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Changes in the community structure and growth of fresh water microalgae as a consequence of diuron exposures

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Abstract : The extensive use of pesticides, particularly herbicides may affect the growth of algal populations, in turn disturbing the balance of the aquatic ecosystem. In this study, the Nile River water microalgae were exposed to different concentrations of diuron (ranging from 0.01 to 0.16 mg/L), to assess its impact on algal growth (including, cell number, community structure, species diversity, redundancy, half-maximal effect concentration (EC_{50}), and growth rate) and photosynthetic pigment contents (chlorophyll (a) and carotenoids). While diuron at low concentration levels of 0.01 and 0.02 mg/L cause slight inhibition on the Nile River water algal growth demonstrated by chlorophyll (a) and carotenoids contents, it drastically decreased the photosynthetic pigments at higher concentration ranges (0.04 to 0.16 mg/L). The EC_{50} value decreased from 56 to 34.7 µg/L with increasing the exposure time from 3 to 7 days, suggesting an acute toxicity in microalgae, induced by low diuron concentration. Furthermore, the growth rate derived from chlorophyll (a) content in the presence of diuron was higher than the corresponding values derived from the total algal counts at the same time intervals. As a consequence of diuron exposure, the phytoplankton composition (Bacillariophyta, Chlorophyta, and Cyanophyta with % composition of 55, 9.5, and 35.5, respectively) was changed, due to the replacement of susceptible algae by more resistance ones. Moreover, the number of taxa and evenness with which individuals are distributed within are reduced and the decline in diversity was observed.

Keywords : Diuron, Toxicity, Nile River water algae, Growth, Pigments, Species sensitivity.

Introduction

The use of herbicides in agricultural field may result in contamination of surface water, which in turn potentiated a risk for ecosystem as well as the drinking water quality[1,2]. Diuron (3- (3,4-dichlorophenyl)-l,l-dimethylurea), one of the most commonly used herbicides, has been used in many urban and industrial areas as an active ingredient in antifouling boat paints and algaecide formulations used in fountains and aquaculture. As a consequence, it was detected in surface waters and sediments of marine and fresh water environments[3]. In the environment, diuron can be transformed abiotically through hydrolysis and photodegradation reactions; however these reactions occur at very low rates under natural conditions[4]. Being frequently detected in surface and ground water, diuron is known as a potential water contaminant by US Environmental Protection Agency [5]. Notably, the application of diuron is partially restricted, however, it is still effective in parts of the world (http://www.pesticideinfo.org/, access date: October 2011).

In aquatic environment, planktonic algae play a vital role as they provide a crucial source of food to large aquatic organisms and oxygen supply. The use of photosynthetic organisms (algae) in toxicity tests with

diuron is very appropriate, because it inhibits photosynthesis via preventing the oxygen generation [6] and blocks the photosystem II (PSII) electron transport[7]. Additionally, it inhibits the growth and photosynthetic pigment contents in *Chlorella vulgaris* and *Ankistrodesmus acicularis*[8,9]. [10]concluded that neither the variation in species sensitivities nor differences in exposure time could adequately predict the low threshold concentration of diuron that caused chronic effects on the freshwater periphyton communities.

Because of their fast growth and short generation times, microalgae can be used in ecotoxicological studies for assessment of acute and chronic toxicities of persistent herbicides[11]. The most widely used test to assess the toxicity of herbicides on algae was mono-specific toxicity tests. The results of single-species toxicity tests have produced large herbicide-dependent sensitivity differences[12]. However, discernment is required when extrapolating results from mono-specific assay to ecosystem impairment and authors have reported the importance of reinforcing the ecological relevance of toxicological studies to improve the ecotoxicological risk assessment[13]. A first step in such reinforcement is to evaluate the toxic effects at the community level by applying the community ecology concepts to ecotoxicology testing[13,14].

Studying the toxicological effects of agricultural herbicides and industrial materials on fresh water periphyton communities are predominately increasing. The ecological importance of periphyton community to the total aquatic primary production in food webs in addition to ecological balance, and species of phytoplankton can be considered as useful indicators of water quality[15]. The use of phytoplankton species (or categories) for water quality assessment has a long history. Different methods have been developed; some of them use phytoplankton abundance, whereas others examine the phytoplankton community structure[16]. The diversity of phytoplankton and their association as a biological indicator for assessment of water quality has been worked out[17]. The diversity indices, the mathematical function deals with the number of species in a biological community, provide important information about rarity and commonness of species in the community[17]. The phytoplankton response to photosynthetic– inhibiting herbicide depends on the species sensitivity, and generally green algae are the most sensitive group. Due to sensitivity difference, competitive interaction could led to the replacement of the susceptible algae by more resistance ones, which would have an impact on community structure[18,19]. Quality and chemistry of water is also indirectly affected by herbicide inhibition of the photosynthetic phytoplankton such as increase in ammonium, dissolved carbon and nitrate levels, and reduction in oxygen and chlorophyll levels [20].Therefore, the aim of the present study was to investigate the impact of diuron on the growth and photosynthetic pigment contents of Nile River water algae as well as diversity, redundancy of the species, and changes in community structure (including the variation in response of different algal type).

