Scholarly Research Journal for Interdisciplinary Studies,
Online ISSN 2278-8808, SJIF 2018 = 6.371, www.srjis.com
PEER REVIEWED & REFERRED JOURNAL, MAY-JUNE, 2019, VOL- 6/51

EFFECT OF ROUNDUP (GLYPHOSATE) ON AQUATIC ECOSYSTEM USING HYDRA AS A MODEL SYSTEM

Seema Borgave[1], Santosh Kshirsagar[2] & Rupendra Bhagde[3]

Affiliations: Post-graduate Department of Zoology, S.N. Arts, D.J.M. Commerce and B.N.S. Science College, Sangamner 422605, Ahmednagar, Maharashtra, India

seemaborgave@gmail.com

Abstract

In the current environmental scenario, one is daily exposed to a wide range of chemicals emitted from various origins and people are hardly aware about these and their long term effects. Roundup is a one of these origins. It is a commonly used herbicide in the area of Sangamner taluka of Ahmednagar district. Farmers use Roundup in large amounts to control different weeds and grasses. Chemical nature of Roundup is Isopropyl-amine salt of Glyphosate (41% w/w) and other relative ingredient (59% w/w). International Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans" (category 2A) in March 2015. Amongst mammals, glyphosate is considered to have "low to very low toxicity". Hydra, a fresh water coelenterate, is known for its tremendous regeneration capacity and has been extensively explored as a model system for aquatic toxicity testing. It has only two cell layers in the body column; all the cells get exposed to the surrounding medium which makes it susceptible to toxicants present in it, though present in very minute quantities. Here we show that commonly used herbicide Roundup hampers the life process even at low concentrations like 1:1000000 dilution. The adhering capacity of the basal discs was drastically reduced in whole and regenerating hydra after treatment. Also, the cells started disintegrating in both, whole and regenerating hydra at 1:1000000 dilutions within 24 hours post-treatment. Thus, heavy use of such herbicides may disturb the aquatic ecosystem. Our study underlines the importance of precautious use of herbicides like Roundup for the protection of environment, especially aquatic ecosystem.

Keywords: *Glyphosate, Hydra, Regenerating hydra, Roundup*

1. Introduction

We are daily exposed to a wide range of chemicals emitted from various origins such as medicines, food additives, petrochemicals, agricultural run-off etc. Such chemicals are usually known to accumulate in the groundwater in the original form or intermediate more toxic forms (Heberer, 2002; Gooddy *et al.*, 2001; Chen *et al.*, 2000; Ternes, 1998; Halling-Sorensen *et al*., 1998; Zaki *et al.*, 1982). Assessment of the short term and long term effects of the potential toxic elements on biological systems is of utmost importance for maintaining the health of the environment. Earlier studies have employed various vertebrate and invertebrate model systems for testing toxicity of different pollutants or agents. However, use of vertebrate model systems often invites ethical concerns which can be easily avoided by using appropriate invertebrate system. Hydra, a freshwater cnidarian, may prove to be useful in this regard (Karntanut and Pascoe, 2002).

