In situ effects of arsenic, aluminium and chromium stresses on algal periphyton of the river Ganga at Varanasi, India

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ABSTRACT

In situ effect of metal stress on periphytic algal communities of a river was studied using chemical diffusing substrates. The metal stress caused the inhibition of periphytic biomass in a concentration-dependent manner. The study indicated differential response of various periphytic groups to different metal treatments. Diatoms exhibited tolerance against arsenic (As) and aluminium (Al) treatment but displayed sensitivity against chromium (Cr) treatment. An increased abundance of cyanobacteria was noteworthy in Cr enrichment, but Al and As were hazardous to these organisms. The relative abundance of green algae also increased in all three test metals. The metal stress lowered the species richness and diversity of periphytic algae, apparently due to the elimination of some of the sensitive species followed by an increased abundance of tolerant forms. Periphytic taxa tolerant to one metal were not necessarily tolerant to other metals or metalloids, and *vice versa*. The metal-induced changes in algal community composition will lead to severe ecological consequences by affecting biological diversity and in turn productivity of aquatic systems. Since algae occupy the aquatic food web base, any harmful effect on these organisms would have repercussions at higher trophic levels. Thus, it seems urgent to incorporate biomonitoring practices and chemical analysis to monitor the river Ganga's ecological health.

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Key words: heavy metal, periphyton, chemical diffusing substrate, arsenic, aluminium, chromium.

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INTRODUCTION

Heavy metal, the widely used term by environmental biologists, refers to a range of environmentally relevant elements with specific gravity >5 g cm^{-3} (Passow *et al.*, 1961; Kaplan, 2013). Some heavy metals, such as arsenic, copper, zinc, nickel, manganese, iron and molybdenum, are required by living organisms, including algae and cyanobacteria, for their metabolic activities (Rai and Gaur, 1981a; Park *et al.*, 2020). However, they are extremely toxic to diverse living organisms, if present at high concentrations (Machado *et al.*, 2015). Metals and metalloids that are not required for the physiological machinery of living organisms, such as cadmium, mercury, arsenic, and aluminium, are toxic to biota at high concentrations (Kaplan, 2013; Barral-Fraga *et al.*, 2016; Negi *et al.*, 2023). The concentrations of these pollutants in waterbodies have risen in recent decades, owing primarily to the increased activity of metal-related and other industries (Zhou *et al.*, 2008).

Efforts have been comprehensively made to understand the toxicity of metals to algae and cyanobacteria using single-species cultures of these organisms (Rai *et al.*, 1981; De Filippis and Pallaghy, 1994). Many of these studies also try to explain the mode of harmful action of metals on the metabolic machinery

of these organisms. A majority of these studies point towards the binding of metals to proteins and their subsequent inactivation, and induction of oxidative stress as some of the major consequences of metal toxicity in these organisms (Gaur and Rai, 2001).

Unlike laboratory studies on metal toxicity to single species cultures of algae and cyanobacteria, similar studies on algal communities in natural conditions are fewer. A majority of such studies on algal communities have been carried out on metals like copper (Arnegard *et al.*, 1998; Serra *et al.*, 2009), zinc (Williams and Mount, 1965; Arini *et al.*, 2012a, 2012b) and cadmium (Duong *et al.*, 2010; Morin *et al.*, 2008b; Arini *et al.*, 2012a, 2012b). In this context, little emphasis has been placed on other metals and metalloids, such as mercury (Pérès *et al.*, 1997), aluminium (Genter, 1995), arsenic (Wängberg, 1995; Tuulaikhuu *et al.*, 2015) and chromium (Singh and Rai, 1990). Some efforts have also been made to study the response of algal communities to mining effluents which are often enriched in these pollutants (de la Peña and Barreiro, 2009; McCauley and Bouldin, 2016).

Arsenic (Ar) contamination of water has assumed tremendous significance nowadays in view of large-scale contamination of waters with this metalloid (Sharma and Sohn, 2009). Arsenate is the most abundant form of arsenic in waters with enough oxygen, whereas arsenite is the most abundant species in reducing environments (Smedley and Kinniburgh 2002; Barral-Fraga *et al.*, 2016). Incidentally, arsenate is an analogue of phosphate and is taken up through phosphate transporters; consequently, these two anions compete with each other for their uptake (Castro *et al.*, 2015). Algae and their communities can convert these two major forms of arsenic in nature (Hellweger, 2005; Debnath and Bhadury, 2017). Aluminium (Al) and chromium (Cr) are other important toxic heavy metals in polluted waters that are widely used in industries (Li *et al.*, 2018). Aluminium has received a great deal of attention, particularly in the context of increased acidification of waterbodies which may mobilize this metal causing toxicity to biota (Sharma and Sohn, 2009). A large number of earlier studies on aluminium toxicity simultaneously also evaluate acidification effects, and such studies do not clearly distinguish the effects of aluminium from that of acidification (Smedley and Kinniburgh, 2002).

