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Studies on hill stream fish photosensitivity with psoralene and retene photosensitizers

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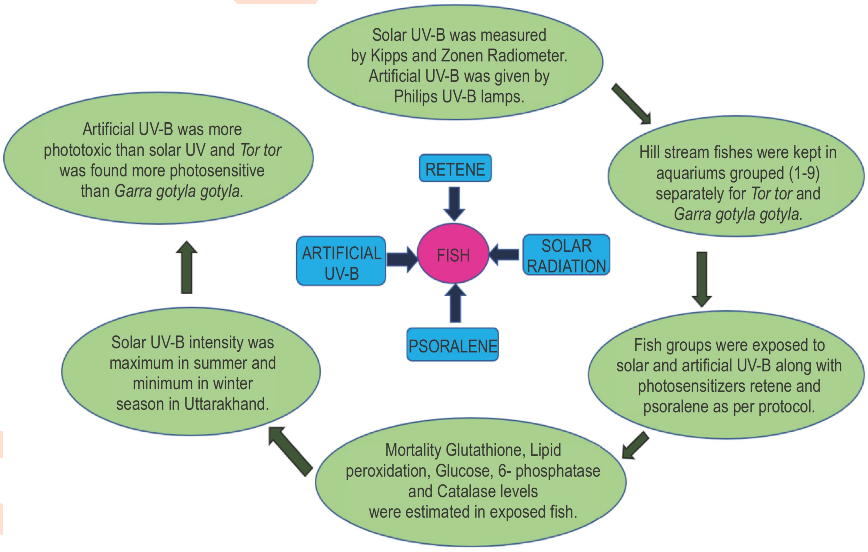
Abstract

Aim : A study was designed to measure the solar UV- B in Uttarakhand, Garhwal region and to observe the effect of retene and psoralene photosensitizers on hill stream fishes, *Tor tor* and *Garra gotyla* in the presence of natural solar ultraviolet and equal intensity of artificial UV-B radiation.

Methodology : Solar UV-B was measured with the help of Kipps and Zonen radiometer having UV-B sensors. Experimental sites selected were Dehradun and Tehri located in Garhwal region of Uttarakhand. The artificial radiation was provided with Philips UV-B Lamps. Mortality, biochemical and enzymological parameters, which included glutathione, glucose-6-phosphatase, catalase and lipid peroxidation were analyzed.



Results : The data of monitoring of UV-B showed that the maximum UV-B intensity was observed during the month of May to August and the minimum was in the month of December to February. Mortality rate of fish exposed to solar radiation, artificial UV-B, psoralene and retene indicated highest with 26% in *Tor tor,* exposed with psoralene+ artificial UV-B. Reduced glutathione, glucose- 6- phosphatase and catalase level decreased in all the groups compared to control. The maximum reduction in GSH level was observed after treatment of artificial UV-B + psoralene in *Tor tor,* while maximum reduction in catalase and glucose-6- phosphatase was observed after retene and artificial UV-B.

Interpretation : *Tor tor* and *Garra gotyla* are important hill stream fishes. Retene and psoralene are natural photosensitizers present in the aquatic ecosystem and become phototoxic by generating oxidative radicals. Artificial UV-B was more toxic than natural solar radiation and *Tor tor* was found more sensitive than *Garra gotyla.* Enhanced UV-B with retene and psoralene photosensitizers affect hill stream fishes and aquatic biodiversity.

Key words: *Garra gotyla*, Phototsensitizers, Psoralene, Retene, *Tor tor*, UV-B radiation