Roundup is a commonly used herbicide in the area of Sangamner taluka of Ahmednagar district. Farmers use the Roundup in large amounts to control different weeds and grasses. It is one of the most effective herbicides so it has a great demand in the local market. Chemical nature of Roundup is Isopropyl-amine salt of Glyphosate (41% w/w) and other relative ingredient (59% w/w). Use of Roundup is recommended to control *Axonopuscompressus*, *Synodondactylon*, *Imperatacylindrica* etc., Kalm grass of tea crop and for weed control in general. It is commonly used for agriculture, horticulture purpose as well as garden maintenance. Glyphosate is effective in killing a wide variety of plants, including grasses, and broadleaf woody plants. It is one of the most widely used herbicides. Maximally Glyphosate is absorbed through foliage and minimally through roots, it means that Glyphosate is only effective on growing plants and cannot prevent seeds from germinating (Gomes *et al.,* 2014). When Glyphosate is applied it is readily transported around the plant to growing roots and leaves and this systemic activity is important for its effectiveness (Dill *et al*., 2010; Duke *et al*., 2008). While growth stops within hours of application, it takes several days for the leaves to turn yellow (Hock *et al*., 2004). The World Health Organization International Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans" (category 2A) in March 2015. Amongst mammals, glyphosate is considered to have "low to very low toxicity". The LD_{50} of glyphosate is 5,000 mg/kg for rats, 10,000 mg/kg in mice and 3,530 mg/kg in goats. The acute dermal LD_{50} in rabbits is greater than 2,000 mg/kg (Glyphosate in Review Midpeninsula Regional Open Space District, 2018). Thus, glyphosate can have carcinogenic effects in nonhuman mammals as well. Based on previous studies, it can be predicated that glyphosate-based herbicides may cause life-threatening arrhythmias in mammals. Some evidences also show that such herbicides cause direct electrophysiological changes in the cardiovascular systems of rats and rabbits (Gress *et al*., 2015). Due to the glyphosate formulations, there is either delayed development or accelerated development, reduced size at metamorphosis, developmental malformations of the tail, mouth, eye and head, histological indications of intersex and symptoms of oxidative stress in some animals (Mann *et al.*, 2009). Some glyphosate-based formulations can cause oxidative stress in bullfrog tadpoles (Glyphosate in Review Midpeninsula Regional Open Space District, 2018). In some freshwater invertebrates, glyphosate has a 48-hour LC_{50} ranging from 55 to 780 ppm. The 96-hour LC_{50} is 281 ppm for grass shrimp (*Palaemonetas vulgaris*) and 934 ppm for fiddler crabs (*Ucapagilator*). These values make glyphosate 'slightly toxic to practically non-toxic' (Glyphosate in Review Midpeninsula Regional Open Space District, 2018). Some other studies suggest that glyphosate may be mutagenic. The glyphosate-based formulations can cause DNA strand breaks in various taxa of animals *in vitro* (Glyphosate in Review Midpeninsula Regional Open Space District, 2018).

Hydra, a fresh water coelenterate, is known for its tremendous regeneration capacity and has been extensively explored as a model system for aquatic toxicity testing. It has only two cell layers in the body column and thus all the cells are exposed to the surrounding medium making it susceptible to toxicants that are present in very minute quantities. Due to its enormous ability to regenerate, it also allows the assessment of teratogenic compounds and the impact of contaminants on stem cells (Gilbert, 2010). Thus, hydra is used as an environmental indicator. The analysis of toxicants is easier in simple animal like hydra thorough the observation of alteration in tentacle morphology, loss of tentacles, change in contractility, loss of regenerative capacity, inability to attach to substratum, altered behavioral patterns and mortality. Importantly, hydra are ubiquitous throughout freshwater environments and relatively easy to culture making them appropriate for use in small scale bioassay systems. It is a very good alternative model for preliminary toxicity screening and can substantially reduce the use of vertebrate animals (Riedl *et al.,* 2018; Zeeshan *et al.,* 2017; Murugadas *et al*., 2016; Quinn *et al*., 2012).

As mentioned earlier, a number of studies point out at a hazardous role of glyphosate on life. Considering the enormous use of this herbicide in the farms, one needs to address the effects of glyphosate on simple aquatic animals like hydra which is a ubiquitously found fresh water animal, an important part of aquatic ecosystem and due to its regeneration capacity considered as an immortal animal. The present study describes the effects of the Roundup on whole and regenerating hydra.

2. Materials and Methods

2.1. Collection of hydra

Green hydra were collected from the local pond from the S.N. Arts, D.J.M. Commerce and B.N.S. Science College campus. Hydra were transferred into hydra medium with the help of a dropper. *Amoeba*, *Paramecium*, Daphnia, Cyclops and other zooplanktons were collected from the same local pond and used as food for hydra.