Chromium exists in various ionic forms in wastewaters including Cr (III) and Cr (VI). Chromium (VI) is considered as most toxic and is also carcinogenic and mutagenic. A major proportion of Cr, present in surface waters, comes from the particulate matter in the sediment (Corbi *et al.*, 2011). Other major sources of Cr contamination in water bodies include the leather industry, plating and electroplating industry, film and photography industry, metal cleaning as well as mining activities (Corbi *et al.*, 2011). Excessive Cr concentration in waters causes diverse effects on flora and fauna by inhibiting a variety of metabolic activities.

Metal contamination of freshwater ecosystems has now become a growing environmental problem worldwide. Periphytic algae are an important component in these ecosystems, where they constitute a major proportion of primary producers at the base of the aquatic food web. Periphyton plays an important role in nutrient cycling and has been acclaimed to be excellent indicator of water quality (Yadav *et al.*, 2018). Although substantial progress has been made in understanding the response of these communities to various metal contaminants, the perusal of literature suggests that aluminium, arsenic and chromium have been little explored with regard to their effects on algal periphyton. Most of the earlier efforts have focused on copper, zinc, cadmium and other metal ions. In light of the above, in the present study, an effort has been made to investigate the effects of arsenic, aluminium and chromium on the periphytic algal communities of the river Ganga at Varanasi, India. We hypothesized that i) the test elements, As, Al and Cr would deleteriously impact the biomass and species diversity of the periphytic community and the extent of these effects would vary across different concentrations of the test metals; ii) individual algal species would respond differentially to different metal treatments and this would led to the differences in community compositions of periphyton growing in the control and various metal treatments. To test the hypothesis, we performed an *in situ* experiment in the river Ganga employing a specially designed substrate, chemical diffusing substrates (CDS). The CDS encompasses a porous substratum, which not only releases the test chemical *via* its porous surface but also provides a substratum for the attachment of periphyton. Diffusing substrates have been proven to be a good system which could be satisfactorily employed for the field study (Fairchild *et al.*, 1985; Pandey *et al.*, 2014; Yadav *et al.*, 2018). Thus, prompted by earlier studies, the present investigations employed CDS for *in situ* study of heavy metal stress on periphyton.

Study area

The present experiment was conducted in the river Ganga at Garhwa Ghat, Varanasi, India. Varanasi city (25°18' N, 83°1' E; elevation 80.71 m asl) lies in the middle stretch of the Ganges basin in the northern part of India. The city has a tropical monsoon climate with total annual rainfall ~1100 mm. During the course of the experiment, the average minimum and maximum atmospheric temperatures were 12.4°C and 32.1°C.

METHODS

Experimental design: chemical diffusing substrates

To study the effect of heavy metal stress (As, Al and Cr) on algal periphyton of the river Ganga, chemical diffusing substrates were constructed. Each CDS was made using a cone-shaped plastic funnel (height 8 cm, diameter 14 cm at the base, internal volume 600 ml) and an unglazed, circular, porous and fired clay tile (diameter 15 cm; thickness 4 mm). The wide opening of the plastic funnel was sealed with the clay tile using an epoxy resin (m-seal, Pidilite Industries, Mumbai, India) while its narrow opening was closed with a replaceable rubber cork (Fig. 1). For metal treatment, solutions of selected test metals; As ($Na_2HAsO_4.7H_2O$), Al ($Al_2SO_4.18\ H_2O$) and Cr ($K_2Cr_2O_7$) were prepared in Milli Q water using their analytical grade salts (Rankem, India). Three different concentrations, low (1 g L^{-1}), medium (2.5 g L^{-1}) and high (5 g L^{-1}) were prepared for each test metal. These three concentrations were chosen on the basis of our laboratory and field trails for 1 month. Based on these trails our group already published several research papers in the past (Pandey *et al.*, 2014; Pandey and Bergey, 2018; Yadav *et al.*, 2018). The CDS constructed earlier were then filled with one of the different metal solutions and deployed in triplicate in the river Ganga. The control CDS were also prepared similarly and were filled with river water (pH 7.8) in place of metal solution.