Materials and Methods

Diuron (98% purity) was purchased from Sigma-Aldrich (Germany). The stock and working solutions of diuron were prepared in ethanol (HPLC grade), because of relatively low water solubility. Ethanol was considered as a good solvent in toxicity test [21]. The final concentration of ethanol does not exceed 0.05% (*v/v*).

Algae collected from raw Nile water, were concentrated via the Sedgwick- Rafter method [22] to form inoculums represented by three taxonomic groups; namely green algae "Chlorophyta", blue-green algae "Cyanophyta", and diatoms "Bacillariophyta"; for bioassay tests. The amount of algae inoculated in bioassay culture flasks was determined at the beginning of each experiment using Chlorophyll (a) content.

The concentrated algae were cultivated in/on a standard algal media supplemented with $NaNO_3$, K_2HPO_4. $MgCl_2$, $MgSO_4$, $CaCl_2$, and $NaHCO_3$ using EPA algal assay procedure bottle test[23]. Sterilized media (600 mL) was added to Erlenmeyer flask (1 L capacity) and then incubated at 24°C ± 2 °C under continuous white fluorescent light with intensity of 33.8 mE/m2/s. Afterward, diuron concentrations at the range of 0.01 to 0.16 mg/L were added, whereas the control flask has the same amount of ethanol in the absence of diuron. All the experiments were carried out in triplicate. The algal growth was determined via taking a known volume of algal suspension, filtered through a 0.45 µm membrane filter, and extracted with 100% acetone after adding 0.5 mL magnesium carbonate solution (1 %) to prevent chlorophyll degradation. Chlorophyll (a) Chl (a) content was calculated according to APHA [24] using the following equations:

$$Ca = 11.85(OD664) – 1.54(OD647) – 0.08 (OD630)$$

Chlorophyll a (μg/L) = Ca × Extracted volume (L) / Sample volume (L)

where: OD 664, 647, and 630 are the absorbance at 664, 647, and 630 nm

The total carotenoid concentrations were determined according to [25] using the following equation:

Carotenoids = 7.6 (OD 480) – 1.49 (OD 510)

where OD 480 and 510 are the absorbance at 480 and 510 nm.

The growth rate was determined according to the following equation:

$\mu/d = (\ln X_2 - \ln X_1)/(T2-T1)$

where X_1, X_2 are the log of chlorophyll (a) or log of total algal counts at time T_1 and T_2 respectively.

Algae were identified microscopically according to the key of fresh water algae[26,27]. A Sedgwick Rafter counting cell was used for phytoplankton count by transferring 1 mL from well mixed samples. The results were expressed as number of organisms/mL according to APHA [24].

The community structure parameters, namely diversity ($\acute{H}$) and redundancy (or dominancy) index (R) were estimated according to[28]. Diversity was estimated using the following equation:

$H` = - \sum^{s}_{I=1} (ni/N) \log (ni/N)$

where: S: number of taxa samples, ni: number of individuals, and N: samples size

Additionally, redundancy (or dominancy) index (R) was estimated for each group using the following equation:

R= H` ( max/s) – H`/ H` ( max/s) - H` (min/s)

where: H` (max/s) and (min/s) are the maximum and the minimum values of H` at given S.

Statistical analysis

Results of Chl (a) content and carotenoids were subjected to Duncan's multiple range tests, to compare between treatments at 5% level of probability [29]. EC_{50} was determined by Probit test according to [30] using the percentage of growth inhibition derived from chlorophyll (a) content.

Results and Discussion

Changes in photosynthetic pigments

Because the effects of diuron on different pigments are at variance, we decided to assess the two major pigment pools, chlorophyll (a) and the carotenoids spectrophotometrically[31]. Results in Table 1 showed that the chlorophyll (a) content of the control culture substantially increased and attained its maximum value (271.3 μg/L) at 8th day post-incubation. A slight inhibition of the algal growth was recorded at 0.01 mg/L treatment with diuron up to the 8th day. Thereafter, the algae restore their activities and exceeded the Chl (a) content of the control at day 10 of incubation. For 0.02 mg/L treatment, a reduction of 40% in correlation to the control was observed at the 5th day. Subsequently, the algal cell recovered by the end of the experiment and approximately reached that of the control. In line to the present finding, previous studies indicated a simulative effect of low concentration of different herbicide on Chl (a) content[32,33]. At the intermediate and higher doses of diuron 0.04, 0.08, and 0.16 mg/L, the Chl (a) content of the algal cells were drastically decreased. The dose-effect relation was significant except for 0.04 and 0.08 mg/L at the 1st and the 5th day of incubation.