2.2. Preparation of treatment solutions

Whole and regenerating hydra were treated with various doses of Roundup. These doses were prepared by serial dilution of Roundup so as to prepare different concentrations ranging from 1:10, 1:100 up to 1:1000000 dilution

2.3. Treatment of whole hydra with Roundup dilutions

Healthy hydra were selected and randomly distributed in equal numbers into various beakers containing 50 ml hydra medium for treatment purpose. Hydra kept in beaker with of medium alone served as control and rest were used for treatment with various dilutions of Roundup. The overall morphology of hydra from control and treated groups was observed for next 72 hours and the changes were noted down. The treatment was repeated four times to confirm the effects of Roundup on whole hydra for calculating the LC50.

2.4. Treatment of regenerating hydra with Roundup

Healthy hydra were selected and cut into two pieces by taking a sharp transverse cut using sharp needles in the middle of the body column. The body column pieces with either hypostome or basal disc were separated and kept in separate beakers for treatment. One beaker kept as a control and rest for Roundup treatment (LC50 dose). Regenerated hydra from both control and treated groups were observed for next 72 hrs. All the experiments were repeated minimum 4 times.

3. Results

Effects of Roundup on whole hydra

Hydra from the control group exhibited normal morphology and sticking behavior throughout the treatment window. The dilutions of Roundup from 1:10 to 1:100000, killed almost 100% of the treated animals by the end of 72 hrs (Table 1 and Figure 1). On the other hand, at 1:1000000 dilution Roundup was found to kill approximately 50 percent of the treated hydra as compared to controls by 72 hrs. However, the treated animals showed impaired attachment and mostly floated on the treatment solution. Thus, 1:1000000 dilution of Roundup was found to be the LC50 dose for hydra in our study.

Table no 1: Effect of Roundup at various dilutions on whole hydra

Treatment window	Control	1:10	1:100	1:1000	1:10000	1:100000	1:1000000
24 hours	100 %	0 %	0 %	0 %	0 %	16.66 %	77.77 %
48 hours	100 %	0 %	0 %	0 %	0 %	1 %	61.11 %
72 hours	100 %	0 %	0 %	0 %	0 %	1 %	50 %

The percentage given here depicts the overall percent of live hydra at the end of the treatment window.

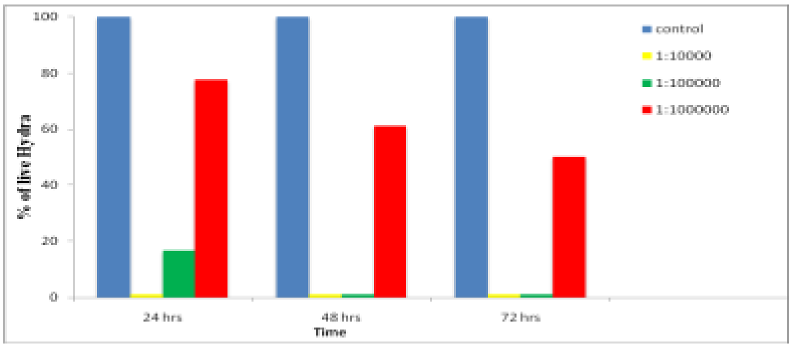

Figure 1: *Histograph depicting the percentage of live hydra after treatment with Roundup for different time intervals*

Figure 2: *Effects of Roundup on whole hydra after 24 hours - (a) Control hydra; (b-f) Roundup treated hydra - (b)1:100 dilution, (c)1:1000 dilution, (d)1:10000 dilution, (e)1:100000 dilution, (f)1:1000000 dilution*

