Potential Removal of Some Insecticides from Water using Microalgae and their Determination by a Validated UV-Vis Spectrophotometric Method

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ABSTRACT

The present study evaluates the efficiency of microalgae *Chlorella vulgaris* and *Ulva lactuca* for removing fenamiphos, imidacloprid and oxamyl from water. The influence of pH, incubation time, insecticide and biomass concentration on the degradation of insecticides were considered valuable factors in the study. The biosorption experiments were performed by adding 100, 500, and 900 mg/L of alga to the insecticide aqueous solution (50, 250, and 450 mg/L). The experiments were performed at different pHs; 5, 7, and 9 with a time course; of 5, 10, and 15 min. UV-Vis spectrophotometric method was conducted for quantifying insecticides. The optimum conditions from the Plackett-Burman test were obtained at 15 min, pH 5, 50 mg/L insecticide concentration, and 900 mg/L biomass that exhibited a removal percentage of 66.20 % and 61.91% for fenamiphos with *C. vulgaris* and *U. lactuca*, respectively, While, 5 min, pH 9, 50 mg/L pesticide concentration and 900 mg/L algae biomass with the removal of 40.76, 28.44% and 70.28-70.07 % of imidacloprid and oxamyl for *C. vulgaris* and *U. lactuca*, respectively. This study proved that the removal of insecticides by fresh water and marine microalgae *C. vulgaris* and *U. lactuca* is both effective and biomass of algae dependent. Thus, *C. vulgaris* and *U. lactuca* showed a potential reduction of insecticides in polluted water samples. This study will open new channels for a more in-depth understanding of how to remove the insecticide pollutants in the aquatic environment based on microalgae technology.

Keywords: Pesticide removal; *Chlorella vulgaris, Ulva lactuca*; Fenamiphos; Imidacloprid; Oxamyl; UV-Vis spectrophotometric method.

INTRODUCTION

Environmental severe impacts on environmental system have been spotted from the inclusive agricultural use of pesticides. Pesticides save at most yield from pests, inclusive rodents, weeds, fungi, insects, and nematodes (Özkara et al., 2016). However, pesticides may show as pollutants in water sources, harming human health so that very toxicity, carcinogenicity, and mutagenicity or occuring aesthetic problems (Chowdhary et al., 2020). Environmental regulations in developed countries have been very rigorous for drinking water treatment over the last few years, essentially relating to pesticide components (Schwarzenbach et al., 2010).

The methods available are numerous available to treat raw water to separate possible harmful organics before offering it for public use (Radjenovic and Sedlak, 2015). There has been widespread use of conventional treatment methods, inclusive chemical coagulation, sedimentation, clarification, and disinfection, but they have not always proved effective (Bhargava, 2016). In order to create more efficient water treatment systems, several innovative methods have been developed. Due to its efficiency capacity and wide application, adsorption is one of the most frequently used methods (Saravanan et al., 2017). It has also been widely used to remove pesticides from drinking water. Adsorption is an alternative to the traditional method for low operating costs, less polluting problems, and most economical for removing heavy metals.

Biosorption is high technique in the removal of pollutants from effluent. Even at low concentrations, this technique is considered a highly efficient method for removing pollutants. Biosorption is a form of adsorption method that depend of solid-state (sorbent) and liquid-state (solvent). Together viable and nonviable biological materials are coveted to take of the pollutants. The dead material does not need any growth media for its growth, in addition to the main advantage of using biomass over life material. This process uses probable sorbents such as bacteria, yeast, fungi, and algae because of their high efficiency and low cost (Fu and Wang, 2011).

DOI: 10.21608/asejaiqjsae.2022.254546

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Received July 05, 2022, Accepted, August 11, 2022.

Around 40000 microalgae have been described or analyzed so far, representing enormous biodiversity (Hu and He, 2008). Among the most remarkable is the green eukaryotic microalga.

According to scientific studies, marine algae, also known as seaweeds, have high pollutant binding capacities because their cell walls contain polysaccharides, proteins, or lipids that contain functional groups such as amino, hydroxyl, and sulphate, that can serve as binding sites for pollutants.*Ulva lactuca*, green macroalgae called green sea lettuce or green laver usually found in coastal areas, it has been widely studied for its ability to accumulate pollutants, as a bioindicator, and as a remediation agent (Areco et al., 2021).

Fenamiphos, ethyl 3-methyl-4-(methylsulfanyl) phenyl isopropyl phosphoramidate, an organophosphorus insecticide, is applied to against a lot of nematode pests. In addition, it can be applied on plant growth phases such as plants and some kinds of cereals (Hsu et al., 2022). However, the critical reverse ecological effects of fenamiphos have cactually beginning to attract attention. LD_{50} rates of insecticide vary among1.0 and 20.0 mg/kg in rats and 55.0 and 95.0 mg/kg in pigs (Qader et al., 2021). Imidacloprid **(**1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-4,5-dihydro-1H-imidazol-2-amine) is neonicotinoids are broad-spectrum insecticides that have excellent systemic and contact activity, making them suitable for use on a wide range of crops, turf, ornamentals, and termites as well as fleas and ticks (Bhandari et al., 2019). In addition, it has been found in soil, groundwater, wetlands, dust, vertebrate prey, and food (Craddock et al., 2019; El-Dewy et al., 2018). Oxamyl, (N, N-dimethylcarbamoyloxyimino-2- (methylthio) acetamide) is a carbamate compound. Oxamyl is systemic and used as an insecticide and nematicide (Ghareeb et al., 2019). Solubility of oxamyl in water high (280 g /L) and is characterized by high acute toxicity (LD50 = 2.5 mg/Kg). It controls nematodes in various plants, vegetables, cotton, Soya beans, potatoes, and other crops. Ground and surface water resources can easily be contaminated by it (Arrington et al., 2016).

This study aimed to estimate the capability of *C. vulgaris* and *U. lactuca* to remove fenamiphos, imidacloprid, and oxamyl from water. In addition, to find out the effect of different physicochemical parameters; pH, incubation time, insecticide concentration, and algal biomass concentration on insecticide removal based on the Plackett-Burman design.

MATERIALS AND METHODS

1. Chemical and reagents

Analytical standard of fenamiphos (99%, (*RS*)-*N*-[Ethoxy-(3-methyl-4-methylsulfanylphenoxy)phosphoryl]propan-2-amine) was purchased from Miles Inc, Co. (Stilwell, Kansas, USA). Imidacloprid (99%, 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-4,5-dihydro-1H-imidazol-2-amine) was purchased from Bayer AG **Co., (**Leverkusen, Germany). Oxamyl (98.89%, methyl (1Z)-2-(dimethyl amino)-N-[(methyl carbamoyl) oxy]-2-oxoethanimido thioate**))** was supplied by Syngenta. Fenamiphos formulation (Nemaphos 40% EC) was obtained from Bridge Trade Co. (Cairo, Egypt). Imidacloprid formulation (Imidor® 35% SC) was obtained from Chema Industries Co. (26, 1st Industrial Zone, New Nubaria city, Behira, Egypt). A commercial formulation of oxamyl (Vydate 24% SL) was obtained from Agrimatco Egypt Co. (Giza, Egypt). The chemical structures of the studied pesticides are presented in Figure 1.

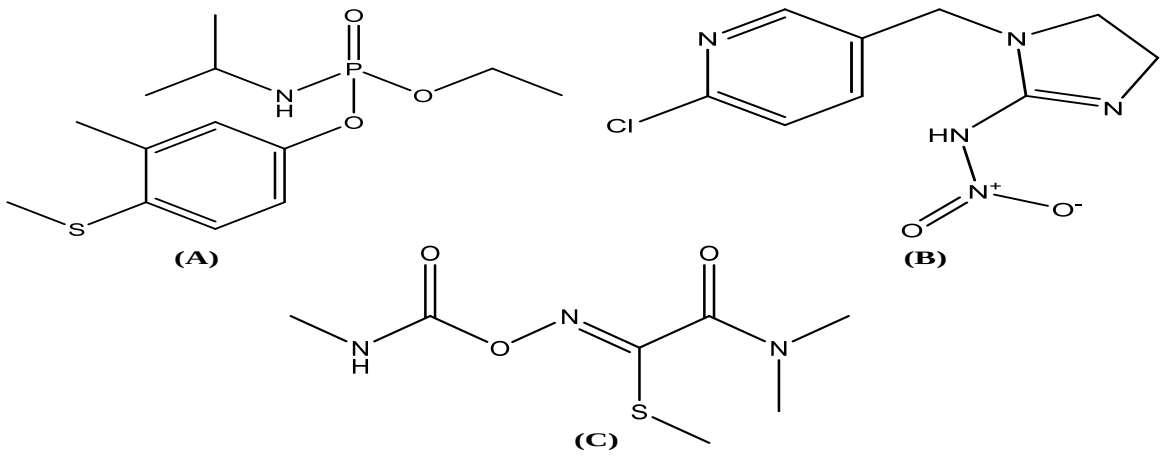

Figure 1. Chemical structures of fenamiphos (A), imidacloprid (B), and oxamyl (C).

All solvents and reagents were used without further purification. In order to prepare pesticide stock solutions, accurately weighted quantities of methanol (HPLC grade) were dissolved in 1 mg/L. To achieve the necessary concentrations for calibration curves, the stock solutions were diluted with methanol to prepare the working solutions for UV-Vis spectrophotometric analysis.

