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Impact of Ethoxysulfuron on Lemna gibba L. and Recovery from Damage after Prolonged Exposure under Rice Cropping Conditions

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ABSTRACT

The aim of the study was to determine the impact of the herbicide, ethoxysulfuron on the growth of Lemna gibba under rice field cropping conditions. During the course of the study, Lemna frond number was decreased from day 0 (1820 µg/L ethoxysulfuron) to day 30 (10 µg/L) resulting in 100% inhibition of growth. The decline in growth continued till day 50, at below the detectable level of residues, symptoms (chlorosis) of ethoxysulfuron toxicity were confirmed by pigment content analysis and toxicity index values. A significant decrease in the pigment content was observed between day 0 and day 50. From day 70, there was a consequent growth recovery and complete recovery on day 180. Our findings indicated that ethoxysulfuron and its metabolites retaining the sulfonylurea group was highly toxic to L. gibba, however, the effects are reversible after prolonged exposure due to the degradation of the metabolites.

Keywords *- ethoxysulfuron, Lemna gibba, toxicity index value, pigment content*

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I. INTRODUCTION

Sulfonylureas are a family of environment friendly herbicides and only require a small quantity to kill weeds, commonly used in rice and cereals as a post-emergence. Sulfonylureas are extensively used in paddy fields and there is a potential risk of contaminating the environment, particularly nearby terrestrial habitats or aquatic ecosystems that can be contaminated by drift or runoff [1]. A determination of the potential impacts of herbicides on primary producers and an assessment of their environmental risks was established in the late 1990s [2]. Determining injury caused to aquatic plant either by herbicide residues or in the form of a metabolite/breakdown product in the environment is a critical issue and is limited by analytical sensitivity, hence biological indicators are preferred. In an earlier study, we evaluated the effect of pretilachlor residues in water and soil under rice field and their influence on aquatic species using *Lemna gibba* (duck weed) as a biological indicator [3].

The growth and reproduction of *Lemna* species (Duck weed) is faster than other vascular plants in aquatic phytotoxicity assessment tests. Consequently, *L. gibba*, a relatively new bio-indicator species [4, 5] is commonly used in phytotoxicity tests due to its small size, high reproductive rate, ease of cultivation, and ease of growth measurement without using any specialized instruments [6]. The importance of duckweed as sensitive indicator of water quality as well as the biological parameters in the reliable ecotoxicological assessment of industrial effluent [7]. The toxic symptoms of herbicides have been assessed by using pigment content analysis in *L. gibba* [8].

Upon literature search, we found that a study related to the effect of herbicides on Lemna species under rice field condition is limited. With this background, the present study was initiated. The aim of the present study was to determine the impact of the herbicide, ethoxysulfuron on the growth of the duck weed, *Lemna gibba* under rice field cropping conditions. The toxicity index and effects on pigment content were also studied.

II. EXPERIMENTAL

2.1. Materials

The reference standard of ethoxysulfuron (purity 99.7%) was purchased from Sigma Aldrich, St. Louis, USA. Ethoxysulfuron formulation (15% water dispersible granule) was purchased from local vendors, and both were used for this study.

High performance liquid chromatography (HPLC) grade acetonitrile, acetone, dichloromethane, methanol, hydrochloric acid, silica gel, sodium sulfate, and orthophosphoric acid were obtained from Merck India Limited. Distilled water was purified using a milli-Q apparatus (Millipore, Bedford, MA, USA). Econofilter 0.2µm Polytetrafluroethylene (PTFE) filter was supplied by Agilent technologies, USA. *Lemna gibba* culture was obtained from BASF, Germany and maintained in the Department of Ecotoxicology, International Institute of Biotechnology and Toxicology (IIBAT), Padappai, Tamil Nadu, India.

2.2. Experimental Procedure

2.2.1. Persistence study

Ethoxysulfuron residues in paddy water under field cropping conditions were investigated by applying the recommended dosage of 120 g active ingredient/hectare (a.i. /ha). The variety of rice seeds used was ADT-43, and it was spread over three plots (10 m^2 each) on an IIBAT agricultural farm in Padappai. The age of the rice crop at the time of herbicide application was 10 days after transplanting. The required water level was maintained by frequent irrigation for upto 180 days. During the experimental period, the water temperature and the intensity of sunlight were measured.