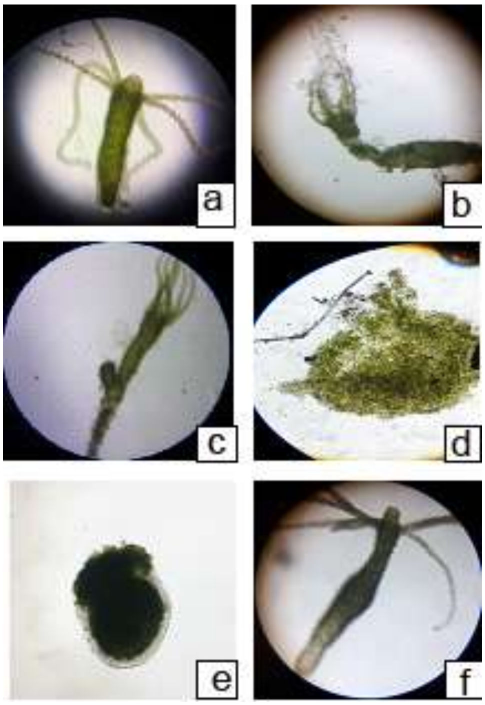

Figure 3: *Effects of Roundup on whole hydra after 48 hours - (a) Control hydra; (b-f) Roundup treated hydra - (b)1:100 dilution, (c)1:1000 dilution, (d)1:10000 dilution, (e)1:100000 dilution, (f)1:1000000 dilution*

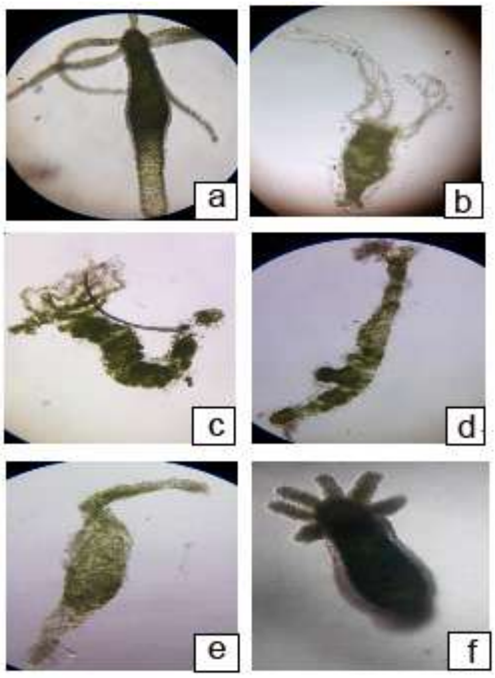

Figure 4: *Effects of Roundup on whole hydra after 72 hours - (a) Control hydra; (b-f) Roundup treated hydra - (b)1:100 dilution, (c)1:1000 dilution, (d)1:10000 dilution, (e)1:100000 dilution, (f)1:1000000 dilution*

Effects of Roundup on regenerating hydra

The overall regeneration process in the regenerating hypostomes and basal disc portions in the control was normal and complete regeneration was achieved by 72 hrs in 100% basal disc pieces. On the hand, the regeneration process was hampered in both, hypostome pieces and basal disc pieces in presence of Roundup (Table 2 and Figure 5).

Table no 2: Effects of Roundup on regenerating hydra

Treatment window	**After 24 hours**		**After 48 hours**		**After 72 hours**	
	Hypostome	**Basal Disc**	**Hypostome**	**Basal Disc**	**Hypostome**	**Basal Disc**
Control	97.61 %	100 %	97.61 %	100 %	97.61 %	100 %
Roundup (1:1000000 dilution)	88.09 %	92.85 %	85.71 %	78.57 %	78.57 %	66.66 %

The percentage is calculated on the basis of regeneration shown in hypostome and basal disc in each category.

