

## Increased tolerance to ultraviolet radiation (UVR) and cotolerance to cadmium in UVR-acclimatized freshwater periphyton

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### *Abstract*

We studied the long-term acclimatization of freshwater periphyton communities exposed to low and high ultraviolet radiation (UVR) intensities that simulate UVR doses received by lowland and high-mountain streams of central Europe. To assess changes induced by UVR, we compared the community structure (species and biomass), function (photosynthetic yield), and tolerance to UVR and cadmium of periphyton growing in microcosms (artificial channels). On the basis of the rationale behind the pollution-induced community tolerance concept, we expected that an increase in UVR tolerance would be through the replacement of more sensitive taxa by more tolerant taxa. After 38 d of exposure, periphyton in the high-UVR treatment was dominated by Cyanobacteria, whereas diatoms dominated periphyton in the low-UVR treatment. Concomitantly, the high-UVR community increased its tolerance to UVR and showed cotolerance to cadmium (Cd). Structural changes contributing to this increased tolerance included an increase in UVR-absorbing compounds, and the formation of cell aggregates that increased self shading. Induction of antioxidant enzymes after UVR and Cd exposure might be involved as defense mechanisms against oxidative stress. These changes reduced the exposure and effects of UVR, resulting in the protection of photosynthesis (high-UVR photosynthetic yield was unaffected). A fivefold reduction in chlorophyll *a* in the high-UVR treatment suggested that acclimatization had high metabolic costs. Additional experiments showed that even though biomass accrual offered some protection against UVR and Cd, the community changes experienced by the high-UVR community contributed the most to UVR tolerance. Periphyton exposed to high UVR may experience simultaneous positive (tolerance to UVR and cotolerance to Cd) and negative effects (biomass reduction can increase accessibility by toxicants).

Among risks posed by global change, the interaction between ozone depletion and climate change may enhance the exposure of aquatic communities to ultraviolet radiation (UVR, 280–400 nm) (Villafane et al. 2007). In aquatic photosynthetic organisms, enhanced UVR can induce a broad range of cellular effects, depending on light quality, intensity, and exposure (Häder et al. 2003). Besides direct biological effects (Helbling et al. 2001), UVR can cause cellular damage indirectly, through increased production of reactive oxygen, which may result in increasing oxidative stress (Helbling et al. 2001; Hernando et al. 2005). Ensuing physiological consequences on photosynthesis and growth (Holzinger and Lutz 2006) suggest that a continued increase in solar UVR can, in the long term, result in reduced primary production and a change in phytoplankton and periphyton composition (Häder et al. 1998).

Algae have evolved a variety of protective strategies to attenuate cellular UV absorption (Häder et al. 1998; Navarro et al. 2007), neutralize oxygen radicals (Ledford and Niyogi 2005), and repair damaged molecules (Jansen et al. 1999). Preventive defense mechanisms of algae include the production of UV-absorbing compounds (Sommaruga and Garcia-Pichel 1999; Tank et al. 2003; Navarro et al. 2007), and reactive oxygen scavengers as well as enzymes that participate in cellular antioxidant-scavenging cycles (Aguilera et al. 2002). Other mechanisms operate to repair damages to DNA or the photosynthetic apparatus (Bouchard et al. 2006; Häder and Sinha 2005) once damages have occurred. However, specific algae differ in their sensitivity to UVR (Donahue et al. 2003; Xue et al. 2005); thus the degree of response by periphyton to enhanced UVR will depend on community composition (Weidman et al. 2005) as well as UVR exposure history. For instance, studies of UVR effects on periphyton document large differences in response by communities from different environments (Kelly et al. 2003; Tank and Schindler 2004).

The pollution-induced community tolerance (PICT) concept predicts a community that has been restructured upon chronic chemical stress to become more tolerant to that chemical (Blanck et al. 1988). Elimination of sensitive species, replacement by more tolerant ones, and individual biochemical acclimatization can all contribute to increase tolerance to a chemical stress (Blanck 2002; Schmitt et al. 2006). On the basis of the rationale of PICT, UVR-induced structural changes are anticipated to increase the tolerance of the periphyton community to enhanced UVR. In their

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conceptual model, Vinebrooke et al. (2004) suggested that stressor-induced shift toward more resistant species may also induce positive cotolerance to other toxicants showing similar damaging mechanisms. To our knowledge, no studies have evaluated the role of UVR exposure on UVR tolerance of periphyton communities, or the occurrence of cotolerance to other environmental stressors.

In this study, we examined the long-term response of stream periphyton growing in microcosms (artificial channels) simulating UVR doses received in low- and high-elevation environments. We predicted that increased UVR induces structural changes in the periphyton community that cause the community to increase UVR tolerance. We further predicted that an increased UVR tolerance will increase the tolerance of the community to other environmental stressors that induce oxidative damage. This latter hypothesis was tested using cadmium because, similarly to UVR, it causes cellular toxicity through oxidative damage (Pinto et al. 2003).

## Methods

*Algal growing conditions on microcosms*—Natural periphyton communities were allowed to colonize indoor microcosms, using an experimental set of six transparent Plexiglas channels (86 cm long  $\times$  10.4 cm wide) supplied with water from a river 20 m from the research facility, the Chriesbach River (NE Switzerland). Water was pumped into a holding reservoir and distributed by gravity into each channel at a flow rate of 4 liters  $\text{min}^{-1}$ , resulting in a water depth of 3 cm and a flow velocity of 2–3  $\text{cm s}^{-1}$ . Outflow water was collected in a reservoir and pumped back to the river. Seventy-two microscope slides (76  $\times$  26 mm) were fixed vertically in each channel and inserted in grooves parallel to the water flow, resulting in four lanes of two-sided glass slides with a total surface area of 1,422  $\text{cm}^2$  for colonization. Dissolved oxygen, conductivity, pH, and temperature of the water were monitored weekly using WTW probes (OXI 340, COND 340i, and pH340i). Mean values of these variables were  $10.9 \pm 2.1 \text{ mg L}^{-1}$  oxygen,  $602 \pm 172 \mu\text{S cm}^{-1}$  conductivity,  $7.74 \pm 0.17 \text{ pH}$ , and  $17.9^\circ\text{C} \pm 1.1^\circ\text{C}$ .

