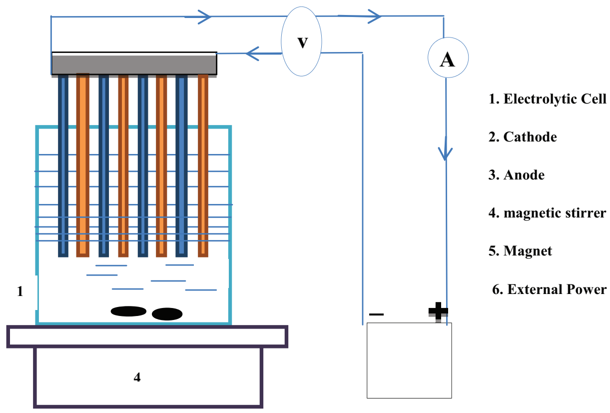

Fig. 1. The schematic diagram of electrochemical reactor.

from the Biology Laboratory of Tehran University. Then this organism was multiplied at the Microbiology Laboratory of Kashan University of Medical Sciences. The reproduction of D. magna was performed by parthenogenesis process [29].

The first group had electrochemical process treatment, while the second group had no electrochemical treatment. After treatments, the contents of the electrochemical cell were poured into containers of the same characteristics in terms of material, volume, and mouth area. Then, 10 one-day-old *Daphnia* were released to containers of both groups, and the status of their mortality was evaluated over specified time durations. To determine toxicity, the numbers of dead *Daphnia* (immobile) were counted and recorded at 168 h. *D. magna* were considered dead, if it remained inactive after spinning the container [30]. Mortality in the controls was considered not more than 10%. The experiments were performed according to bioassay tests in the Standard Methods for Examination of Water and Wastewater [29]. The test was conducted with four replicates in each group. Finally, data were analyzed and lethal concentrations were determined through Probit analysis in SPSS 22 statistical software.

3. Results and discussion

Table 1 shows the physicochemical parameters of river water for the bioassay test. Parameters shown in Table 1 were measured to ensure that diazinon concentrations in the water were the only cause of death of *Daphnia*. Therefore, significant parameters such as pH, dissolved oxygen (DO), and temperature were measured before and during the trial to ensure that they were consistent with the standards (temperature: 22.20 + 0.12, pH: 7.23 + 0.12, DO: 6.97 + 0.12). Results showed that the parameters were maintained according to the standards [31,32].

In this study, 800 *D. magna* were examined in two groups. The first group had electrochemical process while the second group had no electrochemical treatment. The results indicated no mortality at 0.5 mg L^{-1} up to an exposure time of 72 h. The first records of mortality in both groups happened at 96 h. Mortality rate in the second group increased to 9 during the 144-h period. At other concentrations, the increase in mortality rate was greater in the second group than groups treated with the electrochemical process. Statistically, there was a significant relationship determined for status of live *Daphnia* with electrochemical treatment method ($p < 0.001$). For example, with increasing initial concentration of diazinon, there was significant difference observed between the two groups (first and second), mainly within a shorter exposure time. In non-contaminated samples (without addition of diazinon), all *Daphnia* survived up to 168 h (7 d). In addition, observation revealed no mortality within times less than 12 h among the studied concentration range (0–6 mg L^{-1}). This result was recorded in all samples (contaminated or non-contaminated and with or without application of the electrochemical process). The Probit method was used to estimate concentrations that killed 50% of *D. magna*. The Probit analysis procedure produces two chi-square tests for different aspects of the model:

- The Pearson goodness-of-fit chi-square statistic is used to test the null hypothesis that the model adequately fits the data.
- The parallelism test checks to see whether the assumption of equal slopes across factor levels is reasonable. If results of the parallelism test are significant, then there need to be separate analyses for each replicate.

If the null hypotheses of these tests are true, then statistics have chi-square distributions with the displayed degrees of freedom. If the significance value of a given test is small (less than 0.05), then the model does not adequately fit the data. In this case, data do not violate the model assumptions. According to the aforementioned and regarding significance levels determined by Pearson test and parallelism test, it is seen that the significance level of the Pearson test was greater than 0.05; therefore, a significant relationship was determined between test replications. Furthermore, according to significance level of the parallelism test (0.984), which was greater than 0.05, the number of replications was determined as appropriate and logical (Table 2).