Prior to beginning the experiment in the river, a diffusion experiment was carried out at the study site to determine the pattern of the rate of release of various test metal ions through CDS. To meet this objective, the CDS for each different metal treatment was kept in the river using bamboo frame (described later in detail). These CDS diffused metal ions from their clay surface into the water outside. At every week, CDS were sampled for metal solution and the concentration of metal ions remaining inside the solution was measured *via* atomic absorption spectrophotometer (Perkin-Elmer, Analyst 800). Three replicates were considered for each treatment and the experiment lasted 30 days. The data obtained were used to calculate the rate of release of metal ions from CDS.

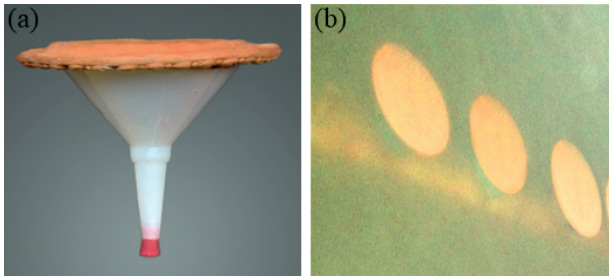

Fig. 1. Chemical diffusing substrate and the experimental set-up deployed in the river Ganga: a) A chemical diffusing substrate. b) Section of the experimental set-up deployed in river.

Experimental set-up

The experiment was set up in the river Ganga in February and left for 4 weeks for colonization and growth of periphytic algae. The CDS were immersed in river water by fixing them in a rectangular wooden frame that was supported on bamboo pillars at its vertices. The bamboo pillars were buried vertically deep in the sediments at the river bottom. The CDS were fixed perpendicularly in the wooden frame in such a way that their clay substrate was lying parallel to the water current. The experimental set-up was deployed in the river at a distance approximately 10 m away from the river bank and at a depth of 10 cm below the water surface. Since a colonization period of 4-6 weeks is considered sufficient to support maximum biomass development in mesotrophic and eutrophic systems (Pandey and Bergey, 2018), the present experiment was conducted for a 4-week duration with sampling at a 7-day time interval. When deployed in water, CDS diffused out metal ions *via* its porous clay substrate, which also served as the substratum for the growth and colonization of periphytic algae in the river Ganga.

Collection and analysis of river water

Important physicochemical parameters of river water were assessed regularly at weekly interval. Water temperature, pH, total dissolved solid (TDS) and electrical conductivity (EC) were measured *in situ* with appropriate portable device (Hanna 151 Hi98509 digital thermometer, Hanna pHepR ® pH tester, 192 ISO Tech System, ITS152 302 TDS-conductivity meter). Sampling was done in triplicate in 2l of polyethylene bottles and the sample bottles were transported to laboratory by storing in an icebox. The collected water samples were analysed for estimation of soluble reactive phosphorus (SRP), total phosphorus (TP), nitrate-nitrogen (NO_3^--N), nitrite-nitrogen (NO_2^--N) and dissolved silica following standard analytical methods (Wetzel and Likens, 2000). Soluble reactive phosphorus was determined by ascorbic acid-molybdenum blue method. Total phosphorus was estimated by the same, ascorbic acid- molybdenum blue method after digesting the samples with persulfate. NO_3^--N was analyzed by cadmium reduction column method and NO_2^--N was estimated by diazotization method. NH_4^+-N in water sample was measured by phenol-hypochlorite method. Flow rate was determined using a low-density styrofoam float.

Collection and study of periphyton

Peripytic algae were sampled at weekly intervals after 7, 14, 21 and 28 days of deployment of CDS in the river