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Introduction

Since the discovery of the Antarctic ozone hole, many studies have been conducted to evaluate the effects of enhanced UV-B on plants and animals. It is widely accepted now that even natural levels of ultraviolet radiation (UV-R) is harmful for some autotrophic organisms (Villafane *et al.,* 2004). It is evident that the broad-spectrum UVR wavelength (100- 400 nm) region of solar radiation is carcinogenic. Studies of human cancer associated with exposure to devices that emit artificial broad-spectrum UV-R, indicate that tumors develop at the same tissue sites in humans exposed to sunlight and in animals exposed to artificial sources (Panich *et al.,* 2016). Off the solar UV radiation energy reaching the equator, 95% is UV-A and 5% is UV-B. No measurable UV-C from solar radiation reaches the earth's surface, because the shortest UV wavelengths are completely absorbed by ozone, molecular oxygen and water vapor in the upper atmosphere (Williamson *et al.,* 2014). The UVR wavelengths to which an individual is exposed vary considerably with latitude, altitude, time of day and season. Mechanistic studies with human tissue have demonstrated that the UVB component in solar radiation causes DNA damage (Lim and Lee, 2016). The 21st century is a time for freshwater ecosystems and aquatic resources as United Nation declared 2005-2015 UN decade for water and their resources. A multitude of stressors, including urbanization and associated habitat alteration and loss, alien invasive species, overharvest, pollution and climate change, have resulted in freshwater fish becoming one of the most threatened taxa globally (Patz *et al.,* 2014).

Aquatic ecosystems are exposed to excessive pollutants and contaminants from various sources like domestic and industrial sewage, agricultural waste processes, heavy metals and others. The excessive discharge of different categories of waste material into the water directly affects aquatic organisms, including fish, which are considered as bio-indicators of environmental contamination. Psoralene and retene are present in resinous wood, plants asbabchi, fig and herbs contaminates the water. Changes in the fish organs, such as gills, are good biomarkers of water contamination (Maisano *et al.,* 2017). Polycyclic Aromatic Hydrocarbons (PAH) occur as natural products in plants, microbes, released from volcanic activity and forest related precursors produced by fungi, plants and animals. Major sources of PAH in surface waters are oil spills, industrial waste, fossil fuel combustion and other pyrolytic processes attributed to anthropogenic activity (Lawal, 2017). Retene, (methyl isopropyl phenanthrene-$C_{18}H_{18}$) is a polycyclic aromatic hydrocarbon present in coal tar fraction with boiling point above 360 ºC. It occurs naturally in the tars obtained by distillation of resinous woods. Retene is derived from degeneration of specific diterpenoids biologically produced by conifer trees. Psoralene, 7H- furobenzopyran- 7- one, occur naturally in the environment and is extracted from certain food sources such as dried ripe fruits of *Psoralea corylifolia*, figs etc. (Gajula *et al.,* 2018) It is highly useful in treatment of skin problems like psoriasis, vitiligo and eczema (Bansal *et al.,* 2013). These chemicals become photo reactive due to ultraviolet radiation. Interactive effect of UV-B radiation and heavy metals as cadmium and nickel on *Spinacea oleracea* and zooplanktons have been observed (Mishra *et al.,* 2014; Kumar *et al.,* 2014).

Garra gotyla is a hill stream fish adapted to live in fast flowing waters and is a bottom dweller having well developed suckers. It plays an important role in maintaining the ecological balance of hill streams and is also kept in aquaria to reduce the growth of algae. Langer *et al.* (2013) while compiling the bibliography of Mahseers of the Indian subcontinent described this group as the "King of Indian Aquatic Systems". *Tor tor* cold water fish is a widely distributed species in South and South-east Asia with a restricted area of occurrence. The species has been reported across the Himalayan region and is an important game fish (Bhatt and Pandit, 2016; Baruah and Sharma, 2018). The studies on fish larvae in the presence of UV radiation available are inadequate although the UV radiation's impact on fish and aquatic arthropods have been documented with retene and riboflavin (Kumar *et al.,* 2013; Kumar *et al.,* 2016). In this study the impact of solar and artificial ultraviolet-B radiation was studied on *Garra gotyla* and *Tor tor* cold water fishes with psoralene and retene photosensitizer by using biochemical and enzymological parameters such as mortality rate, glutathione, lipid peroxidation, glucose- 6- phosphatase and catalase.