Two different light conditions, high and low UVR, were set to simulate summer daily UVR (integrating both UVA+UVB) doses received at high mountain (Davos, Switzerland, 3,500 m above sea level [asl]) and lowland (Dübendorf, Switzerland, 560 m asl) areas, respectively. Photosynthetically active radiation (PAR: 400–700 nm) required for periphyton growth was provided daily from 07:00 h to 21:00 h by three lamps with Philips bulbs (HPL Comfort 400 W). The UVR was provided daily from 10:00 h to 16:00 h by two UV Osram lamps (ULTRAMED 400W/FDA R7S FS1). The three channels exposed to high-UVR conditions were placed directly under these lights, receiving  $686 \pm 40 \mu\text{mol photons s}^{-1} \text{ m}^{-1}$  PAR,  $10.88 \pm 1.9 \text{ mW cm}^{-2}$  UVA, and  $1.03 \pm 0.11 \text{ mW cm}^{-2}$  UVB. A UVR-Plexiglas filter (type XT 20070, transmits 90% of PAR, 32% of UVA, 7% of UVB, and 0% of UVC) was placed on the top of the three channels exposed to low-UVR conditions, thereby resulting in  $3.61 \pm 0.48 \text{ mW}$

$\text{cm}^{-2}$  UVA,  $0.07 \pm 0.007 \text{ mW cm}^{-2}$  UVB, and  $633 \pm 31 \mu\text{mol photons s}^{-1} \text{ m}^{-1}$  PAR. Spectral distributions of these bulbs are available at the manufacturer's Web sites. Light measurements were made at the water surface. The experiment was started 14 June 2006 by pumping water from the river and allowing periphyton to colonize the slides in each channel. The developing communities were examined for a period of 2.5 months. Visual control during the experiment showed minimal colonization by *Chironomidae* larvae ( $<1$  individual per slide) and only under low UVR.

*UVR-absorbing compounds, chlorophyll a (Chl a), community composition, biomass*—For determination of UVR-absorbing compounds and Chl a, two to five glass slides (depending on the periphyton biomass) were scraped and resuspended in 20 mL of river water. Samples were sonicated for 5 min to obtain a homogeneous suspension and a 5-mL aliquot from each sample was filtered using glass microfiber filters (25 mm  $\varnothing$ , Whatman Int.). Each filter was placed in a tube with 5 mL of ethanol (90%), boiled for 10 min at  $85^\circ\text{C}$ , sonicated for 5 min, and then stored at  $4^\circ\text{C}$  for 16 h. Each extracted sample then was filtered (Minisart NML 1.2  $\mu\text{m}$  filter, Sartorius) and the filtered extract analyzed in the 200–800-nm range using a Kontron Instruments UVIKON 930 spectrophotometer (Bio-Tek). An aliquot of the filtered extract also was used for Chl a measurements using a high-performance liquid chromatography system (Kromasystem 2000 with diode array detector 440, Kontron Instruments) according to the method described by Murray et al. (1986).

The relative proportion of UVR-absorbing compounds to Chl a was calculated as the ratio of absorbance intensity over the range of UVR to that of Chl a at 665 nm (Navarro et al. 2007). The area below the range of 280–400 nm was calculated by the sum of light absorbance at any wavelength (1-nm step). The resulting UVR ratio is a dimensionless number, representing a ratio between the absorbance capacities of the UVR-absorbing compounds per absorbance unit of Chl a (Navarro et al. 2007).

For taxonomic analysis, periphyton samples (10 mL from the above suspension) were fixed with Lugol's iodine (100  $\mu\text{L}$ ). An inverted microscope (Zeiss Axiovert 135) was used to count and identify periphyton taxa in a 50-mL Utermöhl's chamber. A quantitative analysis of the periphyton taxa composition was performed by counting up to 600 cells on 1 : 500-diluted subsamples. Depending on the sample, this number was reached after examining between 20 and 40 fields. Results were used to calculate the Shannon index of diversity (Krebs 1999). Biovolume was assessed measuring 10 cells from the two dominant species, *Achnanthydium* spp. and *Chroococcus* (Hillebrand et al. 1999).

*Photosynthetic yield assessment*—Photosynthetic yield was measured by fluorimetry using a portable pulse amplitude modulation fluorimeter (MINI-PAM, Walz) in a nondestructive manner. The most useful derived parameter that measures the efficiency of photosystem II (PSII) photochemistry is the photochemical yield under light,

$\Phi$ PSII. This parameter reflects the efficiency of the photochemical energy conversion process. The theoretical background of measurement and analysis of chlorophyll fluorescence is well established (Maxwell and Johnson 2000; Consalvey et al. 2005). To make the measurements, slides colonized by periphyton were placed horizontally on the bottom of testing channels that were placed in a frame allowing access to the bottom of each channel (transparent). The actual measures were taken by positioning the fiber optics of the MINI-PAM below and in contact with the transparent underside of each channel without disturbing the periphyton. The distance between the optics and the surface of the periphyton community was about 7 mm. For some measurements on certain slides, it was necessary to select regions having different levels of biomass. This selection was done using the momentary fluorescence under illuminated steady state as a coarse and fast biomass indicator (Walz 2003).

*UVR and Cd tolerance*—Tolerance to UVR of periphyton exposed to high and low UVR was assessed as the short-term sensitivity of photosynthetic yield to intense UVR at days 43 and 71. On both days, slides were exposed to higher UVR than that applied during the long-term exposure:  $13.3 \pm 0.071 \text{ mW cm}^{-2}$  UVA and  $1.383 \pm 0.003 \text{ mW cm}^{-2}$  UVB. Slides were placed in different channels, but under similar water flow conditions and PAR as during the long-term exposure. On day 43, the photosynthetic yield of one slide from each channel was measured (at three different positions and results averaged for each slide). Measurements were made at different time intervals over a 24-h period. On day 71, two PAM instruments were fixed under one slide from each treatment and their photosynthetic yield monitored every 5 min for 20 h.