2. Algal cultures

Biomass of microalgae *C. vulgaris* and *U. lactuca* were obtained from National Research Center (33 El Bohouth St, Dokki, Giza, Egypt). First, the algae were washed totally using deionized water, shade dried for 24 h, after that dried at 50ºC in oven while a stable weight was obtained. Afterwards, the dried biomass was crushed with an analytical mill, sieved by sieve shaker to select particles size with an average of 500 mesh, and stocked in polyethylene bottles until used. The characteristics of *C. vulgaris* and *U. lactuca* were conducted by the Near-infrared spectroscopy (NIRS) instrument as shown in Table 1.

Table 1. Characteristics of *C. vulgaris* and *U. lactuca* biomass by NIRS instrument

Characteristics	Percentages (%)	
	C. vulgaris	*U. lactuca*
Ash	10.43	12.2
Carbohydrates	13.46	26.84
Fat	18.84	8.12
Fiber	10.1	11.67
Moisture	11.4	14.6
Nitrogen	13.46	26.84
Protein	35.78	26.56

2.3. Experimental design

Experimental designs allow for studying the impact of various variables with a restricted numeral of experiments. Statistical analysis of results will appear that variables significantly impact and correlate the desired response with the variables by polynomial equations. Plackett-Burman factorial design was used to distinguish the most crucial factors early in the experimentation stage when complete knowledge about the system is generally unavailable. Minitab 19.1 software (Minitab Inc. State College, PA) (Minitab, 2019) was used to generate this experimental design for four factors of the current experiments. The Plackett-Burman factorial design utilized in this study to get together dependent and independent variables using the vassal polynomial model: $Y_1 = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_nX_n$ where Y is the dependent variable, A_0 the constant and A_1 to A_n are the coefficients of the independent values, and X_1 to X_n are the independent factors. Eighteen experimental trials involving four independent variables were produced by Minitab software. The independent variables screened were the concentration of pesticide (X_1), the concentration of biomass (X_2), incubation time (X_3), and pH (X_4). All variable were examined at three levels, including low (-), high (+), and basal (0) as shown in Table 2. Biosorption or pesticide removal was determined as the dependent variable (response data). After creating the design, conducting the experiment, and obtaining the response data (biosorption or removal %), the final data were entered in the worksheet, and statistical analysis was performed through Stat > DOE > Factorial > Analyze Factorial Design.

4. Adsorption experiment

A batch of biosorption experiments was performed by adding 100, 500, and 900 mg/L of alga to the Erlenmeyer flasks containing the insecticide aqueous solution (50, 250, and 450 mg/L). The experiments were performed at different pH (5, 7, and 9). A series of Erlenmeyer flasks containing insecticide solution and alga was stirred (200 rpm) on a magnetic multi-stirrer (Velp Scientifica, Italy) at time course (5, 10, and 15 min) at 27±1.0°C. Samples were withdrawn from the stirrer at certain moments and were filtered (0.22 µm). The supernatant contained the remaining insecticide in water after adsorption on the alga. The detection of insecticide was done by using UV-Vis Spectrophotometer. The maximum absorbencies for fenamiphos, and oxamyl were 220, 252, and 234 nm, respectively.

5. Statistical analysis

Statistical analysis was thorough using the IBM SPSS software version 25.0 (SPSS, Chicago, IL, USA) (IBM, 2017). Means and standard error (SE) were obtained from three independent replications for every treatment. Analysis of variance (ANOVA) was performed, and means particular values were separated ($p \leq 0.05$) with Student-Newman-Keuls (SNK). Minitab 19.1 software (Minitab Inc. State College, PA) (Minitab, 2019) was applied to plan the experiments and modeling. The residuals were plotted (scatter, histogram, and normal probability) in order to verify the model's adequacy.

RESULTS AND DISCUSSION

1. Characteristics of *C. vulgaris* and *U. lactuca*

NIRS has significant potential for analyzing algae samples. The potential applications of this spectroscopic method for rapid and inexpensive analyzing algae are obvious.

Table 2. Experimental design by Plackett-Burman factorial design for removal of pesticides by *C. vulgaris* and *U. lactuca*

Experimental number	pH	Incubation time (min)	Pesticide (mg/L)	Biomass (mg/L)
1	9 (+)	5 (-)	50 (-)	100 (-)
2	9 (+)	15 (+)	50 (-)	100 (-)
3	9 (+)	15 (+)	450 (+)	100 (-)
4	9 (+)	15 (+)	450 (+)	900 (+)
5	5 (-)	15 (+)	450 (+)	900 (+)
6	9 (+)	5 (-)	450 (+)	900 (+)
7	5 (-)	15 (+)	50 (-)	900 (+)
8	9 (+)	5 (-)	450 (+)	100 (-)
9	9 (+)	15 (+)	50 (-)	900 (+)
10	5 (-)	15 (+)	450 (+)	100 (-)
11	5 (-)	5 (-)	450 (+)	900 (+)
12	9 (+)	5 (-)	50 (-)	900 (+)
13	5 (-)	15 (+)	450 (+)	100 (-)
14	5 (-)	15 (+)	50 (-)	100 (-)
15	5 (-)	5 (-)	450 (+)	100 (-)
16	5 (-)	5 (-)	50 (-)	900 (+)
17	5 (-)	5 (-)	50 (-)	100 (-)
18	7(0)	10(0)	250(0)	500(0)

The sign in parentheses indicates the level of each factor as low (-), high (+) and basal (0).

These methods require only one spectrum, available in minutes at most, to provide values for as many analytes (Mulbry et al., 2012). The percentage of the protein content of powder of the microalgae *C. vulgaris* was found to be 35.78%, while the carbohydrate content and fat were 13.46 and 18.84%, respectively (Table 1). The powder contains 13.46% nitrogen and 10.10% crude fiber. The moisture and ash were 11.4% and 10.43%, respectively.

The percentage of the protein content of the microalgae *U. lactuca* was found to be 26.56%, while the carbohydrate content and fat were 26.84% and 8.12%, respectively. The powder contains 26.84% nitrogen and 11.67% crude fiber. The moisture and ash were 14.6% and 12.20%, respectively. Macroalgae, *U. lactuca* contain amounts of cellulose (38–52%), percentage of proteins (up to 30%), and minimal amounts of lipids (1.9%) (Dominguez and Loret, 2019).

2. Optimization of pesticide removal by algal biomass

In order to establish the efficiency of *C. vulgaris* and *U. lactuca* biomass as biosorbents for the tested pesticides, removal experiments were conducted at different parameters, including the pesticide concentration (mg/L), algal biomass (mg/L), incubation time (min) and pH, at three several levels (-, 0, and +) agreement to the Plackett-Burman factorial design. In addition, the minimum number of experimental runs (17) and a center point (medium level) were performed as shown in Table 2. In order to achieve maximum response, this protocol examines the interaction between the variables and determines the optimum concentration of every factor. Tables 3 and 4 represent the styling matrix of the coded variables both by the experimental results for removing fenamiphos, imidacloprid, and oxamyl, by *C. vulgaris* and *U. lactuca*, respectively. In general, the data detecteda major difference in the removal (%) relying on the levels of the four independent variables.

The results of fenamiphos, imidacloprid, and oxamyl removal (%) by *C. vulgaris* biomass at different parameters as shown in Table 3. The results of fenamiphos showed that the highest removal (66.09 and 65.56%) were obtained with experiments 7 and 16, which used the highest algal biomass (900 mg/L) and the lowest pesticide concentration (50 mg/L) with pH 5. Experiments 9, 12, and 17 proved 57.95, 54.66, and 56.33%, respectively. However, trials 3 and 8 exhibited the lowest removal efficiency (7.35-6.29%). The

experiment of the center point (18), which used median algal biomass (500 mg/L) and the median pesticide concentration (250 mg/L) with incubation for 10 min, proved 21.37% removal. The results of imidacloprid showed that the highest removal (40.76%) was obtained with experiment 7, which used the highest algal biomass (900 mg/L) and the lowest pesticide concentration (50 mg/L) with incubation for 15 min. It was followed by trial 16 with a removal of 31.28%. However, trials 3, 8 and 13 exhibited the lowest removal efficiency (7.38, 7.44 and 7.81%, respectively). The experiment of the center point (18) demonstrated 15.65% removal. The removal percentage of oxamyl are shown in Table 3. The highest removal value was obtained with experiment 12 (70.28%).

The results of fenamiphos, imidacloprid, and oxamyl removal (%) by *U. lactuca* biomass at different parameters as shown in Table 4. The results of fenamiphos showed that the highest removal (62.02 and 61.93%) were obtained with experiments 7 and 9 which used the highest algal biomass (900 mg/L) and the lowest pesticide concentration (50 mg/L) with incubation for 15 min. Experiments 12 and 16 proved 58.64 and 59.66%, respectively. However, trials 3 and 8 exhibited the lowest removal efficiency (7.41-4.70%, respectively). The experiment of the center point (18), which used median algal biomass (500 mg/L) and the median pesticide concentration (250 mg/L) with incubation for 10 min, proved 25.0% removal. In general, this algal biomass is not suitable for removing or absorbing imidacloprid, where the removal % was very low and ranged from 5.71% to 28.44%. The results of imidacloprid showed that the highest removal (28.44%) was obtained with experiment 16, which used the highest algal biomass (900 mg/L) and the lowest pesticide concentration (50 mg/L) with incubation for 5 min. However, trials 8 and 15 exhibited the lowest removal efficiency (6.89 and 5.71%, respectively). The experiment of the center point (18) indicated 22.30% removal. The removal percentage of oxamyl are shown in Table 4. The highest removal value was obtained with experiment 12 (70.07%).