Table 1 Changes in the chlorophyll (a) content (µg L^{-1}) of Nile River water algae in presence of diuron (n=3).

Concentration of diuron (mg/L)	Time (days)					
	1st	3rd	5th	7th	8th	10th
0.0	38.6^{e}	66.9^{f}	106.1^{e}	198.3^{f}	271.3^{f}	251.9^{d}
0.01	33.4^{d}	49.9^{c}	80.9^{d}	136.7^{e}	230.9^{e}	272.5^{e}
0.02	29.9^{c}	42.5^{d}	64.9^{c}	101.2^{d}	200.4^{d}	254.0^{d}
0.04	27.3^{b}	37.6^{c}	40.9^{b}	46.8^{c}	52.3^{c}	67.9^{c}
0.08	26.4^{b}	30.1^{b}	36.7^{b}	35.7^{b}	34.6^{b}	32.6^{b}
0.16	22.4^{a}	23.9^{a}	20.1^{a}	14.8^{a}	10.3^{a}	7.2^{a}

Initial Chl(a) content = 25.2 µg/L

Means followed with the same alphabetical in every column are not significant at 5% level, according to Duncan's multiple range test.

Table 2 Changes in carotenoids content (µg L^{-1}) of Nile River water algae in presence of diuron (n=3).

Concentration of diuron (mg/L)	Time (days)					
	1st	3rd	5th	7th	8th	10th
0.0	21.2^{e}	32.8^{e}	48.0^{e}	91.05^{f}	120.2^{f}	161.9^{e}
0.01	18.9^{d}	27.2^{d}	35.7^{d}	73.3^{e}	114.2^{e}	156.6^{e}
0.02	18.3^{d}	20.8^{c}	29.3^{c}	51.7^{d}	98.1^{d}	147.7^{d}
0.04	17.2^{c}	18.1^{b}	16.6^{b}	32.2^{c}	36.0^{c}	41.7^{c}
0.08	16.6^{b}	17.6^{b}	16.7^{b}	23.6^{b}	25.2^{b}	27.1^{b}
0.16	15.1^{a}	13.8^{a}	11.6^{a}	9.4^{a}	7.5^{a}	5.2^{a}

Initial carotenoids content = 17.9 µg/L

Means followed with the same alphabetical in every column are not significant at 5% level, according to Duncan's multiple range test.

The EC_{50} values derived by Probits analysis at 3, 5, and 7 days were 56, 32, and 34.7 µg/L, respectively. The EC_{50} at 5 and 7days approached each other, which signify that the algal cells were sensitive to the toxic effect of diuron at such concentration. On the other hand, the EC_{50} values of diuron decreased with increasing the exposure time, suggesting that the lower concentration level can show an acute toxicity to these microalgae.

Clear dose-response relationship was obtained for the effect of diuron on the carotenoids of fresh water microalgae (Table 2). The results revealed that the concentration of carotenoids decreased as the concentration increased, compared with control. Statistical analysis showed that such variations in carotenoids contents at 0.01 mg/L diuron were not significantly different from the control by the end of the incubation. Meanwhile, at 0.02 mg/L, a slight inhibition of carotenoids content (9 % of the control) was observed. In other treatments, a drastic inhibition was revealed. Previous studies pointed out a general progressive significant decreases in photosynthetic pigments as the concentration of herbicide increased[9,34,35], the findings which are consistent with the present study.

Changes in phytoplankton composition

Counts and the distribution pattern of the algal groups (control and treated) are shown in Fig 1. The initial seed was characterized by three–algal groups, namely "Chlorophyta", "Cyanophyta", and "Bacillariophyta" [36]. The initial inoculums of the Nile River water was represented by 30 species of green algae with total counts 4200 organisms /mL; 8 species with total counts 1120 organisms/mL for blue green algae; and 22 species with total counts of 6520 organisms/mL diatoms (Table 3 and Fig. 1). The total algal counts of treated cultures substantially decreased as the concentration levels of diuron and the exposure time increased. The declines in the total counts were 39.5 and 41.5% in the presence of 0.01 and 0.02 mg/L diuron by the end of day 7th, respectively. The reduction in total algal counts exceeded 85% at higher doses of 0.08 and 0.16 mg/L. The total count of diatoms in the presence of 0.01 mg/L diuron approaches that of the control by the end of the 3rd day and exceeds the control by the end of the 7th day of incubation. The high concentrations of diuron resulted in massive decreases in the diatoms number. The most resistant species of diatoms were *Melosira*