Cd tolerance was assessed as the sensitivity in photosynthetic yield at days 38, 52, and 66. Slides from each channel (i.e., six from each test channel) were exposed to various Cd concentrations in six separate channels, each operating as a recirculating system and illuminated only with PAR light. Each channel was connected to a 20-liter tank, containing 8 liters of synthetic medium ( $0.5 \text{ mmol L}^{-1} \text{ NaHCO}_3$ ,  $0.09 \text{ mmol L}^{-1} \text{ MgCl}_2(6\text{H}_2\text{O})$ ,  $0.5 \text{ mmol L}^{-1} \text{ N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid}$ , pH to 7.3).  $\text{CdCl}_2$  was added to five of these tanks to obtain nominal concentrations of 2, 10, 20, 40, and  $60 \mu\text{mol L}^{-1}$  Cd and allowed to equilibrate for 12 h before exposing the communities. The remaining tank had no Cd added and acted as a control. The tanks were kept in a water bath with river water that maintained a medium temperature similar to exposure conditions. Recirculation of individual channels started 1 h before placing the experimental slides with biofilms in the channels. One photosynthetic yield measurement was made on each slide after 120 min of exposure and related to the corresponding dissolved Cd concentration to derive 50% effective concentration ( $\text{EC}_{50}$ ) values.

Samples to estimate concentrations of dissolved Cd were taken manually in each tank at the end of the exposure period of periphyton (120 min). Each sample (10 mL) was

filtered using a plastic syringe and a filter, both previously rinsed with water from the tank (20-mL syringe;  $0.45\text{-}\mu\text{m}$  filter, Orange Scientific Gyrodisc) and acidified to  $0.01 \text{ mol L}^{-1}$  with  $\text{HNO}_3$ . Dissolved Cd concentrations were measured by inductively coupled plasma–mass spectrometry (ICP-MS; Perkin-Elmer Elan 5000) with rhodium used as an internal standard. The accuracy of the ICP-MS measurements was checked using SLRS-4 reference water (error  $<10\%$ , National Research Council Canada). Short-term tolerance of photosynthetic yield to Cd was expressed as the  $\text{EC}_{50}$  values of measured dissolved Cd. Dissolved Cd concentrations were determined to be  $20\% \pm 4\%$  lower than nominal values.

On day 71, the influence of biomass accrual on periphyton tolerance to UVR was tested. One slide from low-UVR and one from high-UVR channels, both presenting regions with different biomass accrual, were used. In this way, areas presenting high and low biomass were selected ( $588 \pm 21$  and  $1,166 \pm 58$  units of momentary fluorescence for low and high biomass of low UVR;  $338 \pm 10$  and  $717 \pm 43$  for high UVR) and their photosynthetic yield measured every 5 min over 16 h under the same UVR conditions as those described above (UVR tolerance test). On day 72, the influence of biomass accrual on the tolerance to Cd also was assessed ( $648 \pm 11$  and  $1,360 \pm 33$  units for low and high biomass of low UVR;  $252 \pm 10$  and  $467 \pm 19$  for high UVR). In this case, periphyton was exposed in one channel working as a recirculating system with  $40 \mu\text{mol L}^{-1}$  Cd for 3 h, and its photosynthetic yield measured every 4 min.

*Statistical analysis and modeling*—In the results, all errors are expressed as standard deviations. Differences between treatments for the different measured variables were tested using simple and repeated-measures analysis of variance (ANOVA), followed by Fisher's least significant difference post hoc test when significant differences were found ( $p < 0.05$ ). Data were log-transformed if required to meet assumptions of ANOVA, and the homogeneity of variances was tested using the Levene test. All statistical analyses were computed using Statistica 6.0 (Statsoft). The  $\text{EC}_{50}$  values were calculated fitting the dose–response curve (dissolved Cd concentrations vs. photosynthetic yield) values to an exponential decay equation with two parameters (yield =  $ae^{(-b[\text{Cd}])}$ ) using Sigma Plot 8.0 (SPSS).

## Results

*Structure and function of the algal biofilm*—Photosynthetic yield was not affected by long-term UVR exposure (Fig. 1B). Despite both communities showing similar cell numbers at day 66 (Table 1), the high-UVR community had a significantly 5.4 times lower Chl *a* than the low-UVR community. The high-UVR community displayed a significant increase through time in the UVR ratio (Fig. 1C, Table 2), indicating a greater increase of UVR-absorbing compounds than the concomitant increase in Chl *a* between days 38 and 66 (Fig. 1A). In contrast, the low-UVR community showed a significant decrease through time in UVR ratio, meaning a reduction of UVR-absorbing