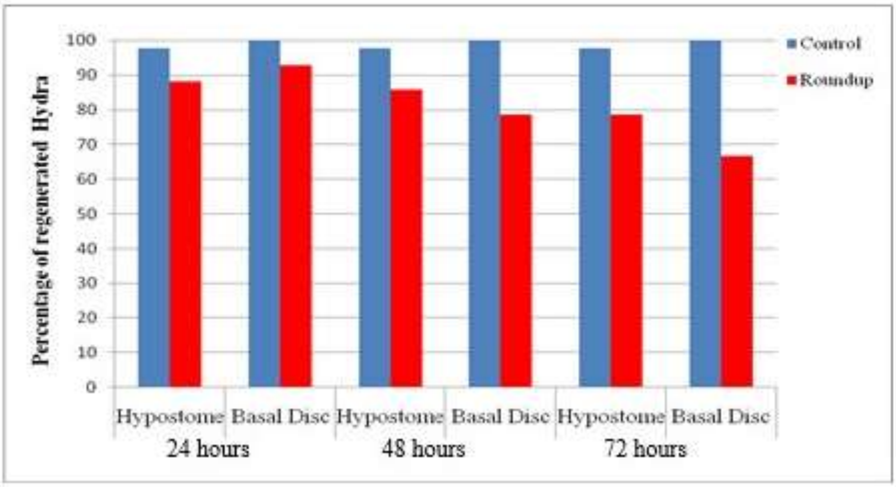

Figure 5: *Histograph depicting the percentage of the regenerated hydra after treatment with Roundup for different time intervals*

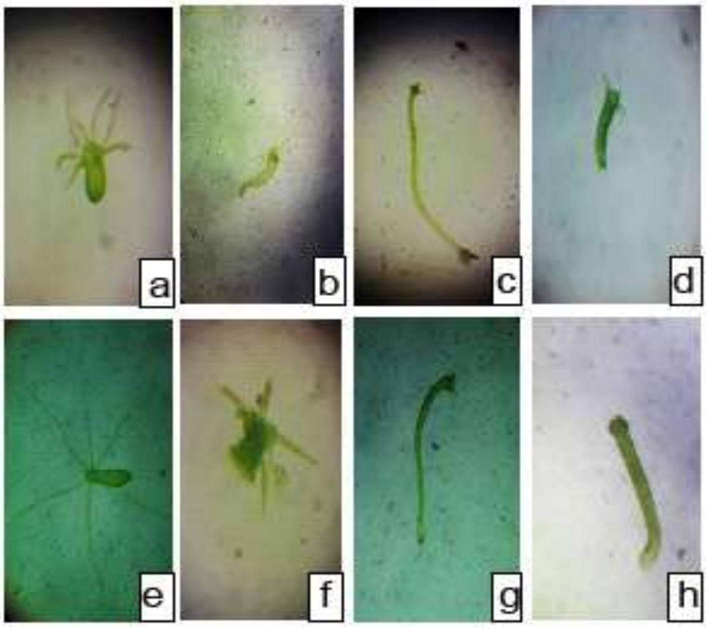

Figure 6: *Regeneration after 24 hours- (a, b) hypostome of control, (c, d) basal disc of control, (e, f) hypostome of 1:1000000 dilution of Roundup, (g, h)basal disc of 1:1000000 dilution of Roundup*

In control, after 24 hours of post-cutting, hypostome starts to regenerate the lost basal region (Figure 6a,b) and there is beginning of the hypostome formation in the basal disc pieces (Figure 6c,d). In some cases disintegration of hydra body was observed in the treated hypostome pieces (Figure 6f), while majority of the regenerating basal discs showed the normal processes (Figure 6g,h).