granulata, Diatoma elongatum, and *Cyclotella comta*. The relative ratio of each algal group was subjected to variation during the incubation period with respect to control and the treatments. With regard to green algae, lower concentrations of diuron (0.01 and 0.02 mg/L) cause slight inhibition. It starts to recover by the end of the 3rd day of exposure; attaining their maximum counts at the 10th day of exposure. On the other hand, high concentration of diuron causes a drastic reduction in the green algal counts. *Eudorina elegans, Gonium pectorale, Mougeotia scalaris,* and *Pediastrum clathratum* were the most dominant species in the treated cultures. Additionally, blue green algae were subjected to considerable decrease in the treated cultures compared with control. The counts in the cultures progressively decreased as diuron doses increased. *Coelospharium kuetzingianum, Anabaena flos- aquae,* and *Cylindrospermum stagnale* were the most dominant species.

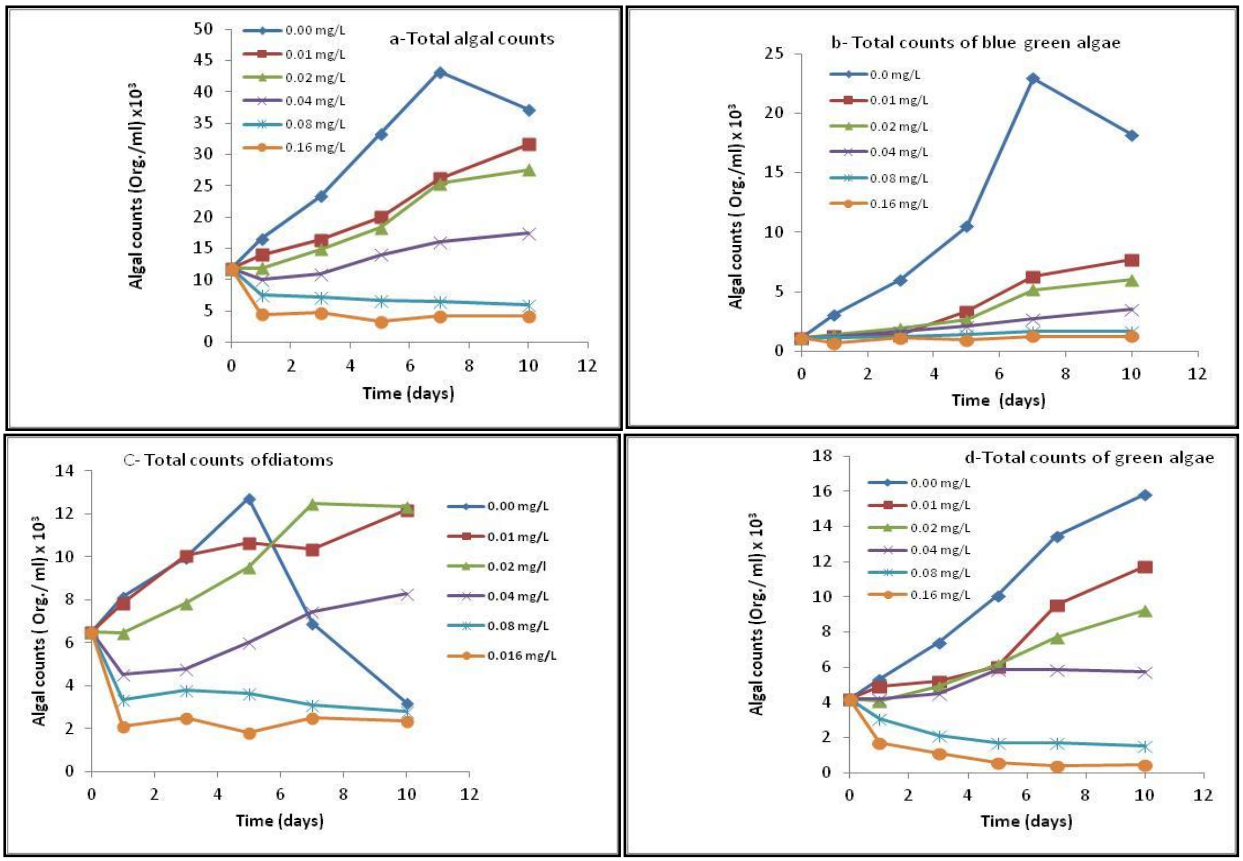

Fig. 1. Changes in algal counts and community structure of Nile River water in presence of diuron

Table 3 Changes in phytoplankton community, diversity, and redundancy ad a results of diuron at the 7th day of incubation.