These models expected which individual parameters conducted in the existence of others on the removal efficiency of pesticides by algal biomass. As seen in these six equations, the pH (X_1), the incubation time (min) (X_2), pesticide concentration (mg/L) (X_3) and algal biomass (mg/L) (X_4) had a significant effect on the pesticide removal (%). However, both the incubation time and algal biomass should be high for the signs of the regression coefficient are positive. At the same time, pesticide concentration and pH should be as low as potential in order to the signs of the regression coefficients are passive in these models.

Based on these quantitative data, the first-order polynomial equations (1-6) and their corresponding coefficients for every response factor in the factorial design test were progressed as follows.

Removal (%) = 63.46 - 1.640 pH + 0.168 incubation time - 0.10486 pesticide concentration + 0.01264 algal biomass - 12.40 ……...(1)

r^2 =0.98 , s = 3.21415

Removal (%) =54.53-0.897 pH +0.145 incubation time -0.10083 pesticide concentration + 0.01520 algal biomass -7.09 ……...(2)

r^2 =0.98 , s = 2.73302

Removal (%) =22.70-1.109 pH +0.300 incubation time -0.02927 pesticide concentration + 0.01230 algal biomass -1.12 ……...(3)

r^2 =0.77, s = 4.87878

Removal (%) =14.36-0.580 pH +0.107 incubation time -0.01258 pesticide concentration + 0.00731 algal biomass +10.42 ……...(4)

r^2 =0.68 , s = 3.48757

Removal (%) = 45.25-0.552 pH + 0.417 incubation time -0.08299 pesticide concentration + 0.02310 algal biomass -5.54 ……...(5)

r^2 =0.85 , s = 9.03199

Removal (%) = 60.35+0.938 pH + 0.123 incubation time -0.10670 pesticide concentration + 0.00570 algal biomass -5.74C ……...(6)

r^2 =0.98 , s = 3.20496

Furthermore, the correlation coefficient of the incubation time is higher in six models (0.168, 0.145, 0.300, 0.107, 0.417 and 0.123 in Equations 1, 2, 3, 4, 5 and 6, respectively), indicating that it has a more significant influence. The goodness of fit of the model was tested using the determination coefficient (R^2). In this condition, (R^2) value was counted to be fenamiphos (0.98and0.98 (equations 1 and 2) for *C. vulgaris* and *U. lactuca*, respectively), imidacloprid (0.77 and 0.68 (equations 3 and 4) for *C. vulgaris* and *U. lactuca*, respectively) and oxamyl (0.85 and 0.98 (equations 5 and 6) for *C. vulgaris* and *U. lactuca*, respectively).

Model of regression with R^2 near to 1.0 is deemed a very high correlation. Thereafter, the present R^2 value appeared a very good suitable through the observed and predicted responses and implicit that the model is dependable for predicting pesticide removal (%). Therefore, these models were used to predict the removal (%) of pesticides. The results in Tables 3 and 4 announce that the models are useful and can theoretically be used to calculate and predict pesticide removal (%).

Additionally, the influence of each factor on the pesticide removal by algal biomass was also evaluated with Pareto charts (Figures 2 and 3 for *C. vulgaris* and *U. lactuca*, respectively). Figures 2A, 2B, and 2C illustrate the Pareto charts for the removal (%) of fenamiphos (A), imidacloprid (B), and oxamyl (C) at $\alpha = 0.05$. These charts provide clear visualization of the effect of the factors and indicate that the incubation time, pesticide concentration, and algal biomass were significant in their effect on the removal or absorption of fenamiphos at $\alpha = 0.05$ (Figure 2A), as announce that magnitude more significant than the limit of the line label. Figure 2B represents the Pareto chart for removal efficacy of imidacloprid. This chart demonstrates that pesticide concentration and the algal biomass had the highest significant effects on the removal efficiency at $\alpha = 0.05$. Another factor (pH) showed significant rates minimal the reference line (2.093 at $\alpha = 0.05$). The Pareto pattern for oxamyl removal in Figure 2C shows that the pesticide concentration had the highest significant effect, followed by the algal biomass. The incubation time and pH were leasted the reference line (2.093 at $\alpha = 0.05$).

Table 3. Removal (%) of fenamiphos, imidacloprid and oxamyl by *C. vulgaris* biomass at different parameters

Experimental number	Experimental removal (%) ± SE of fenamiphos	Predicated removal (%)	Experimental removal (%) ± SE of imidacloprid	Predicated removal (%)	Experimental removal (%) ± SE of oxamyl	Predicated removal (%)
1	39.55^{g}±1.84	45.56	9.72^{ab} ±0.80	13.99	17.03^{c}±0.31	40.52
2	45.45^{h}±1.31	47.24	11.11^{ab} ±3.21	16.99	52.06^{g}±0.38	44.70
3	7.35^{ab}±0.20	5.30	7.38^{a}±0.11	5.28	17.64^{c}±0.29	11.50
4	17.70^{e}±0.68	15.41	14.61^{bc}±0.05	15.12	22.99^{cd} ±0.35	29.98
5	18.86^{e}±1.32	21.97	13.19^{ab}±0.21	19.56	20.72^{cd} ±0.28	32.19
6	13.58^{d}±0.34	13.73	15.01^{bc}±0.04	12.12	22.15^{cd} ±0.28	25.80
7	66.09^{l}±0.50	63.92	40.76^{f}±1.37	31.27	61.90^{h} ±1.10	65.38
8	6.29^{a}±0.48	3.62	7.44^{a} ±0.18	2.28	6.89^{a} ±0.25	7.33
9	57.95^{k}±0.66	57.36	20.83^{bc} ±0.80	26.83	65.48^{hi}±2.06	63.17
10	10.29^{c}±0.33	11.86	8.06^{a}±0.19	9.72	25.13^{d} ±1.81	13.71
11	16.54^{e}±0.31	20.29	11.77^{ab} ±0.65	16.55	32.84^{e}±0.51	28.01
12	54.66^{j}±0.20	55.68	21.11^{cd} ±2.57	23.83	70.28^{i}±0.38	59.00
13	12.97^{d}±0.23	11.86	7.81^{a} ±0.04	9.72	19.27^{cd} ±0.22	13.71
14	49.57^{i}±0.62	53.81	20.14^{cd}±3.15	21.43	35.83^{e} ±2.68	46.91
15	9.17^{bc}±0.31	10.18	9.74^{ab} ±0.41	6.71	11.34^{b}±0.17	9.54
16	65.56^{l}±0.19	62.24	31.28^{e}±1.37	28.26	60.71^{h}±0.69	61.21
17	56.33^{jk}±0.56	52.13	15.88^{bc} ±2.05	18.42	43.10^{f}±4.27	42.73
18	21.37^{f}±0.23	21.37	15.65^{bc} ±0.20	15.65	30.82^{e} ±1.37	30.82

Values in the column with different letters (a-l) are significantly different at $p \leq 0.05$ using one-way analysis of difference (ANOVA) followed by the Student-Newman-Keuls. * Predicted removal calculated from models 1,3 and 5.

Table 4. Removal (%) of fenamiphos, imidacloprid and oxamyl by *U. lactuca* at different parameters

Experimental number	**Experimental removal (%)±SE of fenamiphos**	**Predicated removal (%)**	**Experimental removal (%)±SE of imidacloprid**	**Predicated removal (%)**	**Experimental removal (%)±SE of oxamyl**	**Predicated removal (%)**
1	39.20^{f}±2.30	43.66	9.72^{bcd}±0.48	9.77	65.94^{fg}±0.25	21.96
2	42.05^{fg}±1.97	45.11	7.22^{ab}±0.96	10.85	69.20^{g}±1.00	70.43
3	7.41^{ab}±0.17	4.78	7.82^{ab}±0.14	5.81	16.99^{a}±0.36	19.43
4	15.64^{cd}±0.17	16.94	13.77^{d}±0.04	11.66	27.52^{b}±0.52	22.77
5	17.86^{d}±0.25	20.53	10.06^{bcd}±0.52	13.98	26.27^{b}±0.17	65.86
6	13.87^{bcd}±0.17	15.49	9.80^{bcd}±0.39	10.59	34.30^{c}±3.43	19.43
7	62.02^{h}±0.87	60.86	20.38^{f}±1.92	19.01	67.14^{fg}±1.10	22.77
8	4.70^{a} ±0.65	3.33	6.89^{ab}±0.68	4.74	19.12^{a}±2.31	69.20
9	61.93±2.30	57.27	16.94^{e}±0.16	16.69	65.48^{fg}±2.06	62.11
10	10.57^{bcd}±0.17	8.36	7.10^{ab}±0.37	8.14	20.62^{a}±0.12	21.96
11	16.26^{cd}±0.31	19.08	10.97^{bcd}±1.49	12.91	21.70^{a}±0.51	66.67
12	58.64^{h}±0.39	55.83	10.56^{bcd}±0.96	15.62	70.07^{g}±1.00	18.21
13	9.69^{abc} ±1.01	8.36	7.13^{ab}±1.51	8.14	19.94^{a}±0.17	60.89
14	47.64^{g}±0.50	48.70	11.85^{cd}±1.10	13.17	62.14^{f}±0.55	21.96
15	10.57^{bcd}±0.17	6.92	5.71^{a}±1.58	7.06	20.06^{a}±0.72	22.77
16	59.66^{h}±0.37	59.41	28.44^{g}±1.64	17.94	63.10^{f}±0.69	69.20
17	47.32^{g}±5.52	47.25	10.43^{bcd}±1.37	12.10	62.86^{f}±0.83	65.86
18	25.00^{e}±0.47	25.00	22.30^{f}±0.13	22.30	38.58^{d}±0.37	22.77

Values in the column with different letters (a-h) are significantly different at $p \leq 0.05$ using one-way analysis of difference (ANOVA) followed by the Student-Newman-Keuls. * Predicted removal calculated from models 2, 4 and 6.