2.2.2. Instrumentation

The HPLC method for the determination of residue concentration showed an acceptable recovery 90–110% in the water sample by fortifying two different concentrations of ethoxysulfuron at 100 and 10 µg/L. We established the limit of detection (LOD) and limit of quantification (LOQ) of ethoxysulfuron as 10 µg/L. Triplicate water samples were collected on day 0, 5, 10, 15, 30, 50, 70, 100, 120, 150 and 180. Samples were analyzed for residue by HPLC, and breakdown products were identified by Liquid Chromatography-Mass Spectrometer/Mass Spectrometer (LC-MS/MS). On each occasion, aliquots of collected samples were filtered using a 0.2µm PTFE filter and stored in amber-colored vials at <5 °C until analysis.

2.2.3. Test procedure for *Lemna gibba sudy*

On each sampling occasion, an aliquot of 160-mL water sample was collected in a 500 ml beaker from the rice field water treated with ethoxysulfuron. The untreated sample (control) and treated samples were inoculated with an equal number of *L. gibba* plants (10 fronds) from a 7-day-old preculture under aseptic conditions in triplicates. The test was terminated 7 days after transferring the plants into the test vessels. On each occasion, the number of *Lemna* fronds of treated samples and controls were recorded and the percentage inhibition of yield was calculated. The percentage inhibition of yield (% I_y) at the test concentration was calculated using the following equation: $\% I_y = [(Y_c - Y_t)/Y_c] \times 100$, where I_y is the percentage inhibition of yield based on frond numbers, Y_c is the mean value for yield in the control, and Y_t is the mean value for yield in the treatment group.

2.2.4. Toxicity Index

The following formula was used to calculate the toxicity index (TI) : TI value = concentration of ethoxysulfuron in different occasional samples / Effective concentration (50% growth inhibition) (EC_{50} - 0.24 µg/L) value of ethoxysulfuron, where TI values >1.0 indicate probable toxicity to aquatic plants; TI values > 0.5 indicate potential toxicity to aquatic plants; and TI values >0.1 indicate limited toxicity [9].

2.2.5. Test procedure for Pigment content analysis

For the pigment content analysis, about 200 mg of plant sample was weighed and homogenized in 10 mL of 95% methanol using mortar and pestle. The homogenate was filtered through Whatman filter paper #41, and the absorbance of the filtered solution was measured at different wavelengths of 666, 653, 470 and 436 nm (Ultra Violet -Visible Spectrophotometer, Shimadzu) for chlorophyll a, chlorophyll b, total carotenoid, and carotene content, respectively. The quantities of pigments were calculated according to the formula using different solvents, i.e., acetone, methanol, and diethyl ether [10].

2.2.6. Statistical Analysis

Data were compared using Student's *t*-test at an alpha level of 0.05 to determine the significant difference between treated and control samples. The SAS 9.3 software was used for statistical analysis. Each data point represents the average of three replicates (n=3), unless stated otherwise.

III. RESULTS AND DISCUSSION

3.1. Persistence Study

The herbicide residue in field water was determined on day 0, 5, 10, 15 and 30 were 1820 µg/L (day 0), 1440 µg/L (day 5), 921 µg/L (day 10), 453 µg/L (day 15), and 10 µg/L (day 30). The presence of ethoxysulfuron residues by day 50 was below the detection level. The use of the analytical technique for residue concentration was very difficult below the quantification level. A biological system, viz., *Lemna gibba*, was employed to investigate the effects of the low levels of the residues.

3.2. Estimation of percentage Inhibition of growth and yield on *Lemna gibba*

The potential impact of ethoxysulfuron residue after day 30 was confirmed using the *Lemna* growth inhibition test. During the experimental period, the temperature in the test medium was 27.8–41.6 °C, and the light intensity was recorded with the range of 45,000–60,000 lux under direct sunlight. Ethoxysulfuron caused maximum inhibition (100%) on the growth and yield of *L. gibba* from day 0 to day 50. The percentage inhibition on growth rate and yield by day 70, 100, and 120 were 94%, 80%, 68% and 96%, 79%, 62%, respectively. On day 150, the lowest value of inhibition on growth and yield was 25% and 26%, respectively, indicating recovery of *L. gibba* growth. The complete inhibition observed is accredited to the presence of the parent compound and its breakdown products retaining the sulfonylurea group.