Parameters	Treatments (mg/L)						
	Initial Algal seed	0.00	0.01	0.02	0.04	0.08	0.16
No. of diatoms species	22	9	11	10	9	5	4
% composition	55%	15.9%	39.5%	49%	46%	48%	60%
Diversity	2.526	1.295	1.403	1.394	1.49	1.22	1.04
Redundancy	0.66	1.0	1.0	1.0	0.054	0.0	0.09
No. of green algal species	30	23	18	18	11	4	1
% composition	9.5%	31%	36%	30%	37%	26%	10%
Diversity	3.39	2.906	1.967	2.273	1.511	0.837	0.0
Redundancy	0.00	0.0	0.0	0.0	0.0	1.0	1.0
No. of blue –green algal species	8	8	8	6	5	4	4
% composition	35.5%	53%	24%	20%	17%	26%	30%
Diversity	2.072	1.732	1.626	1.498	1.12	0.983	1.149
Redundancy	1.0	0.73	0.605	0.882	1.0	0.618	1.0

It was reported that green algae and cyanobacteria are more sensitive to type II inhibitors, such as *s*-triazines and phenylurea, than diatoms[37]. Similar observations were made on phytoplankton communities exposed over a long term to metazachlor[38]. As diatoms contain carotenoids and xanthophylls, [39]reported that these pigments have antioxidant properties, which in turn enhance the potential tolerance to oxidative stress caused by pesticides. These findings are in agreement with the current study as percent compositions were in the order of diatoms > blue-green > green at 0.16 mg/L of diuron. This means that the response of diatoms group to diuron was less than the others groups. Conversely, [10,40] observed a decrease in the relative number of diatoms in periphyton communities exposed to diuron concentrations between 0.02 and 10 mg/L.

Changes in phytoplankton properties

When algal counts attained its maximum counts at 7th day of incubation, some of the community properties were summarized in Table 3. The distribution pattern (% composition) of the initial inoculum started with the ratio of 55: 9.5: 35.5 of diatoms: green: blue green algae. This ratio was completely changed to 15.9: 31: 53 within the same groups in the control culture; affected with the experimental conditions after day 7th of incubation. Diversity in turn was reduced in diatoms from 2.53 to 1.29 as a result of species reduction from 22 to 9, while redundancy was raised from 0.66 to 1.0, where some species e.g. *Melosira granulata, Diatoma elongatum* and *Cyclotella comta,* dominated in the culture media. All treatments were characterized by increasing the percentage composition of diatoms, which resist the toxic effect of diuron. The number of diatom species was reduced as the concentration of diuron was increased. The redundancy values were reduced from 1.0 to 0.054, 0.0, and 0.09 at 0.04, 0.08, and 0.16 mg/L diuron, respectively. According to [41], diatoms have cell wall composed predominantly of silica and presumably the passage of chemicals into the cell was delayed by the cell wall. Green algae possess organic cell membranes that permit easier the passage of chemicals. This variation in the structure of cell wall may account for the dominance of diatoms in the River water treated by diuron and their percentage composition increased by extending the exposure time. Green algae were represented by lower percentage composition as the exposure time was extended. Furthermore, the high diversity value was noticed at 0.01 and 0.02 mg/L, whereas the diversity value decreased as the concentration levels increased. Redundancy was almost zero in the control culture as well as in cultures treated with low concentration of diuron, indicating no dominancy. However, at high concentrations (0.08 and 0.16 mg/L), the redundancy value increased to 1.0, where the number of green algae were reduced to 4 and 1, respectively. Blue green algae species were reduced in counts and its percentage composition was reduced from 53% in the control culture to 17% in 0.04 mg/L of diuron. Additionally, the number of species was reduced from 8 to 4 at a concentration rate of 0.08 and 0.16 mg/ L diuron, respectively. The diversity was almost high in all algal treatments as well as the redundancy values, indicating that some genera; including *Coelospharium kuetzingianum, Anabaena flos-aquae,* and *Cylindrospermum stagnale,* were dominating. [42] stated that a high diversity of algal population indicated high water quality, or in other words, high diversity was interpreted to imply lower toxicity. When aquatic communities are stressed, both the number of taxa present and evenness with which individuals are distributed among the taxa are reduced, resulting in lower values of Shannon functions. These findings are in agreement with our study. Also several investigators reported a decline in diversity following the release of pollutants[17,43].