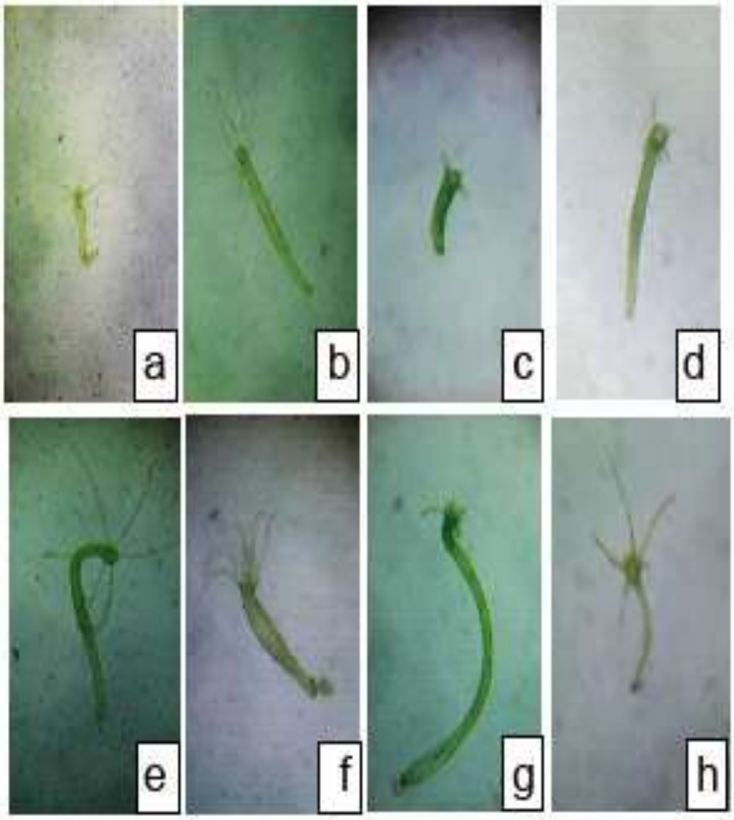

Figure 7: *Regeneration after 48 hours- (a, b) hypostome pieces of control, (c, d) basal disc pieces of control, (e, f) hypostome of 1:1000000 dilution of Roundup, (g, h) basal disc of 1:1000000 dilution of Roundup*

After 48 hours, the normal regeneration of lost structures was observed in the control; both regenerating hypostome regions (Figure 7 a,b) and basal disc regions (Figure 7 c,d). Roundup treated hypostomes with mid-way regenerated basal discs started floating on the water surface indicating loss of attachment power within 48 hours (Figure 7 e,f). The regenerating basal discs exhibited development of the lost hypostome regions that was comparable to the controls however these pieces also lost the attachment power (Figure 7 g,h).

Normal regeneration of hypostome and basal disc was seen in control within 72 hours (Figure 8 a-d). On the other hand, the treated regenerating hydra pieces showed recovery of the lost structures comparable to controls however, the regenerated hypostome and basal disc floated on the water surface. In some cases, the disintegration of the body cells was observe as shown in figure 8 (f & h).

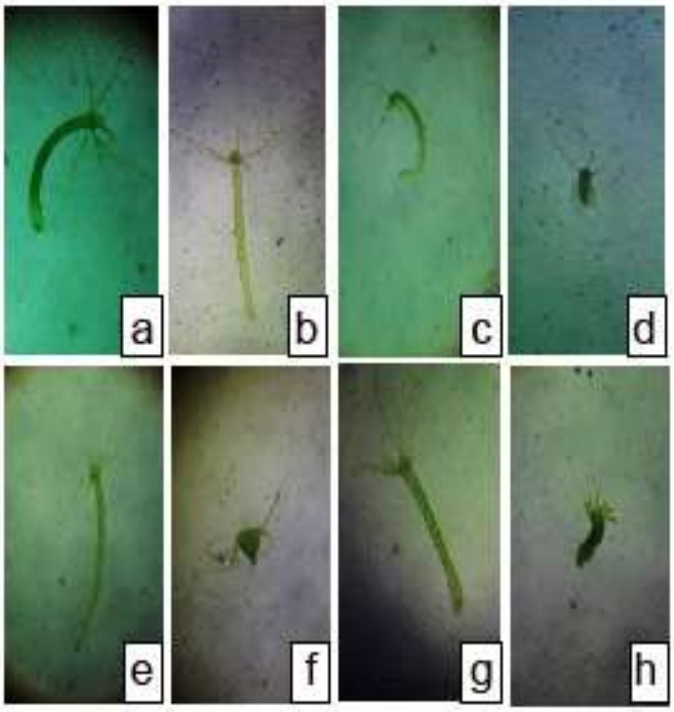

Figure 8: *Regeneration after 72 hours- (a, b) hypostome of control, (c, d) basal disc of control, (e, f) hypostome of 1:1000000 dilution of Roundup, (g, h) basal disc of 1:1000000 dilution of Roundup*