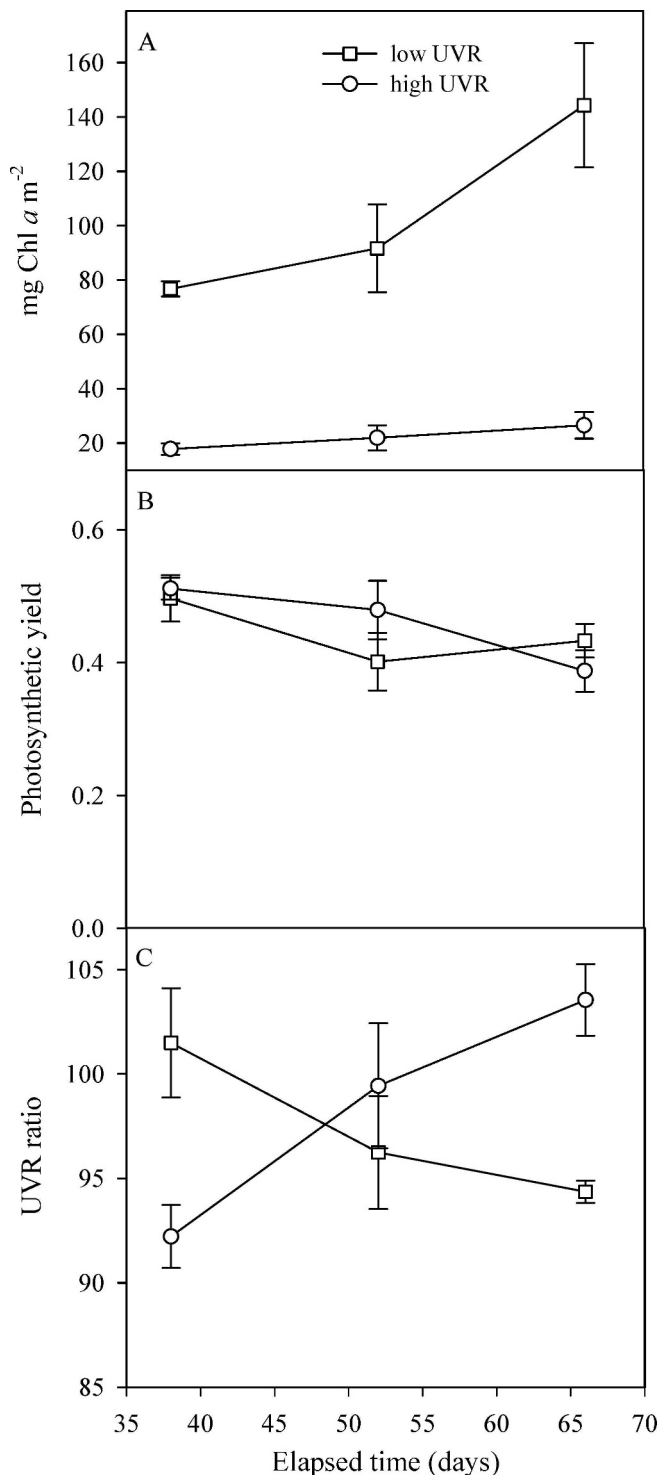


Fig. 1. (A) Chl *a*, (B) photosynthetic yield, and (C) UV ratios of periphyton communities through time.

compounds per unit of Chl *a* (Fig. 1C). In addition, both communities showed significant differences in the UVR ratio at days 36 and 66.

*Achnanthydium* spp. significantly increased in dominance through time in the low-UVR treatment (Fig. 2A, Table 1), whereas *Chroococcus* sp. significantly dominated the high-

UVR community (Fig. 2B, Table 1). In spite of their different composition, both communities showed similar initial diversity indices during long-term exposure (Table 1). At day 66, the high-UVR community showed a significantly lower biovolume ( $6.4 \times 10^{10} \mu\text{m}^3 \text{cm}^{-2}$ ) than the low-UVR community ( $2.5 \times 10^{11} \mu\text{m}^3 \text{cm}^{-2}$ ), in accordance with results shown in Fig. 2C,D and Table 2. Differences in species dominance were also visually detectable (Fig. 2C,D). The low-UVR community had a regular and dense colonization pattern (Fig. 2C), whereas the high-UVR community showed scarce and spotted cell aggregates (Fig. 2D). The low-UVR community had an intense brown color due to the dominance of diatoms, whereas the high-UVR community had an intense green color due to the dominance of Cyanobacteria.

*Tolerance to UVR and the effect of periphyton biomass*—Short-term tolerance of photosynthetic yield to intense UVR was measured on days 43 and 71 of exposure. On day 43, both communities showed a decrease (over 1 h) in photosynthetic yield to 60% of initial yields (Fig. 3A). After 23 h, the high-UVR community recovered and reached values ca. 80% of initial yields, whereas the low-UVR community showed a continued decrease in yield to 20% of initial values. On day 71, both communities showed an initial (first 3 h) decrease in photosynthetic yield to 40% of initial values (Fig. 3B). After 16 h, the photosynthetic yield of the low-UVR community was completely inhibited, whereas the high-UVR community demonstrated UVR tolerance, maintaining photosynthetic yields of ca. 50% of initial values.

To study the effect of biomass on UVR tolerance, the photosynthetic yield of biofilms differing in biomass was assessed under exposure to intense UVR. Biofilms with higher biomass were more tolerant to UVR, demonstrating the role of biomass in UVR protection (Fig. 4A,B). Photosynthetic activity of low-biomass biofilms in the low-UVR treatment was completely inhibited after 60 min of exposure (Fig. 4A), whereas high-biomass biofilms showed values ca. 80% of initial yields. In spite of this initial tolerance, low-UVR high-biomass biofilms showed a slow but constant decrease in yield to values near 0 after 16 h of exposure. The high-biomass biofilm from the high-UVR treatment (Fig. 4B) displayed higher tolerance than the low-biomass biofilm, but only during the first 5 h. After 6 h, the photosynthetic yield of both low- and high-biomass biofilms converged at ca. 40% of initial values and stayed relatively constant through the remaining exposure period, demonstrating their acclimatization to UVR.

*Tolerance to Cd and the effect of biomass*—The high-UVR community showed a similar tolerance to Cd as indicated by similar  $\text{EC}_{50}$  values at days 38, 52, and 66 (Fig. 5A). The low-UVR community showed similar  $\text{EC}_{50}$  values on days 38 and 52, but the  $\text{EC}_{50}$  value clearly increased on day 66. On days 38 and 52, the high-UVR community showed a higher  $\text{EC}_{50}$  value than the low-UVR community. However, at day 66, the low-UVR community displayed higher tolerance (Fig. 5A), indicating that some other factor than UVR exposure also affected cadmium tolerance.

Table 1. List of periphyton taxa growing in the channels and their percentage abundance, Shannon diversity index, and total number of cells. Errors are given as standard deviations.