Ganga. The colonized biofilms on the surface of CDS were removed by scraping an area 40 cm^{-2} with a razor blade. The samples were collected in a plastic test tube and diluted to a constant final volume with distilled water. Each sample was further divided into three parts; the first subsample for biomass (chl *a*) estimation, the second preserved in formalin (4%) for taxonomic analyses and the remaining third for microscopic examination of live algal cells prior to cell enumeration. Chlorophyll *a* was estimated using trochromatic method (Strickland and Parsons, 1968) after extracting the samples in 90% acetone and incubation in the dark for 24 h at 4°C. Taxonomic analysis of periphyton was done by microscopic examination of formalin-preserved subsamples. Approximately 600 algal cell units (containing chlorophyll) were counted and identified to species level. An algal cell unit was designated as each individual cell for unicellular algae, one cell as one filament for large filamentous algae and an area of 10 x10 µm m^{-2} in case of colony-forming algae. For small filamentous cyanobacteria, a 10 µm length of filament was considered as one algal unit (Larson and Passy, 2012; Yadav *et al.*, 2018). The counting was done with a haemocytometer under light microscope at a magnification of 450x and 1000x. Soft algae including cyanobacteria and green algae were directly counted under microscope, whereas diatoms were identified by observing their clean and permanent mounts. The permanent diatom slides were prepared after digesting the sample with H_2SO_4 and concentrated HNO_3 and further mounting in a synthetic resin, Naphrax (Karthik *et al.*, 2010). The taxonomic literature consulted for species identification include Cox (1996), Desikachary (1959), Prescott (1962, 1978), Patrick and Reimer (1966, 1975), Algalbase (http://www.algalbase.org) and ANSP algal image database (http://diatom.ansp.org/algae_image/). The cell count data were converted to cell densities and expressed as number of cells per unit area (cells m^{-2}). The mean cell volume of individual algal taxa was calculated by measuring the cell dimensions of ~10 randomly chosen cells with an ocular micrometre and entering the values in appropriate geometric formulae as given in Hillebrand *et al.* (1999). The total biovolume ($mm^3 cm^{-2}$) for each species was then estimated from the product of its cell density and mean cell volume. Based on the biovolume, the relative abundance of each algal species was calculated. The relative abundances of major algal groups, Chlorophyta, Bacillariophyta and Cyanobacteria, were also assessed as a percentage ratio of the total biovolume of each algal group to the total algal biovolume. Algal diversity and species richness were estimated for each of the metal treated as well as the control samples. Species richness was represented by the total number of species present in individual samples. Diversity was calculated with Shannon diversity index (Shannon, 1948) using the software Past (Version 3.12, Natural History Museum, University of Oslo, Norway). Data were statistically analysed by one-way analysis of variance (ANOVA) to test the significance of the effect of metal treatments and duration of exposure (days) on chlorophyll *a* and other community parameters (species richness, diversity and abundance) of periphyton. Tukey's Honestly Significant Difference (HSD) test was done to compare the mean values of the measured parameters of periphyton between various metal treatments as well as the control.

RESULTS

During the study period, the important physicochemical parameters of Ganga river water were determined at each sampling week (Tab. 1). The measured parameters showed little variations throughout the study period.

The rate of release of different metal ions, As, Al and Cr from CDS was measured every week for a period of one month. The average rate of release of the metal ions, at all tested concentrations, during 30 days of the experimental period has been shown in Tab. 2. Among all the three tested concentrations of various metals taken inside the CDS, in

Tab. 1. Important physicochemical characteristics of river water during the study duration. Data given are mean±SE.

Parameters	Week			
	1	2	3	4
pH	7.80	8.10	7.80	8.10
Conductivity (µs c^{-1})	240	248	250	251
Flow rate (cm-sec^{-1})	23	25	26	24
TDS (mg L^{-1})	0.350	0.270	0.250	0.260
TP (mg L^{-1})	0.062	0.069	0.079	0.082
SRP (mg L^{-1})	0.036	0.042	0.045	0.048
NO_3^--N (mg L^{-1})	0.410	0.420	0.390	0.400
NO_2^--N (mg L^{-1})	0.030	0.020	0.020	0.030
Si (mg L^{-1})	11	10	9.5	12

the present study, the highest rate of release was observed at the highest metal concentration (5 g L^{-1}) followed by medium (2.5 g L^{-1}) and low (1 g L^{-1}).

The biomass of periphytic algae growing on chemical diffusing substrates varied among the control and various metal-treated communities (Fig. 2). A greater amount of chlorophyll *a* was observed in the control communities as compared to various metal-treated communities. Among the three different metal treatments, the chlorophyll *a* concentration was slightly higher in Cr treatment followed by Al and As. A distinct concentration-dependent response of chlorophyll *a* to various metal treatments was also noteworthy. For each test metal, the maximum decline in chlorophyll *a* content occurred at the highest tested concentration of the metal whereas the minimum inhibition was recorded with the lowest metal concentration. The inhibitory effect of various test metals was most prominent during the initial period of the experiment, whereas it was comparatively less effectual in a later period. In general, the mean chlorophyll *a* content increased progressively from week 1 to week 4 in each of the control and metal-treated communities.