4. Discussion and Conclusions

Earlier studies on the effects of insecticides on whole hydra have exhibited immediate death of the animals after treatment. The tentacles and hypostomes of such hydra were disintegrated immediately with lesions on the body of hydra (Kovacevic *et. al.*, 2007; Kalafatic *et. al.*, 1997). In the present study, when hydra were treated with the Roundup at the high concentrations i.e. 1:10 and 1:100 dilutions, the treatment led to cell disintegration immediately. The dose of 1:1000000 dilution was identified as LC50 of Roundup for hydra. At this dose, hydra showed the impaired attachment. In this way, commonly used herbicide Roundup hampers the life process even at low concentrations like 1:1000000 dilution. Thus, heavy use of such herbicides may disturb the aquatic ecosystem.

Regenerating hydra are shown to be highly sensitive to physical and chemical toxic agents like ultraviolet light (Znidaric and Lui, 1974) and insecticides (Kalfatic, 1991; Znidaric, 1983). In our study, regenerating hydra were treated with the Roundup at the dilution 1:1000000, the LC50 dose for whole hydra. In case of hypostome pieces, the regeneration of basal disc like structure took place but the regenerated hydra did not exhibit its attachment power as seen in controls. Due to treatment with Roundup, sometimes the body cells started disintegrating. In case of the basal disc, when the basal disc began to regenerate the lost hypostome, the hydra started floating on the water within the 24 hrs. As compared to the respective controls, the regeneration in both hypostome piece and basal disc was delayed and the attachment power of the basal disc was reduced to a greater extent. In this way, our study identifies LC50 of Roundup for hydra, highlights ill effects of Roundup on whole hydra and regenerating hydra even at very low concentrations. Our study thus underlines the importance of precautious use of herbicides like Roundup for the protection of environment, especially aquatic ecosystem.

Acknowledgements

The authors are thankful to the Principal and Head of the Zoology Department of S.N. Arts, D.J.M. Commerce and B.N.S. Science College, Sangamner for providing the research facilities.

References

Chen, Y., Zhu, X., Zhu, X. (2000). Transformations and hydraulic captures of petrochemical contaminants in a karstfractured aquifer. Environ Geol ***39****, 1304-1308.*

Dill G.M., Sammons R.D., Feng P.C., Kohn F., Kretzmer K., Mehrsheikh A., Bleeke M., Honegger J.L., Farmer D., Wright D., Haupfear E.A. (2010). Glyphosate: Discovery, Development, Applications, and Properties. In Nandula VK. Glyphosate Resistance in Crops and Weeds: History, Development, and Management. Hoboken, N.J.: Wiley. ISBN 978-0-470-41031-8.

Duke S.O. and Powles S.B. (2008). Glyphosate: a once-in-a-century herbicide: Mini-review. Pest Management Science ***64****, 319–325, doi:10.1002/ps.1518.*

Gilbert, S.F. (2010) Developmental Biology Nineth edition, Sinauer Associates, Inc., Sunderland, Massachusettes.

Glyphosate In Review (2018). Prepared by Blankinship & Associates, Inc. 1615 5th Street Davis, CA 95616 for Midpeninsula Regional Open Space District 330 Distel Circle Los Altos, CA 94022.