Species	Low UVR			High UVR		
	Day 38	Day 52	Day 66	Day 38	Day 52	Day 66
<b>Bacillariophyceae</b>						
<i>Achnanthyidium</i> spp.	34.4±18.3	49.3±19.7	56.4±12.0	13.0±4.0	10.2±4.1	15.6±16.0
<i>Amphora</i> sp.	-	-	0.01±0.02	-	0.02±0.04	0.1±0.1
<i>Cocconeis</i> sp.	9.6±6.0	3.7±1.0	6.4±3.8	0.6±0.3	0.7±0.4	0.6±0.7
<i>Cyclotella</i> sp.	0.2±0.1	0.2±0.2	0.01±0.02	0.03±0.05	0.1±0.0	-
<i>Cymbella</i> sp.	0.3±0.2	0.2±0.1	0.03±0.03	0.1±0.1	-	0.1±0.2
<i>Fragilaria</i> sp.	1.9±3.2	0.3±0.1	3.5±3.0	0.4±0.2	0.1±0.2	0.6±0.7
<i>Gomphonema</i> sp.	0.1±0.2	0.03±0.03	0.1±0.2	0.03±0.06	-	0.01±0.02
<i>Melosira</i> sp.	0.9±0.4	0.1±0.1	1.3±0.5	-	0.04±0.1	0.4±0.3
<i>Navicula</i> sp.	6.6±2.4	1.6±1.5	9.8±9.5	9.0±3.2	4.6±2.0	1.9±1.6
<i>Nitzschia</i> sp.	1.3±0.5	0.3±0.3	3.7±1.6	4.5±3.3	3.8±0.6	1.5±0.6
<i>Stephanodiscus</i> sp.	0.5±0.3	-	0.1±0.1	0.1±0.1	-	-
<i>Surirella</i> sp.	0.1±0.1	-	-	-	-	-
<b>Chlorophyceae</b>						
<i>Kirchneriella</i> sp.	-	-	-	-	0.04±0.03	-
Spherical algae	0.5±0.8	0.7±0.3	1.0±0.9	0.2±0.4	0.3±0.4	0.3±0.5
<i>Monoraphidium contortum</i>	-	0.04±0.04	-	0.1±0.1	0.2±0.1	0.0±0.0
<i>Scenedesmus acutus</i>	10.5±4.9	1.4±0.7	1.6±1.0	11.8±5.9	0.9±0.1	1.4±1.5
<i>Scenedesmus armatus</i>	2.8±2.0	1.7±0.5	0.8±0.3	4.0±1.9	2.2±0.6	0.6±0.6
<b>Cyanobacteria</b>						
<i>Aphanothece</i> sp.	-	-	-	-	-	4.3±7.5
<i>Chamaesiphon</i> sp.	-	0.6±1.0	1.1±1.0	-	0.4±0.6	2.7±3.0
<i>Chroococcus</i> sp.	24.8±26.8	39.7±20.1	12.4±7.3	54.8±16.3	76.5±4.5	61.9±38.0
<i>Lynghya</i> sp.	-	-	-	0.3±0.5	-	-
<i>Microcystis</i> sp.	4.9±4.2	-	-	1.0±1.7	-	3.8±3.9
<i>Pseudoanabena</i> sp.	0.7±1.2	-	1.8±3.2	-	-	4.1±4.5
Diversity index	1.69±0.17	1.09±0.11	1.46±0.27	1.42±0.33	0.91±0.08	1.15±0.8
Number of cells (×10 <sup>6</sup> ) cm <sup>-2</sup>	270±81	1,600±580	1,100±800	150±8	890±130	940±600

Table 2. The results of the repeated-measures (3 d) ANOVA of biomass-normalized tolerance to Cd (EC<sub>50</sub>), Chl *a*, UVR ratio, total biovolume (only days 52 and 66), and biovolume of dominant taxa (*Achnanthyidium* spp., and *Chroococcus* sp.).

Analysis	Source of variation	df	MS	F	p
Normalized EC <sub>50</sub> *	UVR intensity	1	1.90	37.7	<0.01
	Time	2	0.03	1.7	n.s.
	UVR intensity × time	2	0.21	9.8	<0.01
Chl <i>a</i>	UVR intensity	1	30,373	337	<0.001
	Time	2	2365	14.3	<0.01
	UVR intensity × time	2	1,470	8.9	<0.01
UVR ratio	UVR intensity	1	4.8	0.49	n.s.
	Time	2	6.6	2.97	n.s.
	UVR intensity × time	2	133.1	59.61	<0.001
Total biovolume*	UVR intensity	1	0.41	33.9	<0.01
	Time	1	<0.01	0.2	n.s.
	UVR intensity × time	1	<0.001	<0.01	n.s.
% <i>Achnanthyidium</i> spp.*	UVR intensity	1	1.20	24.9	<0.01
	Time	2	0.19	1.54	n.s.
	UVR intensity × time	2	0.04	0.36	n.s.
% <i>Chroococcus</i> sp.*	UVR intensity	1	1.57	28.2	<0.01
	Time	2	0.01	0.22	n.s.
	UVR intensity × time	2	0.05	0.74	n.s.

\* Values have been log-transformed (df, degrees of freedom; MS, mean squares; F and p, critical values and probability of significance tests, respectively; n.s., not significant).

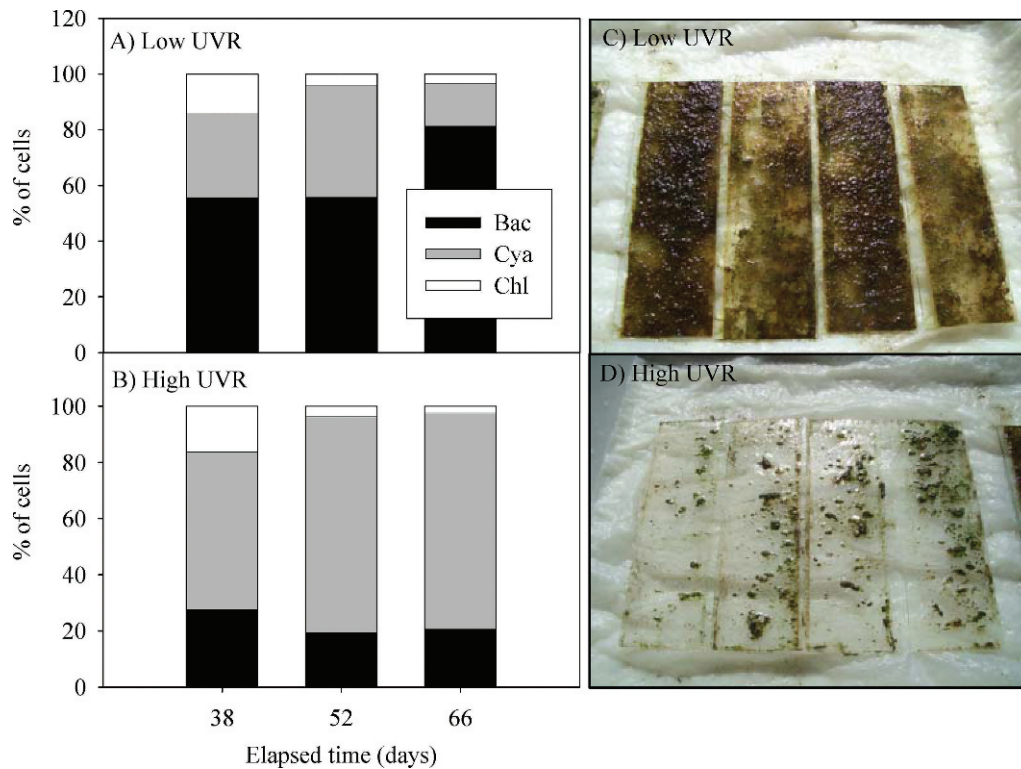


Fig. 2. Structure of (A) low-UVR and (B) high-UVR communities, showing the relative percentage abundance of the main algal groups: Bacillariophyceae (Bac), Cyanobacteria (Cya), and Chlorophyceae (Chl). Pictures of glass slides colonized with (C) low-UVR and (D) high-UVR communities after 63 d of exposure to UVR. Four slides are shown to illustrate the homogeneous colonization in both treatments.