The goodness-of-fit statistics are based on cell counts and the residuals table. Cells in the table represent cross-classification of consistency and fatality. Note that the values of fatality shown are natural logarithms of the actual values.

- Thus, the first row of the table pertains to the group that had no exposure to diazinon.
- The observed responses column reports the number of cases observed in the cross-classification data file.
- The expected responses column reports the number of cases that would be expected in the cell if the model was correct.
- The residuals are a measure of difference between observed and predicted values. Large residuals can indicate cells that are not well fitted by the model.

Table 1
Physicochemical parameters of Saadabad river for Daphnia culturing

Parameter	Concentration
Temperature, °C	21–24
pH	7.4–7.9
DO, mg L^{-1}	6.2–7.1
Alkalinity, mg $CaCO_3$ L^{-1}	128–137
Hardness, mg $CaCO_3$ L^{-1}	290–310

Table 2
The chi-square tests for different aspects of the model

Chi-square tests				
		Chi-square	df[a]	Significance
Probit	Pearson goodness-of-fit test	297.523	411	1.000[b]
	Parallelism test	0.160	3	0.984

[a]Statistics based on individual cases differ from statistics based on aggregated cases.
[b]Since the significance level is greater than 0.150, no heterogeneity factor is used in the calculation of confidence limits.

In this study, the chi-square test used for goodness of fit of the two independent variables; diazinon concentration and exposure time that determined 3.41 and 8.65. According to p value of both variables, it concluded that diazinon concentration and exposure time had a direct influence on the mortality of *Daphnia*. In this model, the impact factors of diazinon concentration, exposure time, and tilt numbers were estimated as 8.6067, 0.0181, and 1.73, respectively. Regarding significance level of the Z test in Table 3, results showed significance level less than 0.05; thus, the Probit model estimated parameters efficiently. As a result, the following model was used for estimation. Hence, the mortality equation of *Daphnia* was as follows:

Probit (p) = Intercept + BX (covariates X are transformed using the base 2.718 logarithm)

The natural response rate is the probability that a Daphnia will die if the consistency is increased. The value of 0.158 means that roughly 15.8% of all Daphnia would live with diazinon (Table 4).

The time-dependent patterns of initial concentrations of diazinon for killing 50% of *Daphnia* (LC_{50}) in treated and untreated samples are shown in Fig. 2. As it can be seen, the lethal concentration declined under increased exposure time, while the initial lethal concentration for treated samples was greater than that for untreated samples. This difference can be attributed to the effect of the electrochemical process. In other words, this process reduced diazinon toxicity.

Results obtained from this study illustrate that the electrochemical process can increase the initial lethal concentration of diazinon from 3.5 to 5.2 mg L^{-1} as 24-h LC_{50}. As illustrated in Fig. 3, the electrochemical process can increase the initial LC_{50} value by 1.22 times and toxicity (LC_{50}) declined over exposure time. In addition, the results of this study show that electrochemical treatment was effective in decreasing toxicity of diazinon. LC_{50} values with application of the treatment were greater than groups without the electrochemical process treatment. In addition, LC_{50} values decreased according to increased exposure time. The results of this study revealed a significant difference in mortality rates of *D. magna* between those in treated and untreated water samples up to 2 mg L^{-1} of pesticide. In other words, the number of live *D. magna* in treated water samples contaminated with diazinon was greater than that in untreated water samples. The 48-h LC_{50} of diazinon calculated in this study for *D. magna* was 3.2 mg L^{-1}. It was reported that toxicity of diazinon (48-h LC_{50} value) on *D. magna* was 2.39 mg L^{-1} [33]. Moreover, according to the previous studies, the 24-h LC_{50} value of diazinon on *D. magna* was 5.21 mg L^{-1} [34], and 3.07 mg L^{-1} [35] which is in agreement with this study. According to EPA classification, compounds with LC_{50} greater than 100 mg L^{-1} were classified as relatively non-toxic, while compounds with LC_{50} of 10–100, 1–10, and less than 1 mg L^{-1} were classified as medium, highly toxic, and extremely toxic, respectively. Therefore, based on the results of this study, diazinon was classified as a highly toxic compound [36]. Electrochemical treatment can decrease the short-term toxicity of diazinon on *D. magna*, but the results of death of all *Daphnia* in treated and untreated contaminated water with a longer exposure time (up to 168 h) and their survival in water free of contamination questions whether or not the electrochemical process was effective in decreasing the long-term toxicity of diazinon. It was reported that toxicity of pharmaceutical wastewater following the electrochemical treatment process could not be efficiently decreased through bioassay using *L. reticulatus* [27]. Nevertheless, there were different indicators used in the studies and this should be taken into consideration.