Materials and Methods

Solar UV was measured with the help of Kipps and Zonen radiometer having UV-B sensors.Sites selected were Dehradun (600 msl) and Tehri (2100 msl). The fish collection was done from four sites of Dehradun (30° 39' N and 78° 10' E), *i.e.,* Sahastradhara, Song, Maldeota and Gular Ghati. Fish fingerlings of 2-3 cm size were collected and cultured by following the method of Tisher and Song lake (2001). Fish were placed in glass aquariums of size 25 x 30 x 45 cm. containing 5-6 l water in each and physico-chemical parameters were maintained for acclimation for about 7-10 days. Experimental protocol was prepared for the morphological, phototoxicological and biochemical studies. Morphological parameters included movement, pigmentation, scale deformation, metallization, sun burn response, mortality etc. The fish were divided randomly into 9 groups containing 5 fish in each group. Group 1 was control, Group 2 was retene (50 ug l^{-1}), Group 3 treated with psoralene (50 ug l^{-1}), Group 4 was exposed to solar UV, Group 5 exposed to same intensity of artificial UV-B, Group 6 was exposed to retene in the presence of solar UV- B, Group 7 was treated with retene in presence of artificial UV- B, Group 8 was treated with psoralene in presence of solar radiation and Group 9 treated with psoralene in the presence of artificial UV-B. Artificial radiation was given with Philips UV-B Lamps. Experiment was performed on *Tor tor* and *Garra gotyla* simultaneously. Solar and artificial radiation was given for 2 hrs (11.00 a.m.-1.00 p.m.) daily. UV-B radiation intensity used was 0.750-800 mw cm^{-2}. Experiment was performed for a period of 60 days between October to November in the year 2018. Retene and psoralene was procured from Sigma Aldrich US.

Fish behavior and mortality was observed during the experiment as per Scott and Sloman (2004). Lipid peroxidation was assayed by measuring malondialdehyde (MDA) content in gills using thiobarbiturc acid by the method of Smith and Anderson (1987). The absorbance was recorded using UV/ visible spectrophotometer at 532 nm. 1, 1, 3 tetramethoxy propane (Wako, Japan) was used as the standard. Reduced glutathione (GSH) was measured using dithiobisnitrobenzoic acid (DTNB) (Beutler *et al.,* 1963), Catalase H_2O_2- H_2O_2 oxidoreductase enzyme activity in the fish tissue was determined by breakdown of hydrogen peroxide using titration method (Takahara *et al.,* 1960). Absorbance was recorded at 412 nm with the help of UV/ visible spectrophotometer. Glucose- 6- Phosphatase was measured following the method (Fishman *et al.,* 1967). Intergroup comparison and statistical inferences were drawn by using Students't' test with P value *0.05, *0.01 (Fisher, 1963).

Results and Discussion

The data revealed the maximum intensity of solar UV-B during May to August and minimum during December to February. Solar UV-B level was higher at Tehri (2100 msl) as compared to Dehradun (600 msl). The highest value (1.106 mw cm^{-2}) of solar UV-B as measured at Tehri in August and minimum (0.376mw cm^{-2}) in December at Dehradun (Fig.1). Mortality rate of fish within 60 days with 2 hrs of exposure to solar, artificial UV-B psoralene and retene indicated highest mortality (26%) in *Tor tor* with psoralene + artificial UV-B exposure. Comparatively lower