Gomes M.P., Smedbol E., Chalifour A., Hénault-Ethier L., Labrecque M., Lepage L., Marc Lucotte M. and Juneau P. (2014). Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: an overview. Journal of Experimental Botany ***65*** *(17), 4691–4703*

Gooddy, D. C., Bloomfield, J. P., Chilton, P. J. (2001). Assessing herbicide concentrations in the saturated and unsaturated zone of a Chalk aquifer in southern England. Ground Water ***39,*** *262-271.*

Gress S., Lemoine S., Seralini G.E., Puddu P.E. (2015). "Glyphosate-based herbicides potently affect cardiovascular system in mammals: review of the literature". Cardiovascular Toxicology ***15*** *(2): 117–26. doi:10.1007/s12012-014-9282-y. PMID 25245870.*

Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P. F., et al. (1998). Occurrence, fate and effects of pharmaceutical substances in the environment- a review. Chemosphere ***36,*** *357-393.*

Heberer, T. (2002). Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett ***131****, 5-17.*

Hock B., Elstner E.F. (2004). Plant Toxicology, Fourth Edition. CRC Press. pp. 292–296. ISBN 978-0-203-02388-4.

Kalafatic M. (1997). Regeneration and asexual reproduction of Hydra obligactis treated with different pesticides. Biologia. ***52,*** *475-480.*

Kalfatic M. (1991) Effect of insecticides (Dimline 25, Torak EC 24 and Gamacide 20) on Hydra (Hydra vulgaris pallas) Int.J.Dev.Bio. ***35,*** *335-340.*

Karntanut, W. and Pascoe, D. (2002). The toxicity of copper, cadmium and zinc to four different Hydra (Cnidaria: Hydrozoa). Chemosphere ***47,*** *1059-1064.*

Kovacevic, G., Željezic, D., Horvatin, K., and Kalafatic, M. (2007). Morphological features and comet assay of green and brown hydra treated with aluminium. Symbiosis ***44,*** *145-152.*

Mann R.M., Hyne R.V., Choung C.B., Wilson S.P. (2009). "Amphibians and agricultural chemicals: Review of the risks in a complex environment". Environmental Pollution. ***157****(11): 2903–2927. doi:10.1016/j.envpol.2009.05.015.*

Murugadas A., Zeeshan M., Thamaraiselvi K., Ghaskadbi S. and Akbarsha M. A. (2016) Hydra as a model organism to decipher the toxic effects of copper oxide nanorod: Eco-toxicogenomics approach. Scientific RepoRts | 6:29663 | DOI: 10.1038/srep29663

Quinn B, Gagné F and Blaise C. (2012) Hydra : A model system for environmental studies. Int. J. Dev. Bio. ***56*** *(6-8), 613-25.*

Riedl V, Agatz A, Benstead R, Ashauer R. (2018). A standardized tritrophic small-scale system (TriCosm) for the assessment of stressor-induced effects on aquatic community dynamics. Environ Toxicol Chem. ***37****(4), 1051-1060. doi: 10.1002/etc.4032. Epub 2018 Jan 5.*

Ternes, T. A. (1998). Occurrence of drugs in German sewage treatment plants and rivers. Water Res ***32,*** *3245-3260.*

Zaki, M. H., Moran, D., and Harris, D. (1982). Pesticides in groundwater: the aldicarb story in Suffolk County, NY. Am J Public Health ***72****, 1391-1395.*

Zeeshan M., Murugadas A., Ghaskadbi S., Ramaswamy B.R., Akbarsha M.A. (2017) Ecotoxicological assessment of cobalt using Hydra model: ROS, oxidative stress, DNA damage, cell cycle arrest, and apoptosis as mechanisms of toxicity. Environ Pollut. ***224****, 54-69. doi: 10.1016/j.envpol.2016.12.042. Epub 2017 Feb 17.*

Znidaric D. and Lui A. (1974) Regeneration of proximal and distal body part in hydra exposed to ultraviolet light. Z Mikrosk Anat Forsch. ***88****(4), 637-648.*

Znidaric D. (1983) Regeneration of Hydra damaged by Zolone PM insecticide, Z.Mikrosk Anant Forsch ***97****(6):1046-1054.*