To study the effect of biomass on tolerance to Cd, the photosynthetic yield of biofilms having different biomass was assessed under exposure to  $40 \mu\text{mol L}^{-1}$  Cd for 3 h. Exposure to low and high UVR showed that high-biomass biofilms were more tolerant than low-biomass biofilms (Fig. 6A,B). Therefore, we normalized the values of tolerance ( $\text{EC}_{50}$ ) using the corresponding biomass values (Fig. 5B), which resulted in a biomass-specific tolerance value per unit of Chl *a* (micromoles of Cd needed to reduce the photosynthetic yield of a micromole of Chl *a* to 50%). After tolerance results were normalized, the effect of treatment and time  $\times$  treatment were significant (Table 2). The post hoc test indicated significant differences in tolerance (high-UVR treatment showed six to eight times higher specific tolerance than the low-UVR treatment) on days 32 ( $\text{df} = 7.24, p = 0.024$ ) and 52 ( $\text{df} = 7.24, p = 0.030$ ) (Fig. 5B). At day 66, the high-UVR community had a nonsignificant higher tolerance than the low-UVR community.

## Discussion

Although the measured variables differed in their sensitivity as indicators of UVR-induced damage, the long-term exposure of periphyton from low-elevation streams (low UVR) to UVR levels simulating doses from high-elevation environments (high UVR) caused major changes in biofilm structure. Our design allowed a continuous supply of algae to colonize the experimental microcosms and may explain the insensitivity of species

richness and diversity measures (Table 1) as indicators of UVR-induced change in community structure. Nevertheless, periphyton exposed to high UVR had strongly reduced biomass, a shift in species composition, and an increase in the ratio of UVR-absorbing compounds to Chl *a*. A decrease in biomass after exposure to UVR has been found in some studies on periphyton (Kiffney et al. 1997; Hodoki 2005), whereas UVR had little effect in other studies (Vinebrooke and Leavitt 1998; Tank and Schindler 2004). In our study, the number of large-sized *Achnanthes* spp. and other diatoms that dominated low-UVR biofilms were reduced in high-UVR biofilms that were dominated by small-sized *Chroococcus* sp. and other Cyanobacteria. This shift to Cyanobacteria dominance has been shown in several studies, even under natural UVR exposure (Vinebrooke and Leavitt 1996, 1999). An increasing UV ratio and constant Chl *a* content suggest that the cellular concentration of UVR-absorbing compounds provided protection to *Chroococcus* sp. and other algae exposed to high UVR. The accumulation and protective role of UVR-absorbing compounds in periphyton has been shown in previous studies (Garcia-Pichel and Castenholz 1991; Navarro et al. 2007). The accumulation of Chl *a* and photoprotective pigments has been related to UVR acclimation in some marine algae (Rech et al. 2005), whereas in other cases pigment accumulation was associated with UVR-induced cell-cycle arrest (Buma et al. 2000). Other factors can also influence algal sensitivity to UVR (Holzinger and Lutz 2006). For instance, plasticity in

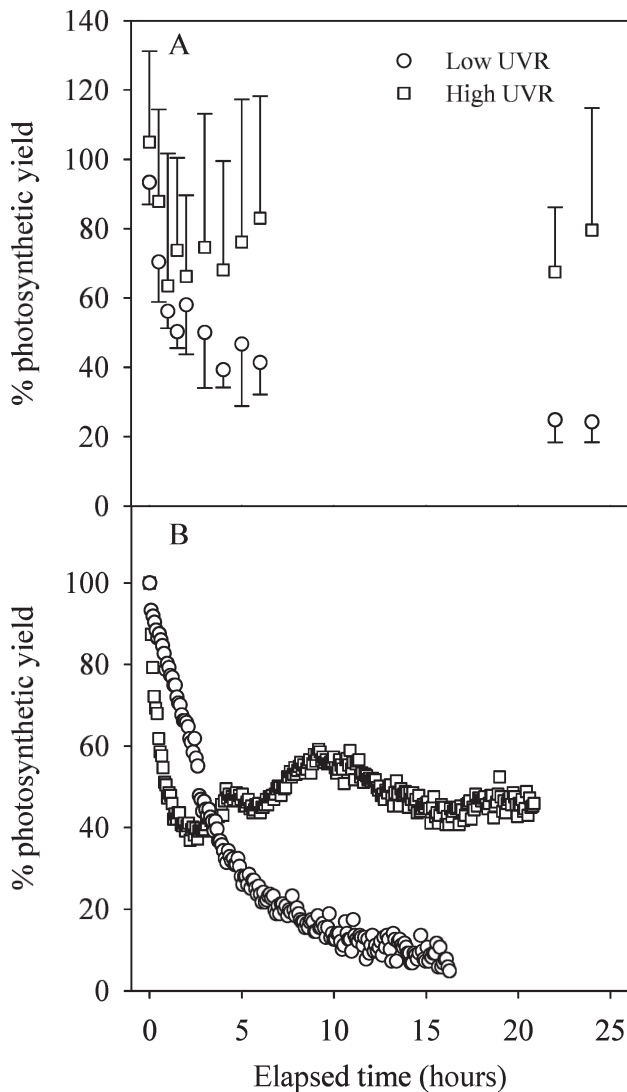


Fig. 3. Graph showing the photosynthetic yield of communities during the UVR short-term tolerance experiments on days (A) 43 and (B) 71. In both cases, the communities were exposed over 24 h to higher UVR intensity than those used during the long-term exposure.

morphology in some Cyanobacteria resulted in self-shading that provided effective protection against short-term exposure to intensive solar UVR (Wu et al. 2005; Helbling et al. 2006). The patchy growth of colonial Cyanobacteria such as *Chroococcus* sp. in the high-UVR community might have reduced the absorbed UV dose and its ensuing effects.