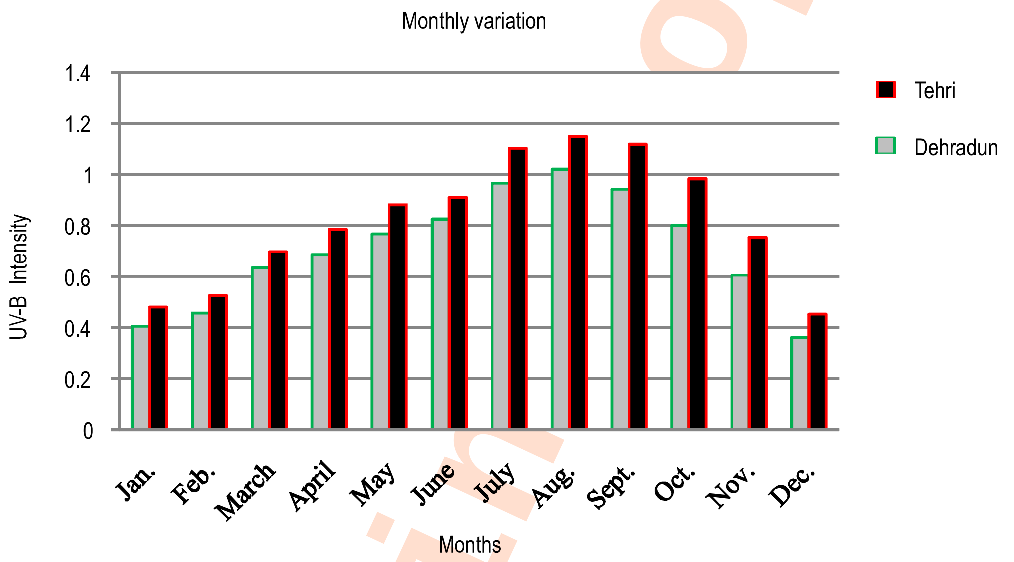

Fig. 1: Monthly variation in solar UV-B level at different altitudes in Dehradun and Tehri during 2017 and 2018.

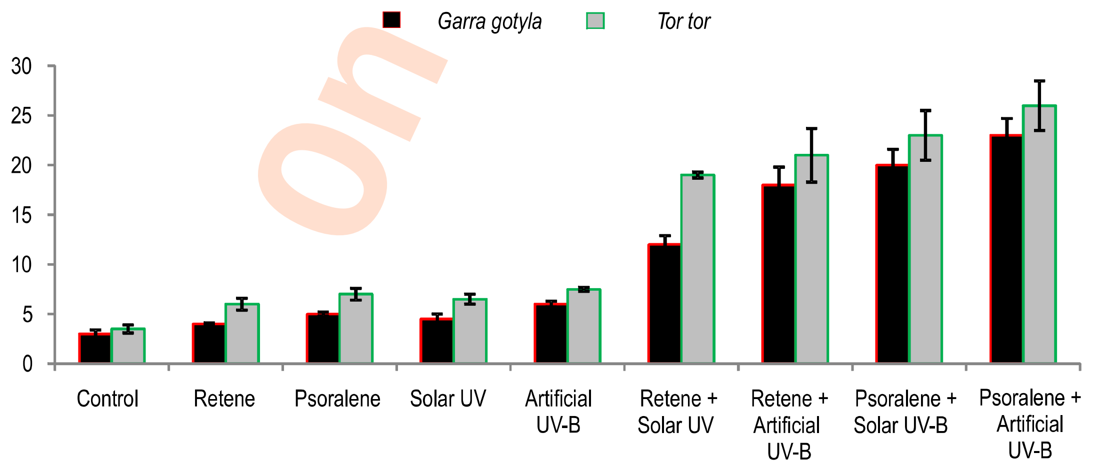

Fig. 2: Mortality rate in hill stream fishes with retene and psoralene along with solar and artificial UV-B.

Table 1: Reduced glutathione (mg g^{-1}) and malondialdehyde (MDA) level µ mole 100 g^{-1} protein in fishes after exposure to solar and artificial UV- B radiation with retene and psoralene photosensitizers

Treatment	Reduced glutathione		Lipid peroxidation (MDA)	
	Garra gotyla	*Tor tor*	*Garra gotyla*	*Tor tor*
Control	980 ± 2.80	975 ± 1.00	6.29 ± 0.31	6.35 ± 0.29
Retene	962 ± 3.31^{NS}	950 ± 2.01^{NS}	7.01 ± 0.62^{NS}	7.09 ± 0.56^{NS}
Psoralene	952 ± 2.19^{NS}	941 ± 2.04^{NS}	7.32 ± 0.48^{NS}	7.49 ± 0.39^{NS}
Solar UV	938 ± 5.13*	920 ± 4.13*	6.76 ± 0.54^{NS}	6.83 ± 0.43^{NS}
Artificial UV-B	892 ± 2.14*	872 ± 2.12*	7.21 ± 0.42*	7.37 ± 0.23*
Retene + Solar UV	798 ± 1.78**	789 ± 1.35**	7.78 ± 0.68*	7.86 ± 0.71*
Retene + Art. UV-B	695 ± 3.27**	718 ± 2.17**	8.08 ± 0.56**	8.19 ± 0.59**
Psoralene + Solar UV-B	753 ± 0.29 **	769 ± 1.29**	7.46 ± 0.63**	7.51 ± 0.66**
Psoralene + Art. UV-B	678 ± 1.13**	643 ± 2.14**	7.93 ± 0.65**	7.99 ± 0.72**