Ethoxysulfuron inhibits the ALS enzyme to stop the synthesis of branched amino acids (valine, leucine, and isoleucine) during the first stage of plant growth and root elongation, resulting in apparent death [11, 12]. These symptoms were observed during the period between 0 and 50 days. The results on day 150 indicate that the degradation of ethoxysulfuron and the breakdown products retaining the sulfonyl urea group occurred slowly [13, 14], hence *L. gibba* also showed slow growth recovery. Analysis of the samples on day 180 showed no sign of inhibition on the growth and yield of *L. gibba*, which had recovered to control levels. The study results demonstrate that the sulfonylurea herbicide ethoxysulfuron at the recommended dose under rice field conditions shows substantial inhibitory effects on the growth rate and yield of *L. gibba*.

3.3. Toxicity index

The Toxicity Index (TI) was calculated to measure the toxic effect of the ethoxysulfuron residue. The TI value was greater than 1.0 until day 30, indicating a probable toxic concentration of ethoxysulfuron in the water sample to *L. gibba*. Data on the ethoxysulfuron residue in the water samples under field cropping conditions, percentage inhibition of growth and yield based on frond numbers of *L. gibba*, and the TI value are presented in **Table 1**. The literature value of toxicity index scores for individual herbicides were highest for the primary producers, *P. subcapitata* and *Lemna gibba* with 95% toxicity index values of 4.7 and 1.9, respectively [15]. The results concluded that the herbicides can substantially increase the estimated toxicity of field water to *L. gibba.*

3.4. Pigment content Analysis

A change in the pigment content of *L. gibba* is one of the first visible symptoms of toxicity. These changes were used as an indicator of photosynthetic damage in plant tissue. The drop of chlorophyll a & b, carotene, carotenoid and xanthophyll contents as well as the growth inhibition on *L. gibba* can be regarded as general responses related with herbicide toxicity. Pigment content of the control plants ranged from 4.41–4.58 µg/g (chlorophyll *a*), 2.4–2.5 µg/g (chlorophyll *b*), 0.95–0.98 µg/g (carotene), 16.4–16.5 µg/g (carotenoid), and 15.6–15.8 µg/g (xanthophyll). A significant decrease in the pigment content was observed between day 0 and day 50. The percentage inhibition on yield on day 50 was 100%, while pigment content on day 50 was 0.68 µg/g (chlorophyll *a*), 0.39 µg/g (chlorophyll *b*), 2.52 µg/g (carotenoid), 0.14 µg/g (carotene) and 2.39 µg/g (xanthophyll), indicating the destruction of photosynthetic pigments by ethoxysulfuron residue. This destruction is due to a deficiency in the electron transport chain, replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecules by H^{+} ion, inhibition of important enzymes for the synthesis of chlorophyll molecules, or peroxidation processes in the chloroplast membrane lipids by reactive oxygen species [16, 17].

Our findings indicated that ethoxysulfuron and its metabolites (**Table 2**) retaining the sulfonylurea group was highly toxic to *L. gibba* [18]. After 180 days, chlorophyll *a*, chlorophyll *b*, carotene, total carotenoid, and xanthophyll content were similar to control levels, indicating that *L. gibba* had recovered growth potential. The pigment content of the control and ethoxysulfuron-treated samples are presented in **Fig. 1(a) and Fig 1(b)**, respectively. A panel of biomarkers like pigment contents, chlorophyll parameters and antioxidative enzyme activities were used to assess the toxicity of herbicide [19]. The loss of photosynthetic pigment content has been reported in different species following exposure to different herbicides [20, 21]. Hence, according to the environmental recommended application of ethoxysulfuron formulation for rice crop (120 g a.i./ha) and adverse effects would be expected on aquatic plants associated with the use of the herbicides in rice crop management.