A combination of mechanisms, including the previously mentioned UVR-absorbing compounds, increased Chl *a* concentrations, and morphological plasticity probably allowed for the maintenance in photosynthetic yield by the high-UVR community. This result, together with the structural changes, suggests that the high-UVR community acclimatized to UVR. Indeed, the high-UVR community showed a higher tolerance than the low-UVR community, as indicated from the short-term UVR tolerance experiments (Fig. 3). During the UVR tolerance tests, photosyn-

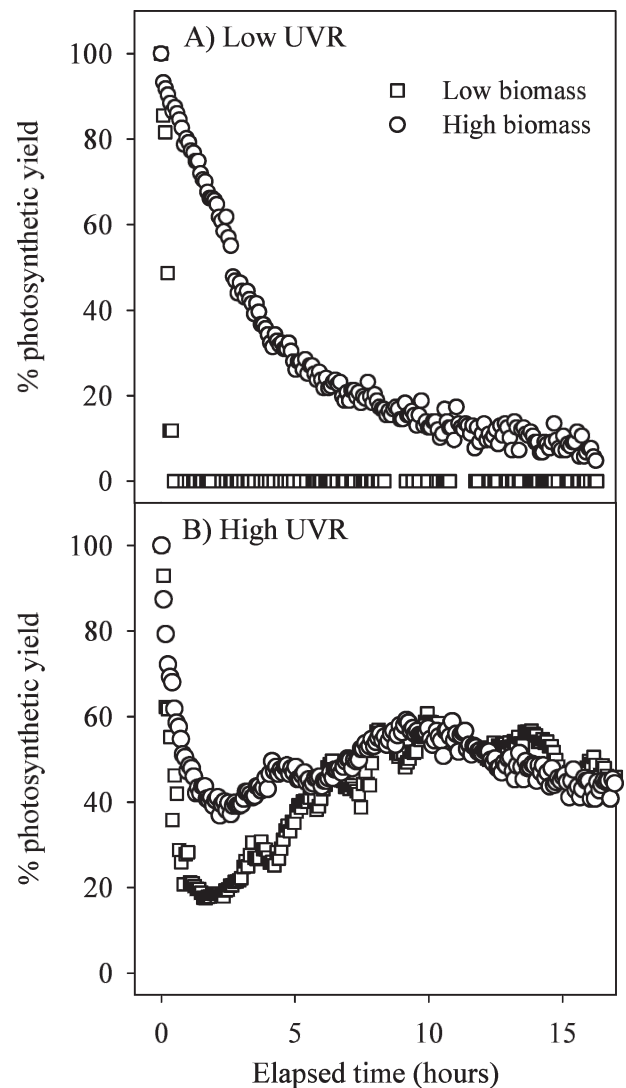


Fig. 4. The effect of biomass on UVR tolerance. The photosynthetic yield of biofilms from (A) low-UVR and (B) high-UVR communities having different biomass was assessed upon exposure to higher UVR intensity than those used during the long-term exposure.

thetic yield initially decreased with time in both communities, and continuously decreased in the low-UVR community but stabilized at values between 40% and 80% of initial ones in the high-UVR community. A decrease in yield has been shown to reflect damage of the PSII reaction center D1 protein (Jansen et al. 1999; Lesser et al. 2002). Thus, in addition to the above mechanisms, repair involving the de novo synthesis or the accumulation of D1 (Vass et al. 2000; Bouchard et al. 2006) may contribute toward explaining the stabilization in yield by the high-UVR community. Alternatively, the fraction in yield that is insensitive to UVR (the remaining yield after exposure to intense UVR, see Fig. 3A,B) might reflect the constitutive tolerance of the species comprising the UVR-acclimatized community.

The adaptive potential of algae to UVR has been inferred from their broad presence in ecosystems having

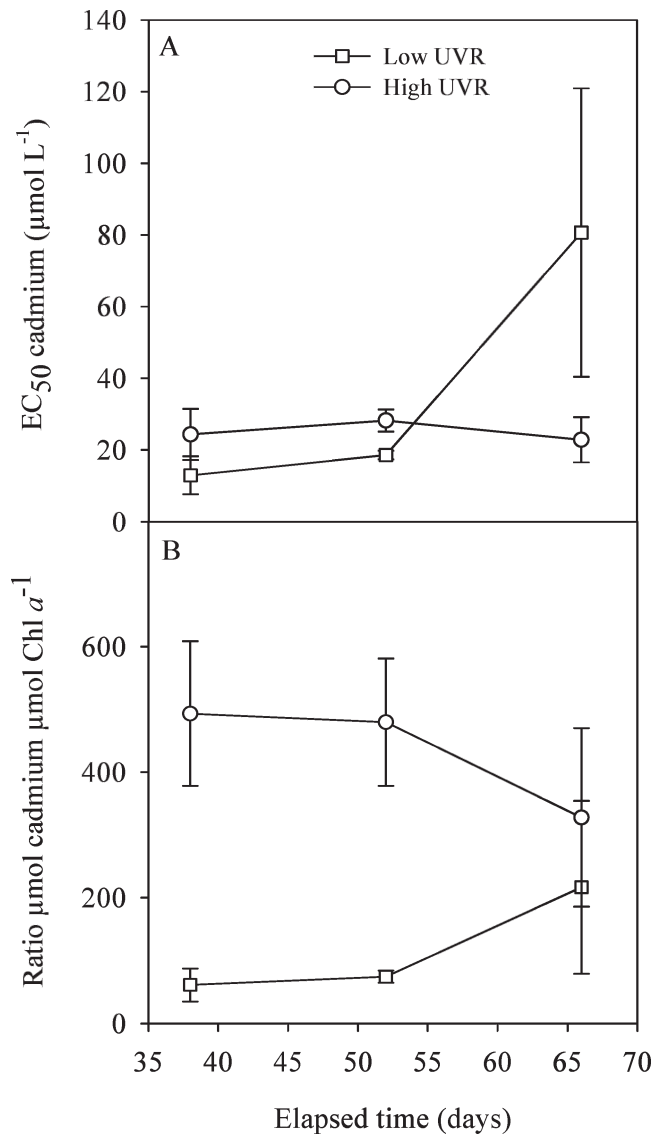


Fig. 5. (A) The tolerance of communities to Cd, expressed as EC<sub>50</sub>. Tolerance was assessed as the short-term sensitivity in photosynthetic yield to various Cd concentrations (0, 2, 10, 20, 40, and 60 µmol L<sup>-1</sup>) after 120 min of exposure. The photosynthetic yield was related to the corresponding dissolved Cd concentration to derive EC<sub>50</sub> values. (B) The specific tolerance of each community, expressed as the micromoles of Cd required to reduce photosynthetic yield of 1 µmol of Chl *a* to 50% of initial values.

high ambient UVR levels (Quesada and Vincent 1997) and from field studies in which UVR intensity was reduced relative to natural levels (Kiffney et al. 1997; Vinebrooke and Leavitt 1999; Tank et al. 2003). Our experiments examining the role of periphyton biomass in UVR tolerance of photosynthesis corroborated the acclimatization of the high-UVR community and its higher UVR tolerance. The biomass accrual in the low-UVR community offered a certain defense against UVR, probably through self-shading. However, the high-UVR community showed recovery and stabilization in photosynthesis, regardless of biomass, thus supporting the hypothesis of acclimatization.