Results are mean ± S.E. of 5 observations in each group. P value *<0.05, **<0.01, NS- Non Significant

Table 2: Glucose- 6 – phosphatase (µ mol^{-1} min^{-1} m l^{-1}) and catalase (units m l^{-1}) level in fishes after exposure to solar and artificial UV-Bradiations with retene and psoralene photosensitizers

Treatment	Glucose- 6 – phosphatase		Catalase	
	Garra gotyla	*Tor tor*	*Garra gotyla*	*Tor tor*
Control	12.12 ± 0.89	12.19 ± 0.85	78.08 ± 1.03	82.05 ± 1.12
Retene	12.16 ± 0.64^{NS}	12.37 ± 0.58^{NS}	77.16 ± 2.34^{NS}	78.22 ± 1.37^{NS}
Psoralene	12.51 ± 0.88^{NS}	12.59 ± 0.88^{NS}	78.22 ± 1.65^{NS}	78.16 ± 1.80^{NS}
Solar radiation	12.16 ± 1.27^{NS}	12.34 ± 1.22^{NS}	75.42 ± 1.63 ^{NS}	79.23 ± 2.04^{NS}
Artificial UV-B	11.19 ± 1.60^{NS}	12.23 ± 1.63^{NS}	71.16 ± 1.13*	75.17 ± 1.21*
Retene + Solar radiation	9.16 ± 0.09*	9.56 ± 0.13*	64.43 ± 2.56*	67.28 ± 1.45*
Retene + Artificial UV-B	8.99 ± 0.31**	8.96 ± 0.33**	60.74 ± 2.24**	63.65 ± 1.76**
Psoralene + Solar radiation	11.08 ± 0.33*	11.53 ± 0.37*	68.73 ± 1.54*	74.45 ± 1.78*
Psoralene + Artificial UV-B	10.62 ± 0.12*	10.75 ± 0.15*	62.15 ± 1. 50*	66.23 ± 1.56*

Results are mean of five replicates ± S.E. in each group. P value **<0.05, **<.0.01, NS- Non significant

mortality rate was observed in *Garra gotyla* than *Tor tor* (Fig. 2). The reduced glutathione level decreased subsequently in all the groups in comparison to control. Non-significant changes were observed after retene and psoralene treatment. Significant changes were observed after retene and psoralene co-treated with solar and artificial UV-B radiation. The minimal value of GSH level was observed after treatment of artificial UV-B + psoralenein *Tor tor* followed by *Garra gotyla* (Table1). Malondialdehyde (MDA) level a marker of lipid peroxidation and cell injury indicated an increase in MDA level in comparison to control. Non-significant change was observed after individual treatment of retene, psoralene and natural solar UV. Significant increase in malondialdehyde level was observed after exposure to artificial UV-B and co-treatment of solar and artificial UV-B radiation with retene and psoralene. The maximum level of MDA was observed in *Tor tor* with photosensitizers and artificial UV-B exposure (Table 1). Glucose- 6- phosphatase activity decreased in all groups in comparison to control. Significant changes were observed after co-treatment of UV radiation and photosensitizers. The maximum reduction in glucose-6-phosphatase activity was observed after treatments with retene and artificial UV-B (Table 2). Catalase level decreased in all the groups in comparison to control.