Tab. 2. The rate of release (µg cm^{-2} d^{-1}) of arsenic (As), aluminium (Al) and chromium (Cr) from chemical diffusing substrates deployed in the river.

Metal treatment	Week			
	1	2	3	4
Cr^{L}	29±2^{a}	25±2^{b}	21±1^{c}	18±1^{d}
Cr^{M}	80±4^{a}	61±3^{b}	52±3^{c}	41±2^{d}
Cr^{H}	221±7^{a}	160±5^{b}	109±6^{c}	85±4^{d}
As^{L}	25±1^{a}	21±1^{b}	19±1^{c}	16±1^{d}
As^{M}	71±3^{a}	55±2^{b}	44±2^{c}	34±2^{d}
As^{H}	201±6^{a}	142±6^{b}	98±5^{c}	76±4^{d}
Al^{L}	23±1^{a}	19±1^{b}	16±1^{c}	14±1^{d}
Al^{M}	64±3^{a}	50±3^{b}	38±2^{c}	29±2^{d}
Al^{H}	185±5^{a}	129± 4^{b}	86±3^{c}	67±3^{d}

$^{a-d}$Data means (mean±SE) are statistically different from each other ($p<0.05$; Tukey's HDS test); L, M, H (superscript) are low (1 g L^{-1}), medium (2.5 g L^{-1}) and High (5 g L^{-1}) concentration of the test metal inside the substrate.

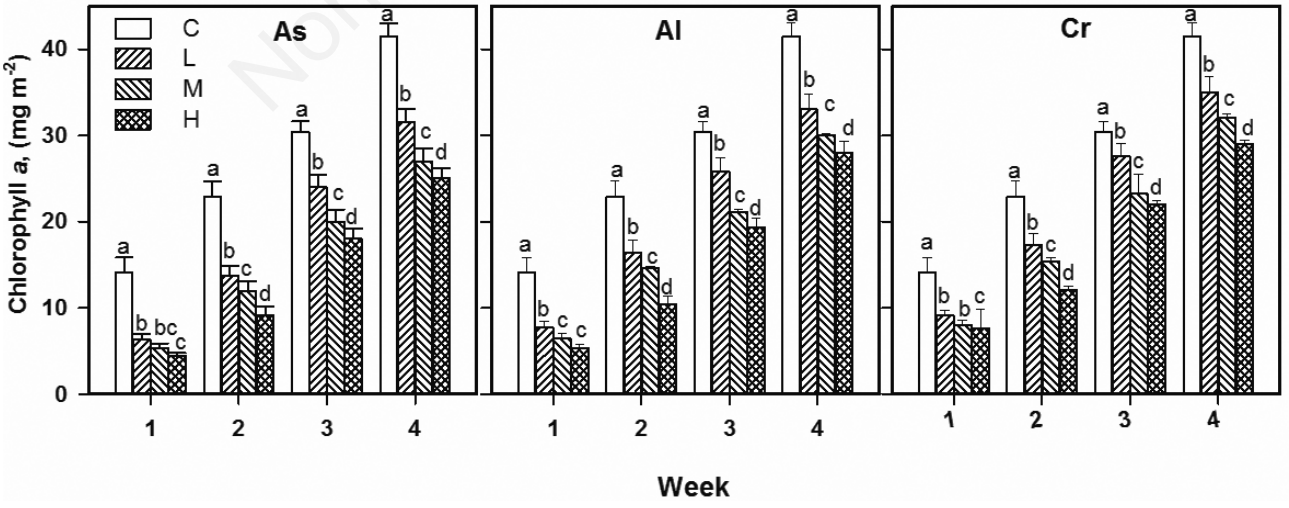

Fig. 2. Biomass of periphytic algal assemblages at low, medium and high concentrations of arsenic, aluminium and chromium after 1, 2, 3 and 4 weeks of deployment of the experiment. C, control; L, M and H are low (1 g L^{-1}), medium (2.5 g L^{-1}) and high (5 g L^{-1}) concentrations of metal inside the chemical diffusing substrates, respectively. Data bars marked with the same letters are not significantly different from each other ($p<0.05$; Tukey's HSD test).