Figures 3A, 3B, and 3C (for *U. lactuca*) illustrate the Pareto charts for the removal (%) of fenamiphos (A), imidacloprid (B), and oxamyl (C) at $\alpha = 0.05$. These charts indicate that pesticide concentration, algal biomass and pH were significant in their effect on the removal of fenamiphos at $\alpha = 0.05$ (Figure 3A) as announce by their magnitude more remarkable than the limit of the line label. Figure 3B represents the Pareto chart for removal efficacy of imidacloprid. This chart demonstrates that the algal biomass and pesticide concentration had high significant effects on the removal efficiency at $\alpha = 0.05$. Other factors (pH and the incubation time) showed significant values minimun the reference line (2.093 at $\alpha = 0.05$). The Pareto pattern for oxamyl removal in Figure 3C shows that the pesticide concentration had the highest significant effect, followed by the algal biomass. The incubation time showed values minimum the reference line (2.09 at $\alpha = 0.05$).

To expression results of the experimental in the shape of surface plots inverts the reactive effects of examined independent variables (Figures 4-9). The three-dimensional response surface diagram is the graphical representation of the regression equation. The removal (%) is produced to shape the prudent pair of the three factors, with one variable permanent at the optimal level. The other two variables vary inwards the experimental scope. The optimum rate of every variable was located based on the hump in the 3D plot. Figure 4 shows that the optimum fenamiphos removal (%) appeared at basal rates of the three variables. Table 3 revealed an optimum response at an incubation time of 15 min, pesticide concentration of 50 mg/L and the algal biomass of *C. vulgaris* 900 mg/L with a response of 66.09%. Figure 6 shows that the optimum imidacloprid removal (%) appeared at basal rates of these three variables. Dissolving the model acceded to the data obtained from Table 3 show an optimum response at an incubation time of 15 min, pesticide concentration of 50 mg/L and algal biomass of *C. vulgaris* 900 mg/L with the removal of 40.76%. Figure 8 appear that the maximum oxamyl removal (%) emerged at basal rates of these three variables. Dissolving the model according to the data gained from Table 3 revealed an excellent response at an incubation time of 5 min, pesticide concentration of 50 mg/L and the algal biomass of *C. vulgaris* 900 mg/L with the removal of 70.28%.

Figure 5 appear that the optimum fenamiphos removal (%) show at basal rates of the four variables. Dissolving the model acceding to the data gained from Table 4 (for *U. lactuca*) revealed an optimum response at an incubation time of 15 min, pesticide concentration of 50 mg/L and the algal biomass of *U. lactuca* 900 mg/L with the removal of 62.02%. Figure 7 shows that the optimum imidacloprid removal (%) emerged at pH 5, incubation time 15 min, pesticide concentration 50 mg/L and algal biomass of *U. lactuca* 900 mg/L with 28.44% removal (Table 4). Figure 9 appear that the optimum oxamyl removal (%) show that basal levels of these four variables. According to the data obtained from Table 4, the optimum conditions were pH 9, incubation time 5 min, pesticide concentration 50 mg/L and the algal biomass of *U. lactuca* 900 mg/L with 70.07% removal.

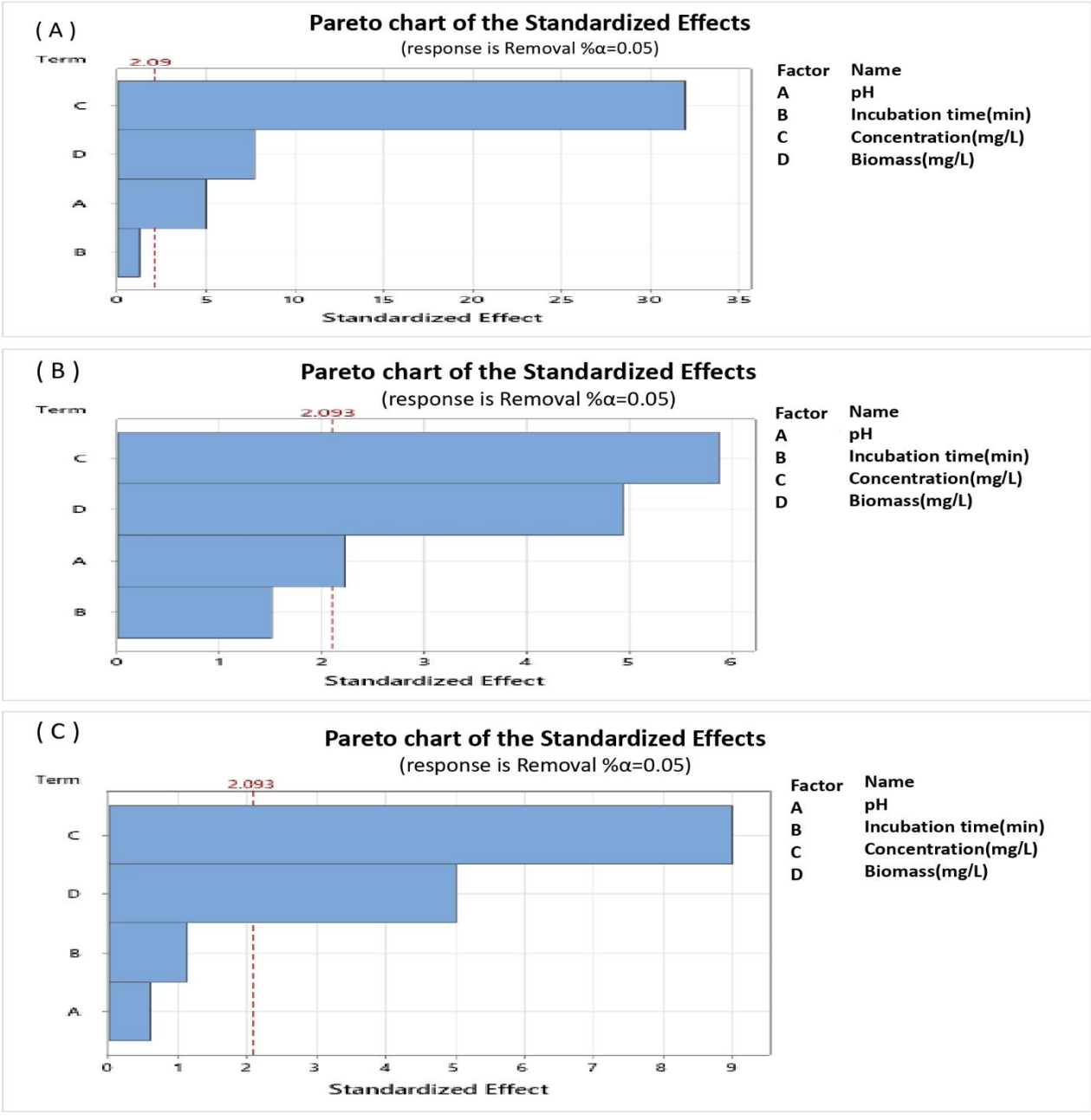

Figure 2. Pareto chart of standardized effect of *C. vulgaris* and incubation time and concentration disappearance of pesticides: (A) fenamiphos, (B) imidacloprid, and (C) oxamyl.