Changes in growth rates

Growth rates derived from chlorophyll content

Table 4 was indicative of the daily changes in the growth rates derived from chlorophyll (a) content. The results showed that the control culture attained the maximum growth rate by the end of the 1st day. In case of 0.01 and 0.02 mg/L diuron, the maximum growth rates were delayed to the intervals between 7-8 days and their values exceeded the control at such interval. This might be linked with the ability of algae to recover from the diuron effect and become adapted. In case of high concentrations (0.08 and 0.16 mg/L), the maximum growth rates were obtained between 3-5 days and 1-3 days, respectively.

Table 4 Growth rates of Nile River algae treated with diuron derived from Chl (a) content.

Conc. of diuron (mg/L)	Growth rates over time (days)					
	0-1	**1-3**	**3-5**	**5-7**	**7-8**	**8-10**
0.00	0.426*	0.275	0.231	0.313	0.313	-0.037
0.01	0.282	0.201	0.241	0.262	0.524*	0.082
0.02	0.171	0.175	0.212	0.222	0.683*	0.119
0.04	0.08	0.160*	0.042	0.067	0.111	0.131
0.08	0.047	0.066	0.099*	-0.014	-0.031	-0.029
0.16	-0.118	0.032*	-0.087	-0.153	-0.362	-0.179
r^{2} **	0.811(s)	0.842(s)	0.836(s)	0.913(s)	0.775(s)	0.57(s)

* = Maximum growth rates (measured as Chl (a) content

(s) = Significant at 5% level.

** = Correlation coefficient of growth rates vs. treatment concentration

Table 5 Growth rates of Nile River algae treated with diuron derived from total algal counts.

Conc. of diuron (mg/L)	Growth rates over time (days)				
	0-1	**1-3**	**3-5**	**5-7**	**7-10**
0.00	0.333*	0.174	0.177	0.131	-0.041
0.01	0.168*	0.079	0.100	0.134	0.063
0.02	0.005	0.106	0.111	0.162	0.028
0.04	-0.161	0.040	0.124*	0.067	0.029
0.08	-0.448	-0.029	-0.03	-0.02*	-0.022
0.16	-0.972	0.030	-0.174	0.112*	0.00

* = Maximum growth rates (measured as chlorophyll (a) content

Growth rates derived from algal counts

Growth rates derived from the total algal counts are given in Table 5. In case of the control culture, the maximum growth rate value was detected by the end of the first day of incubation. In case of low diuron concentrations (0.01 and 0.02 mg/L), the maximum growth rates were attained by the end of the 1st, and 5 to 7 days post-incubation, respectively. Whereas, the presence of intermediate and higher concentration led to growth inhibition with their maximum growth rate obtained between 3 to 5 and 5 to 7 days, respectively.

According to the present study, the growth rate values of Nile River water algae derived from chlorophyll content in the presence of diuron was higher than the corresponding values derived from total algal counts at the same time intervals. Meanwhile, the maximum growth rate values were recorded at different time intervals. Such results tend to show that the inhibitory effects of diuron on mixed algal population counts of River water do not necessary coincide with the magnitude in the changes in chlorophyll content.

Conclusion

The present study pointed out that the high doses of diuron (0.04-0.16 mg/L) cause an inhibitory effect on microalgae growth represented by chlorophyll (a) content and carotenoids . The total algal counts of treated cultures progressively decreased as the concentration levels and the exposure time increased. The declines in the total counts were 39.5 and 41.5% in the presence of 0.01 and 0.02 mg/L diuron by the end of day 7th, respectively. The reduction in total algal counts exceeded 85% at higher doses (0.08 and 0.16 mg/L). This study revealed that diuron can alter the algal community structure by replacement of susceptible species with more resistant ones. The importance of considering both function and structure of Nile River water algae in toxicological assessment as microalgal species could not be equally affected by herbicide exposure. For improving the value of ecotoxicological risk assessments, future research is needed in two ways: first, more information on the effects of pollutants at the community level must be obtained (new tools and new end points), and second, more effort must be directed to reinforce the ecological relevance of toxicological investigation.