The maximum reduction in catalase activity was observed after retene + artificial UV-B. Non-significant changes were observed after individual exposure of retene, psoralene and solar radiation. Significant reduction in catalase activity was observed after artificial UV- B alone and co- exposure of solar and artificial UV- B with retene and psoralene (Table2). Glucose-6-phosphatase is a multicomponent complex located in the endoplasmic reticulum. Hydrolysis of glucose-6-phosphatase involved in the coupled function of different membrane translocase that mediate penetration and removal of inorganic phosphate and glucose (Fortes *et al.,* 2016). Significant decrease in glucose-6- phosphatase and catalase was observed after treatment of retene and psoralene photosensitizers with solar and artificial UV- B radiation. Result are supported by the studies in fish, *Cyprinius carpio and Oncho rhyncus* regarding oxidative stress and antioxidative enzyme defense (Kolesova *et al.,* 2018). Result on increase in lipid peroxidation and reduction in glutathione (GSH) content are supported by the studies that increased malondialdehyde (MDA) content is associated with increase in oxidative stress in UV-B irradiated RBC and copepods (Won *et al.,* 2014).

Surface UV radiation is influenced by increased stratospheric ozone due to reduction in ozone depleting substances and changes in ozone and cloudiness induced by increasing concentrations of greenhouse gases. Models suggest that in the first half of 21st century recovery of ozone and changes in cloudiness will result in decreased surface erythemal irradiance by 2- 10% at mid latitudes, and by 20% at northern and 50% at southern high latitudes. These reductions are dominated by the projected increase in stratospheric ozone (Mitchell *et al.,* 2010). The extent and duration of periods of ice and snow cover on oceanic and inland waters have been decreasing in recent decades, altering the underwater light environment and clear weather potentially resulting in direct exposure of the aquatic environment to higher UV radiation (Clark, 2013). Fishes are susceptible to UV- B radiation with sensitivity varying within groups and species (Kumar and Aara, 2016). Difference in photosensitivity was also recorded among the fishes, *Barilius bendelisis* and *Nemacheilus rupicola* (Chowdhary *et al.,* 2018). Eggs and larvae also have limited behavioral capabilities to avoid UV-B exposure due to their reduced mobility and some species cannot detect UV-B radiation at early developmental stages (Olson *et al.,* 2006). Studies with numerous fish species have shown that UV-B radiation can be detrimental to fish, especially at embryo and larval stages.

The most severe effects of UV-B radiation impair larval development and decrease recruitment (Asta *et al.,* 2011). Indian Mahseer, *Tor tor* is an important food and game fish of Himalayan region of India. Solar UV-B level is high at mountain / high altitude (Bilbao *et al.,* 2015; Kumar *et al.,* 2017). Increase in ultraviolet radiation, pollution and destruction of natural breeding grounds of the fish leads to mortality of brood and juvenile fish. Retene and psoralene are natural chemical present in the plants. Psoralene belong to class of chemical compounds known as furocoumarins. Psoralene is naturally present in plant *Psoralea corylifolia* and *Ficuscarica.* Furocoumarins are the main focus of researchers because of their photo reactivity. Combination of furocoumaris and UV exposure leads to generation of oxidative radicals, reactive oxygen resulting in phyto- photo dermatitis (Melough *et al.,* 2018).

Aquatic animals are naturally exposed to retene and psoralene due to their presence in water bodies. Fish larva exposed to artificial UV-B with psoralene showed maximum mortality indicating that enhanced solar UV-B exposure could be lethal to fish fauna. Artificial UV-B had a strong damaging effect than natural solar radiation and become highly toxic in the presence of photosensitizers. *Tor tor* was more sensitive than *Garra gotyla gotyla* due to enhanced UV-B. Retene and psoralene photoproducts generate reactive oxygen species which leads to cell injury and mortality. Solar terrestrial UV- B monitoring data is useful for future planning of weather, climate and biodiversity conservation. The results indicate that retene and psoralene photosensitizers along with enhanced ultraviolet radiation directly and indirectly affect hill stream fishes and aquatic ecosystem of mountain region as intensity of UV-B radiation is high at high altitude.