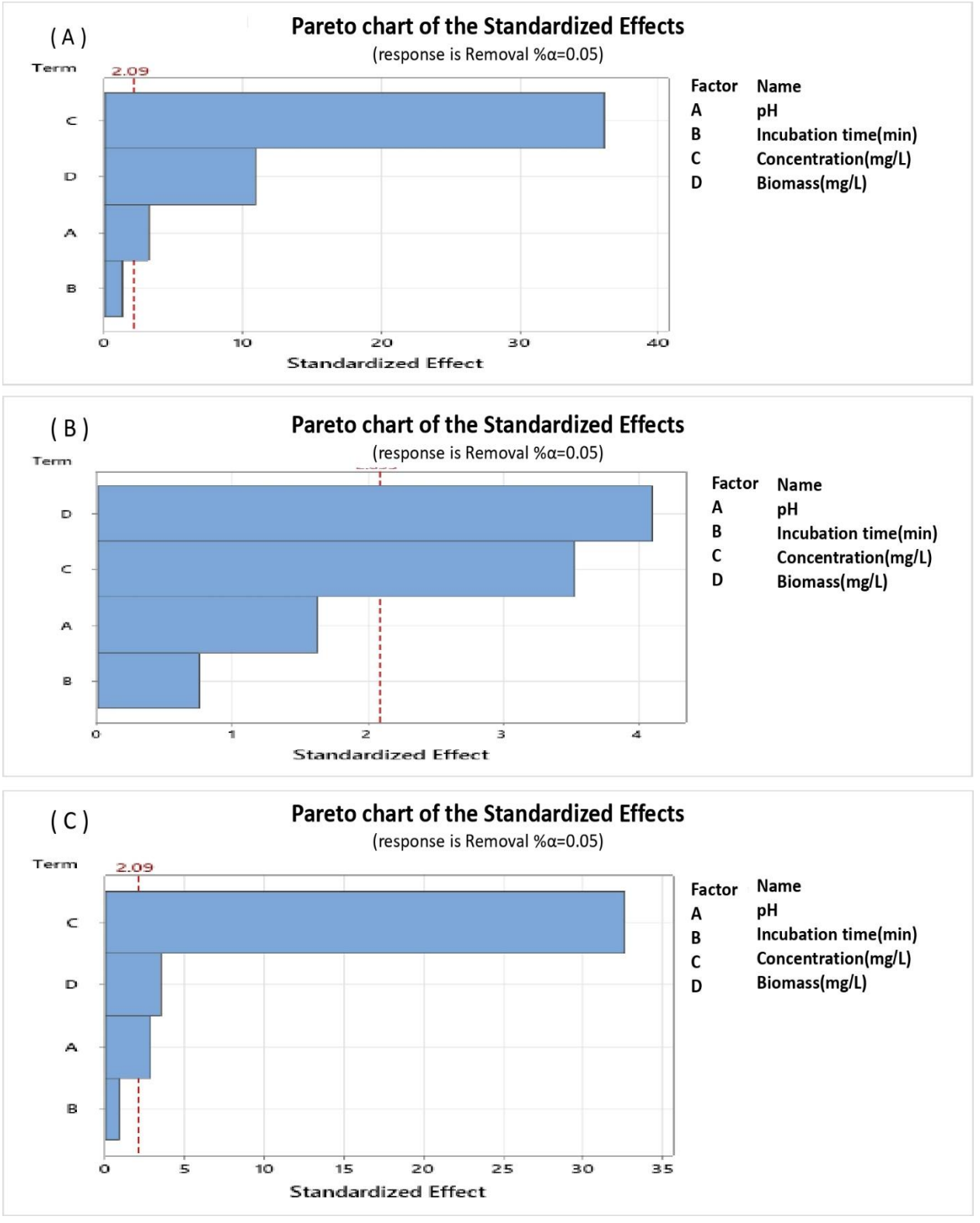

Figure 3. Pareto chart of standardized effect of *U. lactuca* and incubation time and concentration disappearance of pesticides: (A) fenamiphos, (B) imidacloprid, and (C) oxamyl.

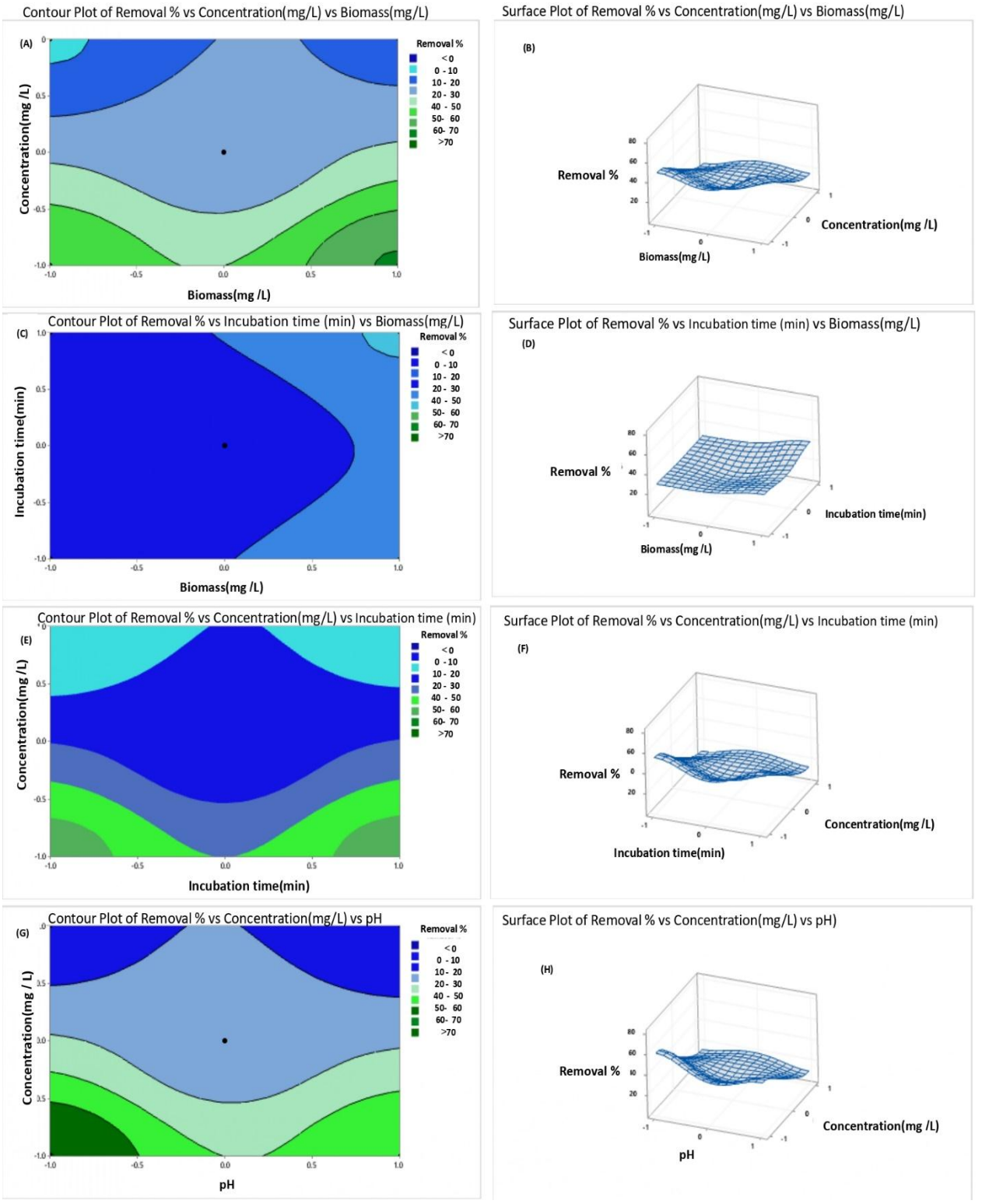

Figure 4. 3D-response surface plots (left) and two-dimensional contour plots (right). (A and B): Impact of concentration and biomass (*C. vulgaris*) on removal of fenamiphos. (C and D): Impact of incubation time and biomass on removal of fenamiphos. (E and F): Effect concentration of fenamiphos and incubation time and biomass on removal of fenamiphos.

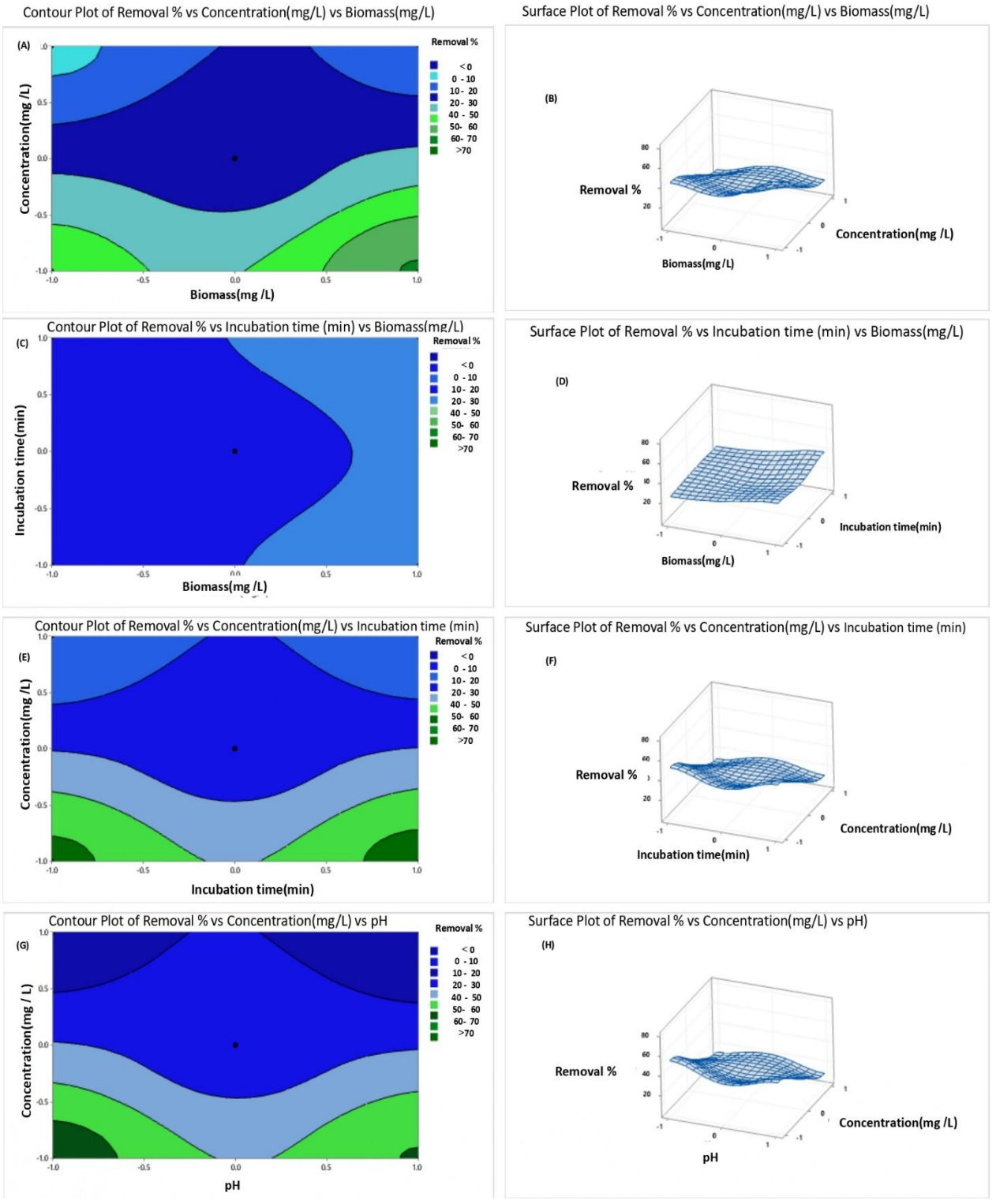

Figure 5. 3D-response surface plots (left) and two-dimensional contour plots (right). (A and B): Impact of concentration and biomass (*U. lactuca*) on removal of fenamiphos. (C and D): Impact of incubation time and biomass on removal of fenamiphos. (E and F): Effect concentration of fenamiphos and incubation time and biomass on removal of fenamiphos.