References

1. Fairchild, J.F. (2011). Structural and Functional Effects of Herbicides on Non-Target Organisms in Aquatic Ecosystems with an Emphasis on Atrazine. In: Herbicides and the Environment, Kortekamp, A. (Ed.), In Tech Open, Rijeka, Croatia, 383-404.
2. Rioboo, C., Prado, R., Herrero, C., & Cid, A. (2007). Population growth study of the rotifer *Brachionus* sp. fed with triazine-exposed microalgae. *Aquatic Toxicology, 83*, 247-253.
3. Smith, R.; Middlebrook, R.; Turner, R.; Huggins, R.; Vardy, S.; Warne M. (2012). Large-scale pesticide monitoring across Great Barrier Reef catchments – Paddock to Reef Integrated Monitoring, Modelling and Reporting Program. *Marine Pollution Bulletin*, *65*, 117-27.
4. Coelho-Moreira, J. S., Bracht, A., de Souza, A.C.D., Oliveira, R.F., de Sa-Nakanishi, A.B., de Souza, C.G.M.; Peralta, R.M. (2013). Degradation of diuron by *Phanerochaete chrysosporium*: role of ligninolytic enzymes and cytochrome P450. *BioMed Research International*, Volume 2013 (2013), Article ID 251354. DOI 10.1155/2013/251354
5. EPA, (2013), United States Environmental Protection Agency http://www.epa.gov/espp/litstatus/effects/diuron efed chapter. pdf.
6. Wessels, J.S.C.; Van der Veen, R. (1956). The action of some derivatives of phenylurethan and of 3, phenyl-1-1,1-dimethylurea on Hill reaction. *Biochemica et Biophysica Acta, 19*, 548–549.
7. Giacomazzi, S.; Cochet, N. (2004). Environmental impact of diuron transformation: A review. *Chemosphere, 56*, 1021–1032.
8. El-Dib, M.A.; Abd El-Aty, A.M. (2012). Physiological responses of *Ankistrodesmus acicularis* to diuron. *Desalination and Water Treatment, 44*, 334-339.
9. Faywz, K.A.; Abd El Fattah, Z. (2007). Alteration in growth and physiological activities in *Chlorella vulgaris* underthe effect of photosynthetic inhibitor diuron. *International Journal of Agriculture and Biology, 9*(4), 631–634.
10. McClellan, K., Altenburger, R., Schmitt-Jansen, M. (2008). Pollution-induced community tolerance as a measure of species interaction in toxicity assessment. *Journal of Applied Ecology*, 45, 1514–1522.
11. Moreno-Garrido, I., Lubián, L.M., Jiménez, B., Soares. A.M.V.M., Blasco, J. (2007). Estuarine sediment toxicity tests on diatoms: sensitivity comparison for three species. Estuarine, Coastal and Shelf Science, 71, 278-286.
12. De Lorenzo, M.E.; Scott, G.I.; Ross, P.E. (2001). Toxicity of pesticides to aquatic microorganisms: A review. *Environmental Toxicology and Chemistry*, *20*, 84–98.
13. Schmitt-Jansen, M.; Veit, U.; Dudel, G.; Altenburger, R. (2008) An ecological perspective in aquatic ecotoxicology: Approaches and challenges. *Basic and Applied Ecology*, *9* (4), 337-345.
14. Clements, W.H.; Rohr, J.R. (2009). Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. *Environmental Toxicology and Chemistry*, *28*, 1789–1800.
15. Celekli, A.; Kulkoyluoglu, O. (2007). On the relationship between ecology and phytoplankton composition in a Karstic spring (Cepni, Bolu). *Ecological Indicators*, 7, 497-503.
16. Pasztaleniec, A.; Poniewozik, M. (2010). Phytoplankton based assessment of the ecological status of four shallow lakes (Eastern Poland) according to Water Framework Directive — a comparison of approaches. *Limnologica, 40*, 251–9.
17. Shanthala, M.; Hosmani, S.; Hosetti, B. (2009). Diversity of phytoplanktons in a waste stabilization pond at Shimoga Town, Karnataka State, India. *Environmental Monitoring and Assessment*, 151, 437-443.
18. Lürling, M., & Roessink, I.(2006), On the way to cyanobacterial blooms: Impact of the herbicide metribuzin on the competition between a green alga (*Scenedesmus*) and a cyanobacterium (*Microcystis*). *Chemosphere*,65,618-626.
19. Magnusson, M., Heimann, K., Ridd, M., Negri, A.P. (2012). Chronic herbicide exposures affect the sensitivity and community structure of tropical benthic microalgae. Marine *Pollution Bulletin, 65*, 363-372.
20. Juttner, I.; Peither, A.; Lay, J. P.; Kettrup, A.; Ormerod, S.J. (1995). An outdoor mesocosm study to assess ecotoxicological effects of atrazine on a natural plankton community. *Archives of Environmental Contamination and Toxicology*, 29, 435-441.
21. Abou-Waly, H.F. (2000). Effect of organic solvents on growth of freshwater algae. *International Journal of Environmental Studies*, 57 (4), 411–418.