Findings showed that *D. magna* could survive up to 168 h in raw water and electrochemically treated samples with zero pesticide concentration. Hence, it can be concluded that natural death caused by physiological and environmental conditions was not the main reason for *Daphnia* mortality. On the other hand, application of the electrochemical process did not intensify the rate of mortality of *Daphnia*. Therefore, the presence of diazinon was considered as the main cause of death. As the number of dead *Daphnia* in the each case group was less, the treatment process can be accepted as the main inhibitory factor on *Daphnia* mortality.

Electrochemical treatment was able to decrease diazinon toxicity at all concentrations tested in this study, but effectiveness of this reduction was typically considered at low concentration up to 2 mg L^{-1}. This finding was confirmed by a study that surveyed application of the electrochemical process for diazinon removal under similar conditions [28]. Based on the results of that study, the electrochemical process decreased the diazinon concentration from 2.5 mg L^{-1} to 0. Accordingly, the electrochemical process, with a current

Table 3
The parameter estimates of Probit analysis (level of the z test)

Parameter estimates								
	Parameter		Estimate	Standard error	Z	Significance	95% Confidence interval	
							Lower bound	Upper bound
Probit[a]	Time		–0.399	0.060	–6.660	0.000	–0.516	–0.281
	Intercept[b]	1	1.733	0.166	10.459	0.000	1.568	1.899
		2	1.751	0.166	10.529	0.000	1.584	1.917
		3	1.733	0.166	10.438	0.000	1.567	1.899
		4	1.733	0.166	10.443	0.000	1.567	1.899

[a]Probit model: Probit (p) = Intercept + BX (covariates X are transformed using the base 2.718 logarithm).
[b]Corresponds to the grouping variable repeat.

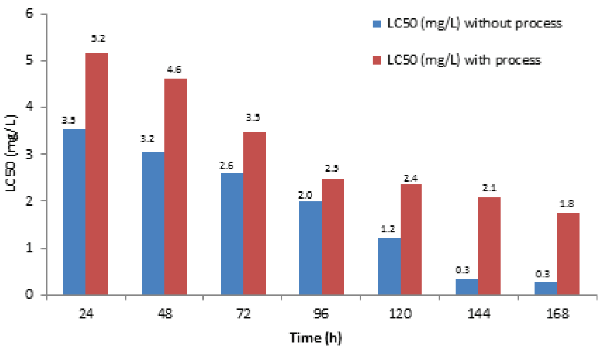

Fig. 2. Comparison of initial concentration for diazinon for 50% *Daphnia* death in treated and untreated samples.

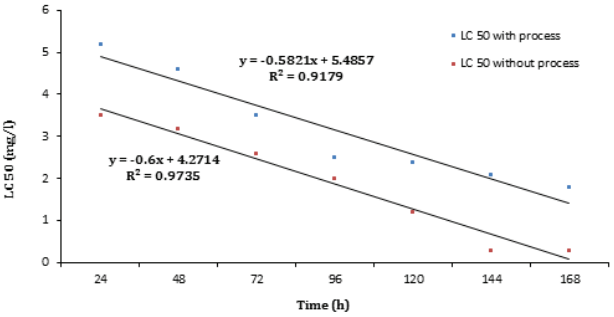

Fig. 3. LC_{50} with/without the electrochemical process in terms of exposure time.

Table 4
The Natural Response Rate Estimate of probit analysis

	Estimate	Standard error
Probit	0.000	0.158

Note: Control group is not provided.

density of 9.55 mA cm^{-2} and reaction time of 15 min was able to decrease diazinon concentration from 2 mg L^{-1} to 0 and reduce short-term toxicity.

4. Conclusion

Increase in the death of *Daphnia* up to 100% within long-term exposure (168 h) for the second group (without electrochemical process treatment) and their survival in control groups and the first group (with electrochemical process treatment) showed that the electrochemical process could increase removal efficiency of the pesticide diazinon and decrease its short-term toxicity. However, long-term toxicity remains a threat to an aquatic environment.

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