3.5. Statistical Analysis

The statistical significance between control and treated samples is attributed to the presence of trace levels of residues of ethoxysulfuron and its breakdown products retaining the sulfonylurea group in the water samples. The deterioration in total chlorophyll, carotene, carotenoid, and xanthophyll content as well as growth inhibition can be regarded as an overall response related to ethoxysulfuron toxicity. After prolonged exposure by day 180, growth rate, yield, and pigment content of *L. gibba* was recovered and similar to control samples. The recovery of *L. gibba* growth was due to the degradation of sulfonylurea group in the water samples. The investigation of the effects and potential recovery of *L. gibba* was exposed to a sulfonylurea herbicide metsulfuron-methyl for 120 days under rice field condition [22].

IV. CONCLUSION

In conclusion, the results indicate the toxic effect of ethoxysulfuron residues on the aquatic species, *Lemna gibba* and the recovery after long term exposure due to the degradation of the metabolites. The present investigation also highlights the successful application of *L. gibba* as a potential plant biomarker for assessing the impact of herbicides on aquatic floating plants since this species is affected by a wide range of environmental contaminants found in water bodies.

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REFERENCES

[1] J. M. Dabrowski, R. Schulz, Predicted and measured levels of azinphosmethyl in the Lourens River, South Africa - Comparison of runoff and spray drift, *Environmental Toxicology and Chemistry*, *22*, 2003, 494-500.

[2] J.F. Fairchild, D.S. Ruessler, P.S. Haverland, A.R. Carlson, Comparative sensitivity of Selenastrum capricornutum and Lemna minor to sixteen herbicides, *Archives of Environmental Contamination and Toxicology*, *32*, 1997, 353–357.

[3] R. RajaRajeswari, S. Sathiyanarayanan, A. Ramesh, and S. Ayyappan, Evaluation of bioavailability of residues of pretilachlor in soil and water under paddy cropping condition and their influence on Lemna gibba, *The Joural of Agriculture and Environment*, *14*, 2013, 102-110.

[4] United State Environmental Protection Agency, OPPTS 850.4400, Aquatic plant toxicity test using Lemna spp., tiers I and II, *EPA 712-C-96-156, EPA*, Washington DC, 1996.

[5] OECD guidelines for the testing of chemicals, revised proposal for a new guideline 221, *Lemna* species growth inhibition test, *OECD*, Paris, France, 2002.

[6] W. Wang, Literature review on duckweed toxicity testing, *Environmental Research*, *52*, 1990, 7-22.

[7] S. Radic, D. Stipanicev, P. Cvjetko, I.L. Mikelic, M.M. Rajcie, S. Sirac, B. Pevalek-Kozlina and M. Pavlica, Ecotoxicological assessment of industrial effluent using duckweed (Lemna minor L.) as a test organism, *Ecotoxicology*, 2009, DOI 10.1007/s10646-009-0408-0.

[8] D.M.M. Dos Santos, R.A. Pitelli, and D.A. Banzatto, Effect of the herbicides on the chlorophyll content of *Spirodela punctate*, *Planta Daninha*, *17*, 1999, 175-182.

[9] W. Battaglin, and J. Fairchild, Potential toxicity of pesticides measured in Midwestern streams to aquatic organisms, *Water Science and Technology*, *45*, 2002, 95-103.

[10] H.K. Lichtenthaler, and A.R. Wellburn, Determination of Total Carotenoids and Chlorophylls A and B of leaf in different solvents, *Biochemical Society Transactions*, *11*, 1985,591-592.

[11] H.M. Brown, Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides, *Pesticide Science*, *29*, 1990,263-281.

[12] J.V. Schloss, Recent advances in understanding the mechanism and inhibition of acetolactate synthase, In: Stetter J (ed) *Chemistry of Plant Protection* (Springer-Verlag, Berlin, 1994) .

[13] M. Mohammad, K. Itoh, K. Suyama, and H. Yamamoto, Recovery of Lemna species after exposure to sulfonylurea herbicides, *Bulletin of Environmental Contamination and Toxicology*, *76*, 2006, 256-263.

[14] M. Mohammad, K. Itoh, and K. Suyama, Effect of herbicides on Lemna gibba and recovery from damage after prolonged exposure, *Archives of Environmental Contamination and Toxicology*, *58*, 2010, 605-612.