22. APHA, (1965). Standard methods for the examination of water, sewage and industrial wastes. (11th edn). New York.
23. EPA, (1971). Algal assay procedures bottle test, National Eutrophication Research Program, National Environmental Research Centers Environmental Protection Agency, Corvalis, OR.
24. APHA, (2005). Standard methods for the examination of water and wastewater, 21th ed., American Public Health Association, Washington DC.
25. Timothy, R.P.; Yoshiaki, M.; Carol, M.I. (1985). A manual of chemical and biological methods for seawater analysis, Pergamon Press, New York.
26. Streble, H.; Krauter, B. (1978). das leben in wassertropfen. microflora des subasser. Einbestimmungsbuchmit 1700 abbildung (Stuttgart).
27. Palmer, C.M. (1980). Algae and water pollution, Castle House Publishing Ltd, England.
28. Washinton, H.G. (1984). Diversity, biotic and similarity indices – a review with special relevance to aquatic ecosystems. *Water Research*, *18*(6), 653–694.
29. Duncan, D.B. (1955). Multiple range and multiple *F*-test, Biometrics, 11, 1–42.
30. Finney, D. (1971). Probit analysis. Cambridge Univ. Press, Cambridge pp 1-33.
31. Nestler, H., Groh, K.J., Schonenberger, R., Behra, R., Schirmer, K., Eggen, R.I.L., Suter, M.J.F. (2012). Multiple-endpoint assay provides a detailed mechanistic view of responses to herbicide exposure in *Chlamydomonas reinhardtii*. *Aquatic Toxicology, 110*, 214–224.
32. Abd El-Aty, A.M.; El Dib, M.A. (2009). Uptake and the effects of cyanazine on *Scenedesmus obliquus* and *Anabaena fl os-aquae. Desalination*, *249*, 1294–1297.
33. Saygideger, S.D., Okkay, O. (2008). Effect of 2,4- dichlorophenoxyacetic acid on growth, protein and chlorophyll-a content of *Chlorella vulgaris* and *Spirulina platensis* cells. *Journal of Environmental Biology, 29*, 175-178.
34. Deng, X., Gao, K., Sun, J. (2012). Physiological and biochemical responses of Synechococcus sp. PCC7942 to Irgarol 1051 and diuron. *Aquatic Toxicology*, 122– 123, 113– 119.
35. Negri, A.P., Flores, F., Mercurio, P., Mueller, J. F., Collier, C. J. (2015). Lethal and sub-lethal chronic effects of the herbicide diuron on seagrass. *Aquatic Toxicology*, 165, 73–83.
36. Gad-Allah, T.A.; Badawy, M.I.; Abd El-Aty, A.M.; Ali, M.E.; Yoon, Y. (2012). Evaluation of algal count and disinfection by-products levels in drinking water treatment plants in greater Cairo. *Journal of Applied Sciences Research, 8* (11), 5504-5511.
37. Lockert, C.K., Hoagland, K.D., Siegfried, B.D. (2006). Comparative sensitivity of freshwater algae to atrazine. *Bulletin of Environmental Contamination and Toxicology,76*(1),73–79.
38. Mohr, S., Feibicke, M., Berghahn, R., Schmiediche, R., Schmidt, R. (2008). Response of plankton communities in freshwater pond and stream mesocosms to the herbicide metazachlor. *Environmental Pollution, 152*(3), 530–542.
39. Pinto, E., Sigaud-Kutner, T.C.S., Leitao, M.A.S., Okamoto, O.K., Morse, D., Colepicolo, P. (2003). Heavy metal-induced oxidative stress in algae. *Journal of Phycology,39*(6), 1008–1018.
40. Tlili, A.; Bérard, A.; Roulier, J.L.; Volat, B.; Montuelle, B. (2010). $PO4^{3-}$ dependence of the tolerance of autotrophic and heterotrophic biofilm communities to copper and diuron. *Aquatic Toxicology, 98*, 165–177.
41. Okay, O.S., Gaines, A . (1996). Toxicity of 2,4-D acid to phytoplankton. *Water Research*, *30*, 688 -696.
42. Nelson, J.H., Stoneburner, D.L., Evans, E.S., Pennington, J.R.N., Meisch, M.V. (1976). Diatoms diversity as a function of insecticidal treatment with a controlled- release formulation of chloropyrifos. *Bulletin of Environmental Contamination and Toxicology, 15*, 630-634.
43. El-Dib, M.A.; Abou-Waly, H.F.; El- Naby, A. M. (2000). Impact of pentachlorophenol on growth and community structure of Nile River water microalgae. *International Journal of Environmental Health Research, 10*, 239-250.

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