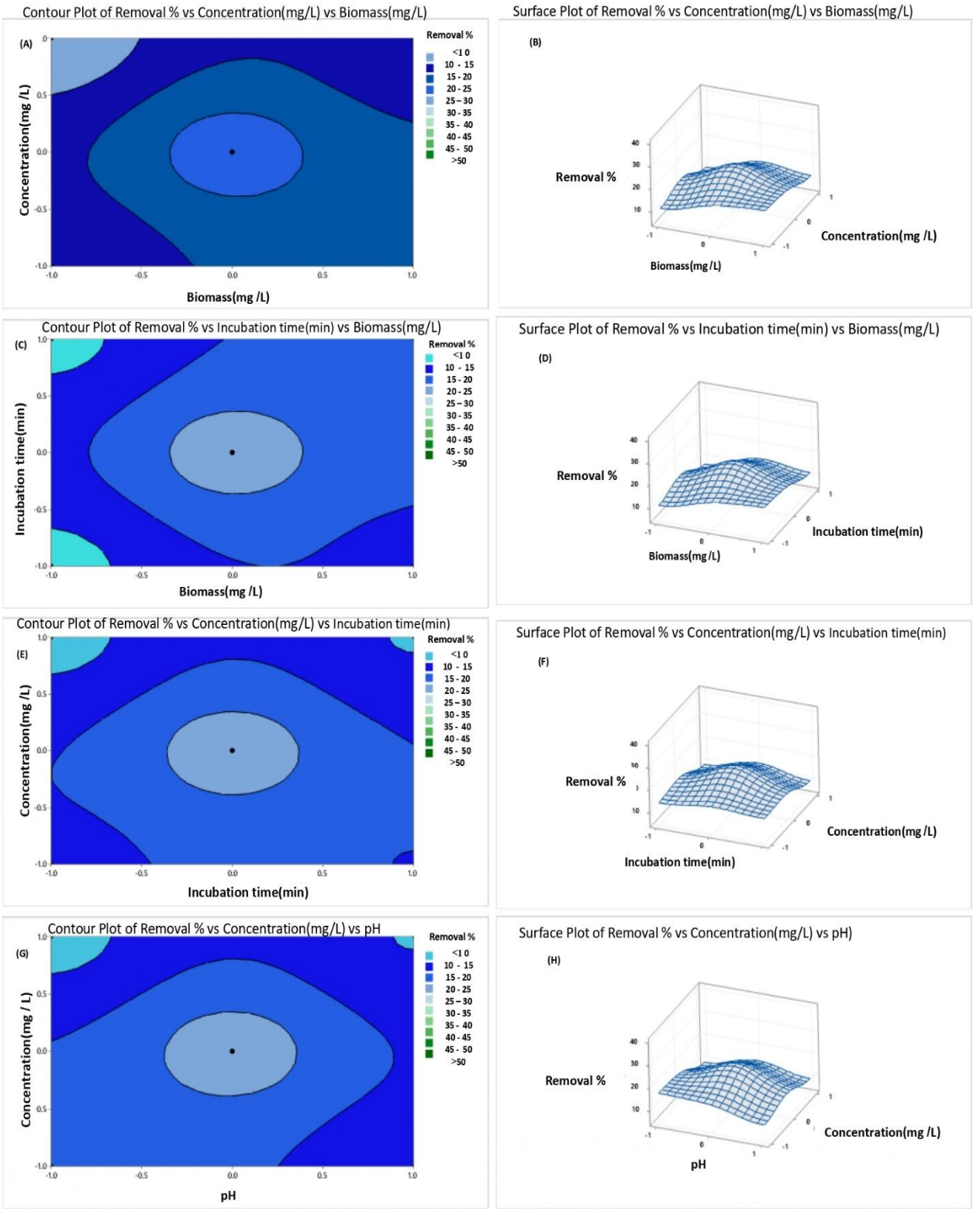

Figure 6. 3D-response surface plots (left) and two-dimensional contour plots (right). (A and B): Impact of concentration and biomass (*C. vulgaris*) on removal of imidacloprid. (C and D): Impact of incubation time and biomass on removal of imidacloprid. (E and F): Effect concentration of imidacloprid and incubation time and biomass on removal of imidacloprid.

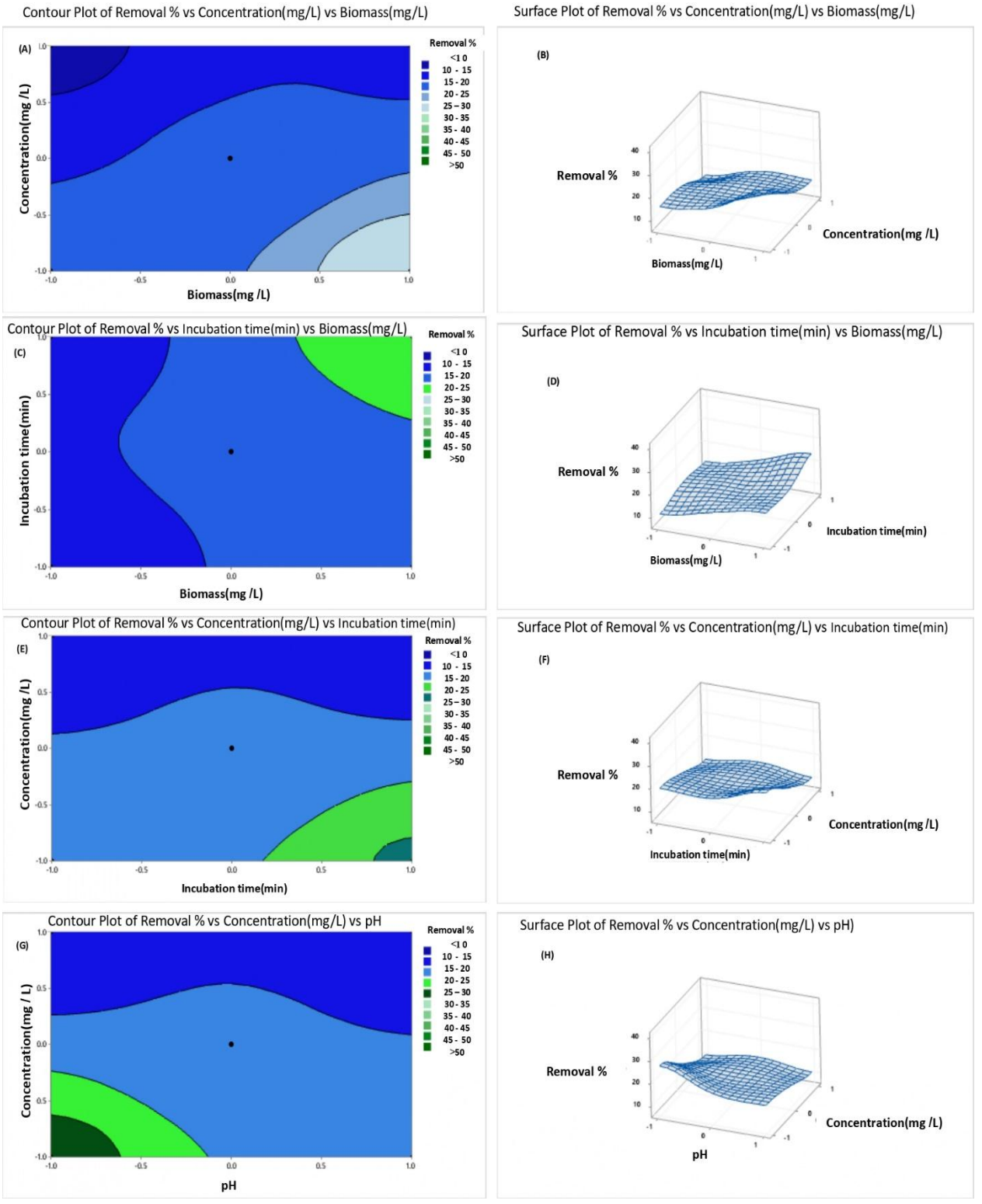

Figure 7. 3D-response surface plots (left) and two-dimensional contour plots (right). (A and B): Impact of concentration and biomass (*U. lactuca*) on removal of imidacloprid. (C and D): Impact of incubation time and biomass on removal of imidacloprid. (E and F): Effect concentration of imidacloprid and incubation time and biomass on removal of imidacloprid.