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References

Asta, J., B. Pal, D. Arne, A.E. Stefan, R. Jorg, M. Krsitin, F.H. Michael, B.G. William and M. Johan: Solar radiation and human health. *Rep. Prog. Phys.,* **74**,170- 176 (2011).

Bansal, S., B. Sahoo and V. Garg: Psoralene- narrow band UV-B phototherapy for the treatment of vitiligo in comparison to narrow band UV-B alone. *Photodermatol. Photoimmunol. Photomed.,* **29**, 311-317 (2013).

Baruah, D. and D. Sarma: Mahseer in recreational fisheries and ecotourism in India. ICAR- Directorate of coldwater Fisheries Research, Bhimtal, Nainital, Uttarakhand, India, **22**, 1-9 (2018).

Beutler, E., O. Duron and B.M. Kelly: Improved method for the determination of glutathione. *J. Lab. Clin. Med.,* **61**, 882-888 (1963).

Bhatt, J.P. and M.K. Pandit: Endangered golden mahaseer Tor *putitora Hamiltion*: A review of natural history. *Rev. Fish Biol. Fishe.,* **26**, 25-38 (2016).

Bilbao, J., R. Roman, C. Yousif, D. Mateos and A. De Miguel: UV and global irradiance measurements and analysis during the Marsaxlokk (Malta) campaign. *Adv. Science Res.,* **12**, 147- 155 (2015).

Chowdhary, N., R. Aara, V. Shukla and S. Kumar: Photosensitivity of hill stream fishes with retene photosensitizer. *Ind. Scien. Res.,* **8**, 187-191 (2018).

Clark, G.F.: Light-driven tipping points in polar ecosystems. *Global Change Biol.,* **19**, 3749-3761 (2013).

Fisher, R.A.: Statistical Methods for Research Workers. 4th Edn., Oliver and Boyd, London. **14**, 429- 430 (1963).

Fishman, W.H., S. Goldman and J. De Llells: Localization of Beta-glucuronic dose in endoplasimic reticulum. *Nature,* **163**, 457 (1967).

Fortes, C., S. Mastroeni, R. Bonamigo, T. Mannooranparampil, C. Marino, P. Michelozzi, F. Passarelli and M. Boniol: Can ultraviolet radiation act as a survival enhancer for cutaneous melanoma? *Eur. J. Cancer. Prev.,* **25**, 34-40 (2016).

Gajula, H., V. Kumar, P.D. Vijendra, J. Rajasheka, T. Sannabommaji and G. Basappa: Distribution of psoralene in different organs of *Psoralea corylifolia* L. *Pharmacognosy and Phytochemistry,* **7**, 300-302 (2018).

Kolesova, A. S., N. Rui, S. Ashtiani, M. Rodina, J. Cosson and O. Linhart: Oxidative stress and antioxidant enzyme defence system in seminal plasma of common carp (*Cyprinus carpio*) and rainbow trout (*Onchorhynchus mykiss*) during spawning season. Czech *J. Anim. Sci.,* **63**, 78-84 (2018).

Kumar, S. and P. Kumari: Biochemical changes in fish after anthracene and ultraviolet radiation. *Sustain. Environ. Res.,* **2**, 237 (2013).

Kumar, S. and R. Aara: Solar ultraviolet radiation and climate change, impact on hill stream fishes of Himalayan region India. *AABES, London (UK). Dec.* 1- 2 (2016).

Kumar, S., N. Choudhary and R. Aara: Impact of temperature change and dissolved organic carbon on phototoxicity of photosensitizer on cyclops in Doon valley. *Mount. Res.,* **11**, 15- 21 (2016).

Kumar, S., R. Aara and N. Choudhary: Monitoring of occupational and solar terrestrial ultraviolet radiation inUttarakhand. *Ind. Life Sci.,* **6**, 19- 25 (2017).

Kumar, S., D. Malik, and S. Nagar: Phytoplanktonic variation in Asan Reservoir at Dehradun, Uttarakhand. *Sustain. Environ. Res.,* **3**, 39 (2014).

Lawal, A.T.: Polycyclic aromatic hydrocarbon. A review. *Cogent Environ. Sci.,* **3**, 1- 89 (2017).