[15] J.B. Belden, R.J. Gilliom, J.D. Martin, and M.J. Lydy, Relative toxicity and occurance patterns of pesticide mixtures in streams draining agricultural watersheds dominated by corn and soybeen production, *Integrated Environmental Assessment and Management*, *3*, 2007, 90-100.

[16] F. Van Assche, and H. Clijsters, Effects of metals on enzyme activity in plants, *Plant cell Environment*, *13*, 1990, 195-206.

[17] L.M. Sandalio, H.C. Dalurzo, M. Gomez, M.C. Romero-Puertas, and del RioLA, Cadmium-induced changes in the growth and oxidative metabolism of pea plants, *Journal of Experimental Botony*, *52*, 2001, 2115-2126.
[18] Ron Marks, Public Release Summary Evaluation of the new active Ethoxysulfuron In the product Hero® selective herbicide, *Australian Pesticides and Veterinary Medicines Authority* (Australia, 2008).
[19] L. Geoffroy, C. Frankart, and P. Eullaffroy, Comparison of different physiological parameter responses in Lemna minor and Scenedesmus obliquus exposed to herbide flumioxazin, *Environmental Pollution*, *131*, 2004, 233-241.
[20] M.T.K. Tsui, and L.M. Chu, Aquatic toxicity of glyphosate-based formulations : comparison between different organisms and the effects of environmental factors, *Chemosphere*, *52*, 2003, 1189–1197.
[21] M.L. Martin, and A. Ronco, Effects of mixtures of pesticides used in the direct seeding technique on non-target plant seeds, *Bulletin of Environmental Contamination and Toxicology*, *77*, 2006, 228-236.
[22] R. RajaRajeswari , and A. Ramesh, Effects of metsulfuron-methyl on aquatic plant (Lemna gibba L) and recovery from after prolonged exposure under rice cropping conditions, *bioRxiv*, 2013, DOI 10.1101/000638.

Table 1 Correlation of residue concentrations of ethoxysulfuron under cropping conditions with percentage inhibition on growth rate, yield, and toxicity index value

Occasion (Days)	Residue (µg/L) in field water A	% Inhibition on growth rate based on frond number B	% Inhibition on yield based on frond number C	Toxicity Index (TI) value D
0	1820 ± 6.43	100 ± 0.0	100 ± 0.0	7583 ± 8.62
5	1440 ± 4.73	100 ± 0.0	100 ± 0.0	6000 ± 8.54
10	921 ± 3.06	100 ± 0.0	100 ± 0.0	3838 ± 6.56
15	450 ± 3.61	100 ± 0.0	100 ± 0.0	1875 ± 5.03
30	10 ± 1.53	100 ± 0.0	100 ± 0.0	42 ± 3.61
50	BDL	100 ± 0.0	100 ± 0.0	N/A
70	N/A	94 ± 3.61	96 ± 4.04	N/A
100	N/A	80 ± 2.08	79 ± 1.53	N/A
120	N/A	68 ± 2.52	62 ± 3.06	N/A
150	N/A	25 ± 2.65	26 ± 2.08	N/A
180	N/A	0 ± 0.0	0 ± 0.0	N/A

BDL - below detectable limit; Limit of detection (LOD) & Limit of quantification (LOQ) - 10 µg/L; N/A - not applicable.

TI = Concentration / EC_{50} value of ethoxysulfuron on *Lemna gibba*, where EC_{50} - 0.24 µg/L

A, B, C & D represents average and standard deviation of triplicate measurements

Table 2 LC-ESI-MS/MS fragmentation ions of ethoxysulfuron and its breakdown products

Compound name	Molecular mass	Molecular ion	Fragment ions
Ethoxysulfuron	398	399	279, 199
Metabolite - 2-(2-hydroxyethoxy)phenyl[(4,6-dimethoxypyrimidin-2yl)carbamoyl]sulfamate	414	415	215, 295
2-hydroxyphenyl[(4,6-dimethoxypyrimidin-2yl)carbamoyl]sulfamate	370	371	279, 199
4,6-dimethoxypyrimidin-2-amine	155	156	139, 100
1-(4,6-dimethoxypyrimidin-2yl)urea	198	199	156,182