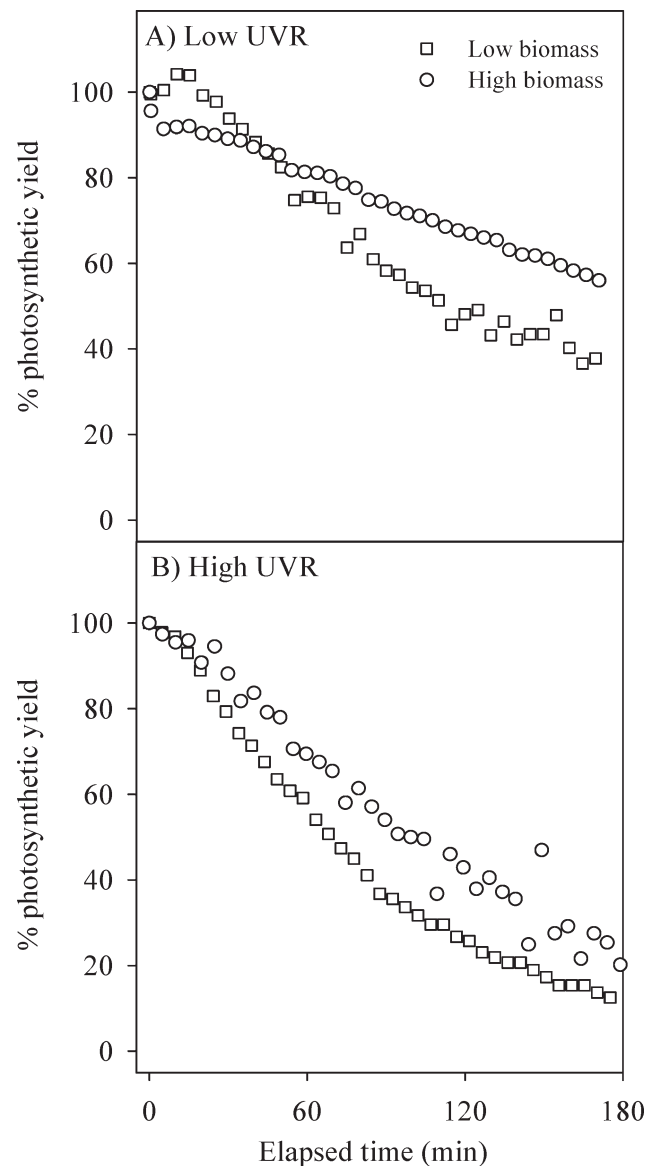


Fig. 6. The effect of biomass on Cd toxicity. The photosynthetic yield of biofilms from (A) low-UVR and (B) high-UVR communities having different biomass was assessed upon exposure to 40 µmol L<sup>-1</sup> Cd for 3 h.

To our knowledge this is the first experimental demonstration of UVR-induced community tolerance. The increase in UVR tolerance allowed the high-UVR community to maintain photosynthetic yield even at UVR intensities higher than that used during the long-term exposure.

In support of our hypothesis, long-term exposure to UVR also increased cotolerance to Cd, indicating common tolerance mechanisms for both stressors in algae (Blanck 2002; Soldo et al. 2005). The fact that the high-UVR community displayed cotolerance to Cd (without previous exposure to Cd) indicates the occurrence of constitutive defense mechanisms in Cyanobacteria. Induction of antioxidant enzymes after UVR and Cd exposure (Pinto et al. 2003) might be involved as a defense mechanism against oxidative stress. An increased tolerance to Cd in the high-

UVR community was detected at days 38 and 52, whereas higher tolerance to Cd occurred at day 66 in the low-UVR community. Since tolerance dynamics followed a similar pattern as biomass accrual dynamics, we normalized EC<sub>50</sub> values using the corresponding biomass values. Results showed that Cd tolerance by the high-UVR community was significantly higher (two to eight times) than that of the low-UVR community. Experiments assessing the influence of biomass on Cd tolerance confirmed the protective role of biomass. However, other than for UVR (Fig. 4), mechanisms conferring increased tolerance to Cd were not sufficient for maintaining photosynthetic yield under prolonged Cd exposure (Fig. 6). Other studies have previously shown that biomass accrual imparts some protection from metal toxicity (Ivorra et al. 2000; Navarro et al. 2002). The lower metal sensitivity in high-biomass periphyton has been attributed to a reduced diffusion through thicker biofilms (Ivorra et al. 2000) and to dilution of the metal (Hill et al. 2000). Therefore, one important implication arising from the results is the importance of considering biomass on functional measurements in periphyton communities.

Our results on UVR-induced cotolerance to Cd are of particular significance regarding the consequences of multiple stressors on freshwater periphyton communities. In their conceptual model, Vinebrooke et al. (2004) suggested that the sign and strength of correlations between tolerances to stressors can strongly influence biodiversity and ecosystem functioning. Hence, knowledge of UVR tolerance and cotolerance to other stressors contributes to assessing and predicting risks associated with the exposure of algal communities to UVR in combination with other stressors. Moreover, since our results showed that different algae show different UVR tolerance, one possible explanation arises for the apparently contradictory UVR effects on periphyton found in the literature. On the basis of our results, it is expected that different periphyton communities exposed to enhanced UVR will respond differently depending on the species present. In our study, it was clear that the species present in the lowland community possessed adequate UVR tolerance variability to acclimatize to high-UVR conditions.

In summary, as expected on the basis of the PICT rationale, our findings showed that long-term exposure to UVR induced UVR community tolerance and Cd cotolerance through changes in the community. Even if these changes allow for the maintenance in photosynthetic activity, they have a cost in the form of lower biomass. Consequently, we can anticipate that, although lowland periphyton communities are equipped to maintain ecologically relevant functions when exposed to enhanced UVR, the costs of acclimatization will increase the bioavailability and accessibility to other pollutants.

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