Lenger, S., N.K. Triphati and B. Khajuria: Morphometric and meristic study of golden Mahaseer (*Tor putitora*) from Jhajjar stream (Jandk), India. *Res. J. Ani. Veteri. Fish. Scie.,* **1**, 1-4 (2013).

Lim, Y. S. and K. S. Lee: Ultraviolet radiation: DNA damage, repair, and human disorders. *Mole. Cell. Toxicol.,* **13**, 21- 28 (2016).

Maisano, M., T. Cappello, A. Natalotto, V. Vitale, V. Parrino, A. Giannetlo, S. Oliva, G. Mancini, S. Cappello, A. Mauceri and S. Fasulo: Effects of petrochemical contamination on caged marine mussels using a multi-biomarker approach: Histological changes, neurotoxicity and hypoxic stress. *Marine. Environ. Res.,* **128**, 114- 123 (2017).

Melough, M.M., E. Cho and O.K. Chun: Furocoumarins: A review of biochemical activities, dietary sources and intake, and potential health risks. *Food. Chem.Toxicol.,* **113**, 99- 107 (2018).

Mishra, S., T.K. Nailwal and S.B. Agrawal: Study on individual and interactive effects of supplemental UV-B radiation and heavy metals on *Spinacea oeracea. J. Environ. Biol.,* **35**, 333-340 (2014).

Mitchell, D.L., A.A. Fernandez, R.S. Nairn, R. Garcia, L. Paniker, D. Trono, H.D. Thames and I. Gimenez-Conti: Ultraviolet A does not induce melanomas in a Xiphophorus hybrid fish model. *Proceed. Nat. Acad. Sci.* USA, **107**, 9329- 9334 (2010).

Olson, M.H., M.R. Colip, J.S. Geriach and D.L. Mitchell: Quantifying ultraviolet radiation mortality risk in bluegill larvae: Effects on nest location. *Ecol. Appl.,* **16**, 328- 338 (2006).

Panich, U., G. Sittithumcharee, N. Rathviboon and S. Jirawatnotai: Ultraviolet radiation-induced skin aging: The role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. *Stem Cells Int.,* **14** , 21- 28 (2016).

Patz, J.A., H. Frumkin, T. Holloway, D.J. Vimont and A. Haines: Climate change: Challenges and opportunities for global health. *Ameri. Med. Assoc.,* **312**, 1565-1580 (2014).

Scott, G. R. and K. A. Sloman: The effects of environmental pollutants on complex fish behavior: Integrating behavioural and physiological indicators of toxicity- A review. *Aquat. Toxicol.,* **68**, 369- 390 (2004).

Smith, R.C. and R.P. Anderson: Methods for determination of lipid peroxidation in biological sample. *Free. Rad. Mad.,* **3**, 341-344 (1987).

Takahara, S., H.B. Hamilton, J.V. Neel, T.V. Kobara, Y. Ogura and E.T. Nishimura: Hypocatalaasemic. A new genetic carrier state. *J. Clin. Invest.,* **39**, 610-619 (1960).

Tisher, C. and Songlake: Culturing Dephnia in eight easy steps. *Aquamen.,* **10**, 26- 34 (2001).

Villafañe, S. Elena, E. Virginia, Barbieri and H. Walter: Annual patterns of ultraviolet radiation effects on temperate marine phytoplankton off Patagonia, *Argentina. Plank. Res.,* **26**, 2, 167–174 (2004).

Williamson, C.E., R.G.R.M. Zepp, Lucas, S. Madronich and A.T. Austin: Solar ultraviolet radiation in a changing climate. *Nature Climate Change,* **4**, 434-441 (2014).

Won, E.J., Y. Lee, J. Han, U.K. Hwang, K.H. Shin, H.G. Park and J.S. Lee: Effects of UV radiation on hatching, lipid peroxidation and fatty acid composition in the copepods *Parayclopina nana. Comp. Biochem. Physiol. Toxicol. Pharmacol.,* **165**, 60-66 (2014).