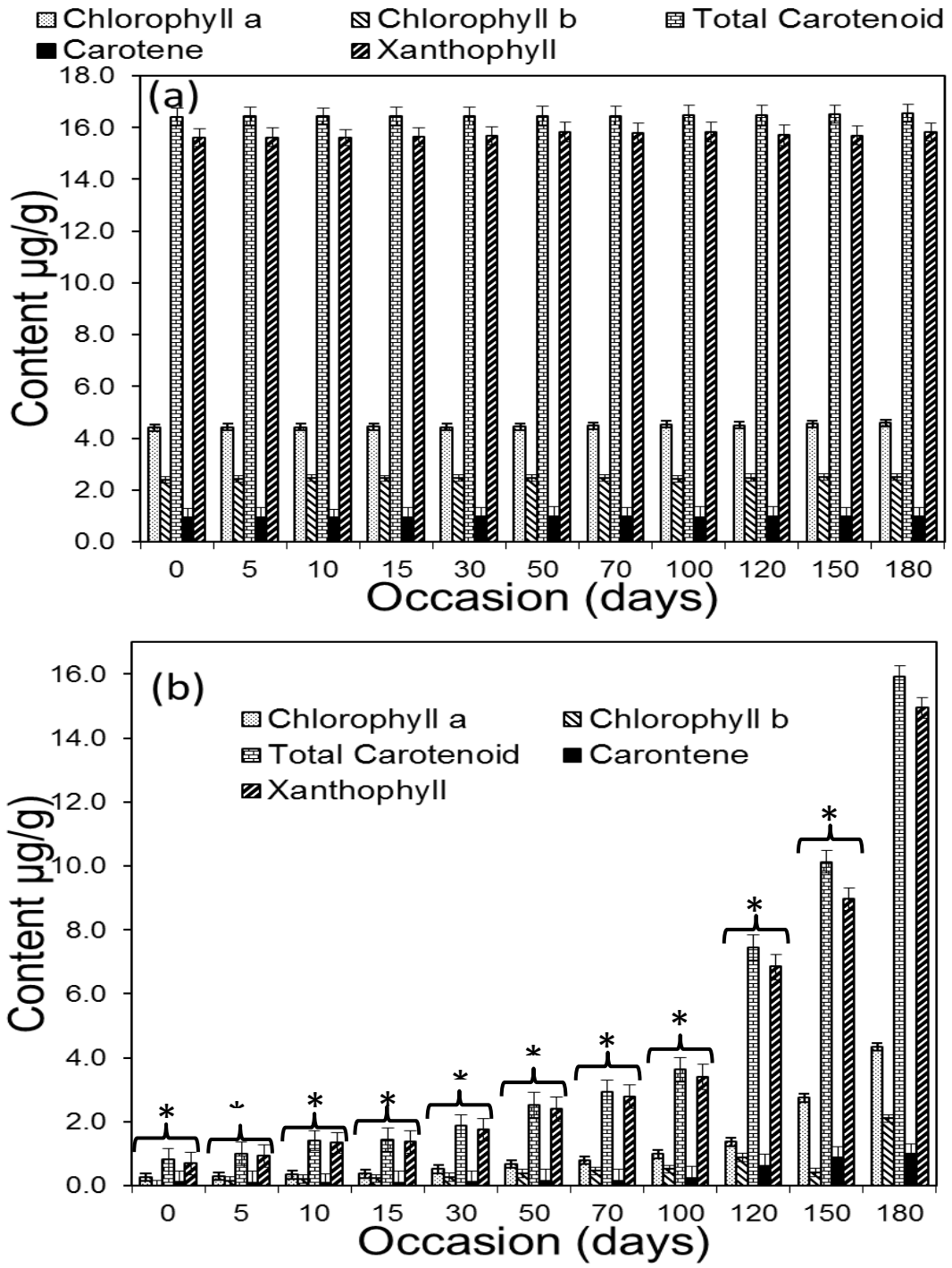

Fig. 1a) Pigment content in control samples and **b)** pigment content in ethoxysulfuron-treated samples. Relative chlorophyll *a*, chlorophyll *b*, carotene, total carotenoid, and xanthophyll content in the aquatic plant *Lemna gibba* exposed for 7 days to field water samples collected at predetermined intervals over a 6-month period. Error bars indicate standard deviation. Values are the mean of three replicates. * indicates significantly different (α<0.05) from the control.