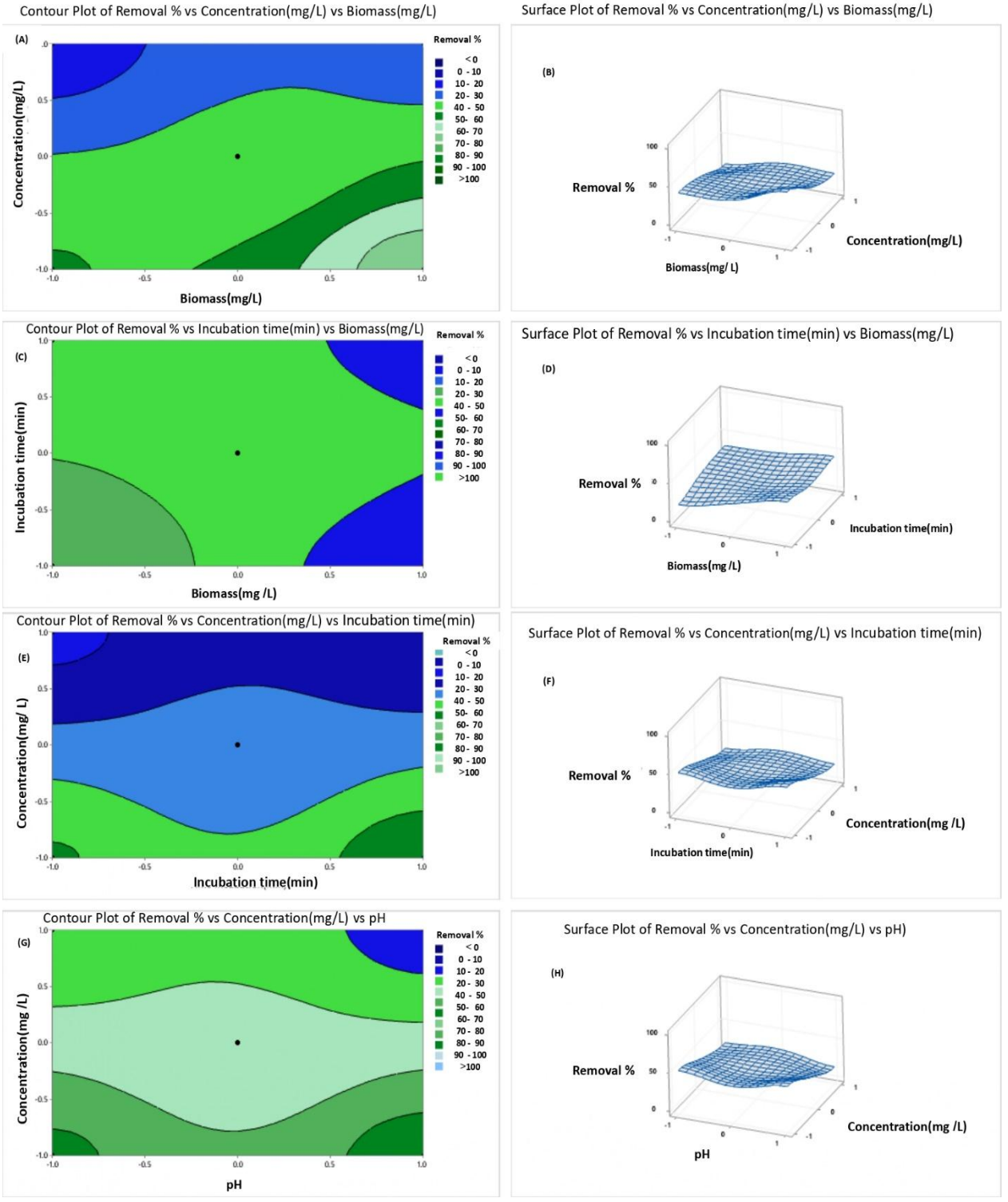

Figure 8. 3D-response surface plots (left) and two-dimensional contour plots (right). (A and B): Impact of concentration and biomass (*C. vulgaris*) on removal of oxamyl. (C and D): Impact of incubation time and biomass on removal of oxamyl. (E and F): Effect concentration of oxamyl and incubation time and biomass on removal of oxamyl

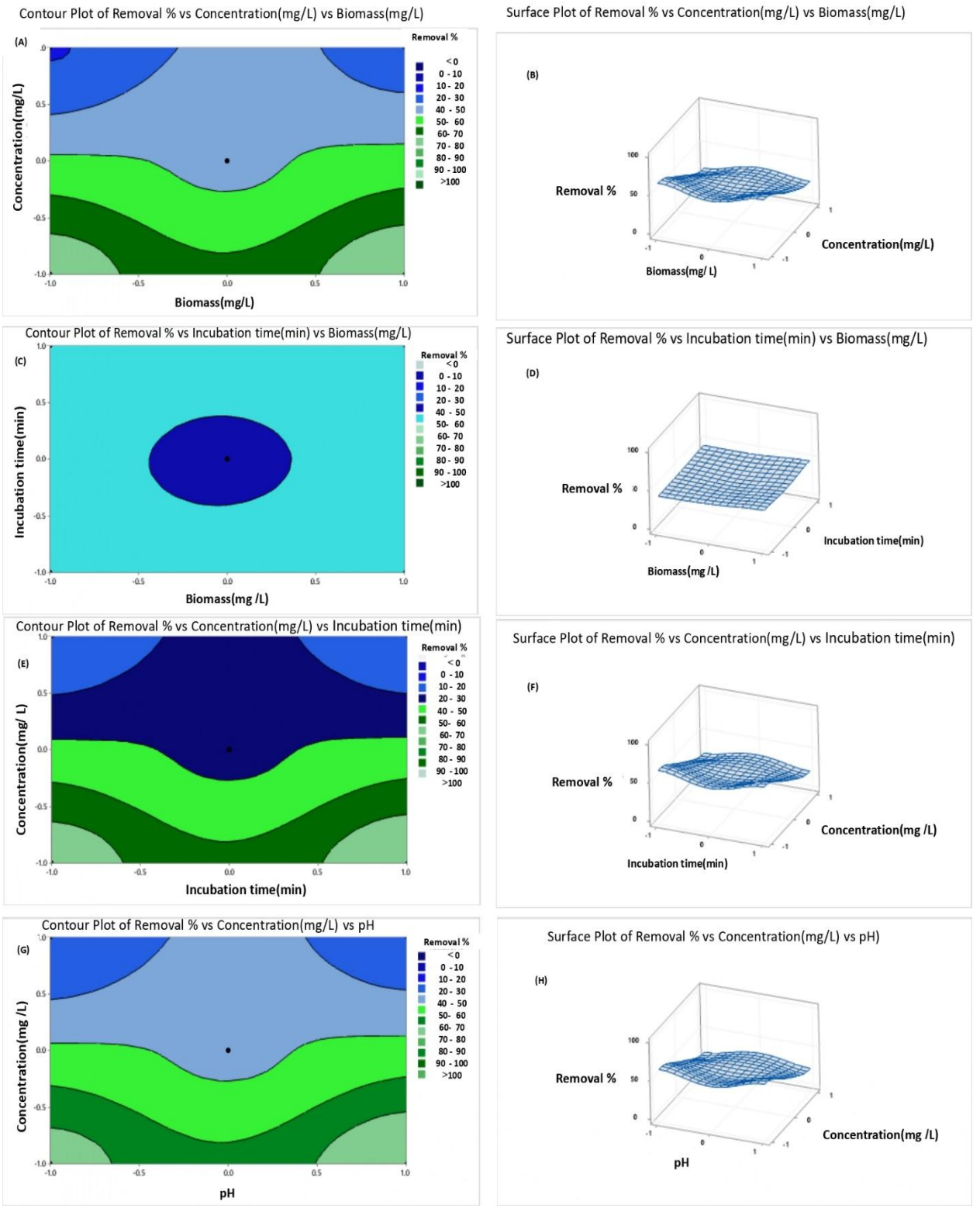

Figure 9. 3D-response surface plots (left) and two-dimensional contour plots (right). (A and B): Impact of concentration and biomass (*U. lactuca*) on removal of oxamyl. (C and D): Impact of incubation time and biomass on removal of oxamyl. (E and F): Effect concentration of oxamyl and incubation time and biomass on removal of oxamyl

Phycoremediation indicates to the incorporation of the words “phyco” denoting “algae” and “remediation” that denotes, “to treat or bring back to the original state” (Yang et al., 2015). The motif involves taking a complex pollutant from the environment and using or transforming it into a nontoxic image (Quintana and Ciurana, 2011). The algae comprise both the microalgae as well as the marine macroalgae more commonly known as the seaweeds. The algae are distributed widely throughout the earth and have adapted to a diversity of habitats. This has also allowed the algae to develop wide tolerance to environmental cases including nutrient rates. As a result of this advantage, algae have become widely used in bioremediation of wastes, resulting in water that has been treated. Algae were widely used in wastewater treatment because of their proven ability to remove nitrogen, phosphorus, chemical oxygen demand, and metal ions from wastewater (Wang et al., 2010).

The microalgae refer to all algae too small to be adequately seen without a microscope and often include both eukaryotic microalgae and prokaryotic cyanobacteria. Approximately 45,000 to 100,000 kinds of algae live on earth (Pandey et al., 2018). A primary producer in the food chain, microalgae are highly adaptable to their environment. Many microalgae have appeared significant features over bacteria and fungi in degrading organic pollutants. In particular, they can overcome the necessity of carbon and other nutrients and contribute to the sequestration of CO_2. The use of microalgae has appeared promise as bioremediation technology (Zhang et al., 2016). Pesticides, toxic elements, and oils can be removed from wastewater by microalgae (Ummalyma et al., 2018).

Concerning used microalgae to degradation pesticide, factors like the size, density, morphology, and metabolic activities of algal particles affect pollution uptake and removal (Subashchandrabose et al., 2013). In particular, algae have a high surface area to biovolume ratio, which makes them more likely to sorb pesticides and interact with them later on.

Different techniques are participated in pesticide uptake by microalgae, inclusive bioadsorption, bioaccumulation, and biodegradation. Bioadsorption is a passive process (Årdal et al., 2014). Different mechanisms are involved in bioadsorption, such as electrostatic interaction, surface complexation, ion exchange, absorption, and precipitation (Bilal et al., 2018; Hussein et al., 2017). It was found that 87-96% of a number of pesticides (atrazine, simazine, molinate, isoproturn, carbofuran, propanil, dimethoate, metoacholar, pendimethalin, and pyriproxin) were removed by bioadsorption by cultivated algae. Therefore, the surface-active groups and properties of microalgae mainly affect the efficiency of pesticide adsorption (Ata et al., 2012). In the 2,4-D adsorption by *Gracilaria verrucosa*, hydroxyl, carboxyl, and amine groups are the dominant active surface groups (Ata and Toker, 2012). As a sulfated polysaccharide, microalgae have a cell wall composed of carbohydrates, fibrils, and intercellular spaces, which could facilitate the adsorption of organic contaminants from the water (Qiu et al., 2017). In order to remove a pesticide, there are two aspects to consider: first, the optimal conditions of the biome, and second, its survival and activity. A second factor is a pesticide's chemical structure and factors associated with microalgae, including number of microalgae, contact with the substrate, pH, temperature, salinity, nutrients, light quality, strength, water availability, oxygen tension, and redox potential, and the presence of alternative carbon substrates and electron acceptors (Rath, 2012).

Barton et al. (2004) proved the transformation of methyl parathion in Anabaena sp. was demonstrated through reduction method. It was transformed to O, O-dimethyl O-p-nitroephenul thiophosphate and subsequently reduced. Both Scenedesmus sp. and Chlorococcum sp. were could convert α-endosulfan, an endocrine damaging insecticide, to endosulfan sulphate, endsulfandiol, ß-endosulfan, endosulfan aldehyde and endosulfan ether. C. vulgaris, C. pyrenoidosa and Scenedesmus obliquus were used to degrade the herbicide diclofopmethyl by absorbing and subsequently hydrolyzing it to yield diclofop into the algal cells (Cai et al., 2007). However, in some status, the transformed compounds from organophosphorus pesticides (e.g. fenamiphos) may be high toxic than the parent compound (Cáceres et al., 2008). Biosorption to C. vulgaris using short and long time exposures have been appeared to promote the removal of a mixture of diverse pesticides, inclusive atrazine, molinate, simazine, isoproturon, propanil, carbofuran, dimethoate, pendimethalin, metolachlor, and pyriproxin (Hussein et al., 2017). Abdel-Razek et al. (2019) explored the potential of using a consortium of microalgae and cyanobacteria (*C. vulgaris*, *Scenedesmus quadricuda* and *Spirulina platensis*) for removal malathion from water samples taken from varying combinations of urban wastewater and agricultural drainage water in Egypt. The fastest algal growth was observed in a treatment containing the microorganism consortium and malathion cultured in water samples taken from agriculture drainage and urban wastewater. Microalgae were able to separate malathion from wastewater samples with up to 99% efficacy.

Fenamiphos was degraded by various green algae (*Scenedesmus* sp. MM1, *Scenedesms* sp. MM2, *Chlamydomonas* sp., *Stichoccus* sp. and *Chlorella* sp.) (Cáceres et al., 2008). All the types tested were can transform fenamiphos to its primary oxidation product, fenamiphos sulfoxide (FSO). At the same time, the plurality of these cultures were can hydrolyze FSO to fenamiphos sulfoxide phenol (FSOP). Fenamiphos sulfone phenol, FSOP, and FSO were detected in the culture extracts of these algae. The capacity of these algae to detoxify fenamiphos can be gainfully used in removal this pesticide and its toxic metabolites.

Kurade et al. (2016) proved the actual removal of diazinon from the aqueous phase by a freshwater *C. vulgaris*. *C. vulgaris* showed the highest diazinon removal capacity (94%) at 20 mg/L. The growth of *C. vulgaris* was significantly affected above 40 mg /L of diazinon, showing > 30% growth inhibition after 12 days of cultivation. Significant increase of the microalgal growth in the exponential growth phase indicated a less/non-toxic nature of the diazinon by-products. Biochemical properties, including carotenoid, chlorophyll and antioxidant enzymes of *C. vulgaris* were influenced by diazinon at comparatively high concentrations. The degradation level constant and the half-life of diazinon (0.5-100 mg /L) ranged through 0.2304-0.049 day and 3.01-14.06 day, respectively. Gas chromatography/mass spectroscopic study proposed the formation of a less toxic by-product, 2-isopropyl-6-methyl-4-pyrimidinol because of microalgal metabolism of diazinon. This study solved that *C. vulgaris* is highly tolerant of diazinon, that could be involved in the removal of traces of diazinon from wastewater and has potential method in the removal of such synthetic toxins by algae.

Accelerated biodegradation of the oxamyl, applied at the recommended dose in soil cultured by banana plants and coupled with *Meloidogyne incognita*, was spotted using algal bioassay studies (El-Ansary et al., 2021). Algae *C. vulgaris, Scenedesmus obliquus, Anabaena oryza* and *Nostoc muscorum* have been used to rate the degradability increase of oxamyl by an accelerated biodegradation method. All oxamyl-degrading types appeared a highly operative in enhancing removal of oxamyl compound. Memorable, the alga *S. obliquus* was the most effective one for oxamyl removal. The m residue in the plant was 25%, and oxamyl degradation in polluted soil by algae was 100% and had an active promoting on plant health.

CONCLUSION

The effectiveness of dry biomass of microalgae *C. vulgaris* and *U. lactuca* for removal of pesticides from the water was demonstrated using UV-Vis spectrophotometric method. The characterization of the algal biomass was achieved by NIRS instrument. Screening and optimization of different factors on removing fenamiphos, imidacloprid, and oxamyl by these microalgae were premeditated in detail using Plackett-Burman design analysis. The proximate optimum conditions of removal (%) depended on the pH, the incubation time (min), pesticide concentration (mg/L) and algal biomass (mg/L). Algal biomass and pesticide concentration were the greatest significant factors. Consequently, the dry biomass of the microalgae *C. vulgaris* and *U. lactuca* can be used to remove fenamiphos, imidacloprid, and oxamyl from polluted water very rapidly and diminish environmental contamination.

Ethical approval

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this article. Also, the related datasets are available from the corresponding author on reasonable request.

Conflict of interest

The authors confirm no known conflicts of interest associated with this publication.

Author's contributions

All authors contributed to the study's conception and design. They performed material preparation, data collection, and analysis. Azza G. A. Reyad performed the experiments, data collection, and analysis. Moustafa A. Abbassy, Entsar I. Rabea and Gehan I. Kh. Marei shared in the experimental section, statistical analysis and wrote the draft of the manuscript. Mohamed E. I. Badawy performed UV-Vis spectrophotometric analysis of pesticide residues and revised the data. All authors participated in manuscript writing and proofreading and approved the final manuscript.

Funding

This research did not receive any grant or specific funding from public, commercial, or not-for-profit funding agencies.

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الملخص العربي

كفاءة إزالة بعض المبيدات الحشرية من الماء باستخدام الطحالب الدقيقة وتقديرها بطريقة التحليل الطيفي بالأشعة الفوق بنفسيجية

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تتركز الدراسة الحالية على تقيم كفاءة الطحالب الدقيقة *Chlorella vulgaris* و *Ulva lactuca* في إزالة المبيدات الحشرية وهى الفيناميفوس والإيميداكلوبريد والأوكساميل من الماء. تم دراسة تأثير الأس الهيدروجيني زمن التحضين وتركيز المبيدات الحشرية وتركيز الكتلة الحيوية على تحلل المبيدات الحشرية وكانت عوامل قيمة في الدراسة. أجريت تجارب الادمصاص الحيوي بإضافة ١٠٠ و٥٠٠ و٩٠٠ ملغم / لتر من الطحالب إلى محلول المبيد (٥٠ و٢٥٠ و٤٥٠ مجم / لتر). أجريت التجارب على درجات من الأس الهيدروجيني مختلفة؛ ٥ و٧ و٩ وزمن تحضين؛ ٥ و١٠ و١٥ دقيقة. تم قياس كمية المبيدات الحشرية بواسطة طريقة التحليل الطيفي للأشعة فوق البنفسجية. الظروف المثلى تم تحديدها باستخدام اختبار Plackett-Burman test عند ١٥ دقيقة، أس هيدروجيني٥، و٥٠ ملجم/ لتر من المبيدات الحشرية، و٩٠٠ ملجم / لتر من الكتلة الحيوية التي أظهرت نسبة إزالة ٦٦,٢٠٪ و٦١,٩١٪ للفينـاميفوس مع C. vulgaris و U. lactuca، على التوالي، بينما، ٥ دقائق، أس هيدروجيني٩، ٥٠ ملجم / لتر من المبيدات الحشرية و٩٠٠ ملجم/ لتر من الكتلة الحيوية للطحالب مع إزالة ٤٠,٧٦ و٢٨,٤٤٪ و ٧٠,٢٨-٧٠,٠٧٪ من إميداكلوبريدو أوكساميل لـ C. vulgaris و U. lactuca، على التوالي. أظهرت هذه الدراسة أن إزالة المبيدات الحشرية من المياه العذبة عن طريق الطحالب البحرية الدقيقة C. vulgaris و U. lactuca فعالة وتعتمد على الكتلة الحيوية للطحالب. وهكذا، أظهر C. vulgaris و U. lactuca انخفاضًا محتملاً في المبيدات الحشرية في عينات المياه الملوثة. ستفتح هذه الدراسة افاق جديدة لفهم أكثر تعمقًا لكيفية إزالة المبيدات الحشرية الملوثة في البيئة المائية بناءً على تقنية الطحالب الدقيقة.

الكلمات المفتاحية: إزالة المبيدات؛ فينـاميفوس؛ اميداكلوبريد؛ اوكساميل؛ طريقة التحليل الطيفي للأشعة فوق البنفسجية.