The periphytic communities growing on chemical diffusing substrates comprised individuals belonging to three different groups, Bacillariophyta, Chlorophyta and Cyanobacteria. Fig. 3 shows the relative abundance of these various groups growing on the control and various metal-treated CDS after 1, 2, 3 and 4 weeks of deployment of the experiment. The diatoms were the dominant algal group during the entire study period in the control as well as in the metal treatments As and Al. In Cr treatment also, the members of Bacillariophyta dominated the community structure, however only up to three weeks. During the fourth week, cyanobacteria dominated the community structure in all three different concentrations of Cr; low, medium and high respectively. In each CDS, the relative abundance of diatom was higher initially in week 1, which gradually and consistently declined with the passage of time in subsequent weeks. In general, the percent relative abundance of diatoms increased in As and Al treatments, whereas it decreased in Cr treatment. The relative proportion of diatoms also varied across different concentrations of test elements and in As treatment, their response was found to be concentration dependent. The greater the concentration of As inside CDS, the greater the relative abundance of diatoms. An increase in diatom abundance was also found in Al treatments, where by the end of the experiment their relative abundance increased from 51.4% to 77%, 60.80% and 57.45 % in low, medium and high concentrations, respectively. Contrary to these, in Cr treatment, the relative abundance of diatoms decreased from 86.8% in the control to 58.8%, 72.4% and 81.1% in low, medium and high concentrations and during the first week and from 51.4% to 17.4%, 24.2% and 36.3% by the end of the experiment. The green algae contributed the least to community structure, whereby in the control CDS they comprised only 3% to 14% of the community, during the entire study period. However, their relative proportion increased significantly, at all three concentrations of Cr up to 15 days of experiment, and in all the tested concentrations of As and Al during the entire study period. The relative abundance of cyanobacteria was low initially in week 1, but increased progressively over time till the last week. Cyanobacteria, similarly to diatoms, responded differentially to the various test metals. The relative abundance of cyanobacteria decreased in the metal treatments As and Al, whereas it increased in Cr treatment, at all three tested concentrations. The decreased abundance of cyanobacteria in response to As and Al was found to be concentration dependent. The decline was recorded maximally at the highest concentration of metal, (As and Al) treatments followed by medium and low. Similarly, an increased abundance of cyanobacteria was also found to be concentration dependent and found to be maximum at the low treatment of Cr during all the sampling weeks.

Fig. 4. shows the relative abundance of major diatom taxa after 4 weeks of metal exposure. Individual species responded differentially to different metal treatments. The relative abundance of *Aulacoseira granulata* increased moderately in As treatment, whereas it increased abruptly in Cr treatment. The species, however, exhibited decreased abundance in all three tested concentrations of Al treatment. The decline of the species was concentration-dependent and observed maximum at the highest tested concentration of Al, whereas minimum decline occurred at the lowest concentration. Another species of *Aulacoseira*, *A. pusilla* increased in all three different metal treatments at all three different tested concentrations, except the highest concentration of Al. The diatom taxon *Ulnaria ulna* also responded positively to various metal treatments. The percent contribution of *Ulnaria ulna* increased notably with an increase in As concentration, however, its relative abundance decreased consistently with increasing concentration of Cr inside CDS.

Different species of *Nitzschia*, such as *Nitzschia palea* and *Nitzschia recta* responded similarly to Al and Cr stress, whereas displayed variable responses to the As stress. The relative contribution of both species decreased abruptly in all the tested concentrations of Al and Cr. However, in As treatment the relative abundance of *Nitzschia palea* increased at various concentrations whereas that of the *Nitzschia recta* decreased significantly. *Navicula recens* also responded differentially to different metal treatments showing increased percentage in Al whereas decreased in Cr. The relative cell density of *Cyclotella meneghiniana* increased at low and medium concentrations of Cr treatment whereas decreased at high Cr concentrations. The species also demonstrated differential responses to As and Al treatments showing an increased abundance in As whereas a decreased abundance in Al treatment.

Various species of Chlorophyta responded to different metal treatments, differentially as well as in species-specific and concentration-dependent manner (Fig. 5). The species specific response of *Scenedesmus* sp, *Scenedesmus quadricauda* and *Scenedesmus bijugatus* was observed in the present investigation. The relative percent of *Scenedesmus quadricauda* increased at all the tested concentrations of various metal treatments, whereas that of the *Scenedemus bijugatus* increased only in Al, and it declined in As and Cr. *Ankistrodesmus falcatus* decreased at all three concentrations of As, Al and Cr. Differential response of *Cladophora glomerata* was observed in various metal treatments. The species was completely absent in As treatments, whereas it exhibited an increased abundance in Al treatments. In Cr treatments, also the species was not present initially up to the third week; however, during the fourth sampling week, a decreased abundance of the species was observed.

The response of cyanobacteria to different metal

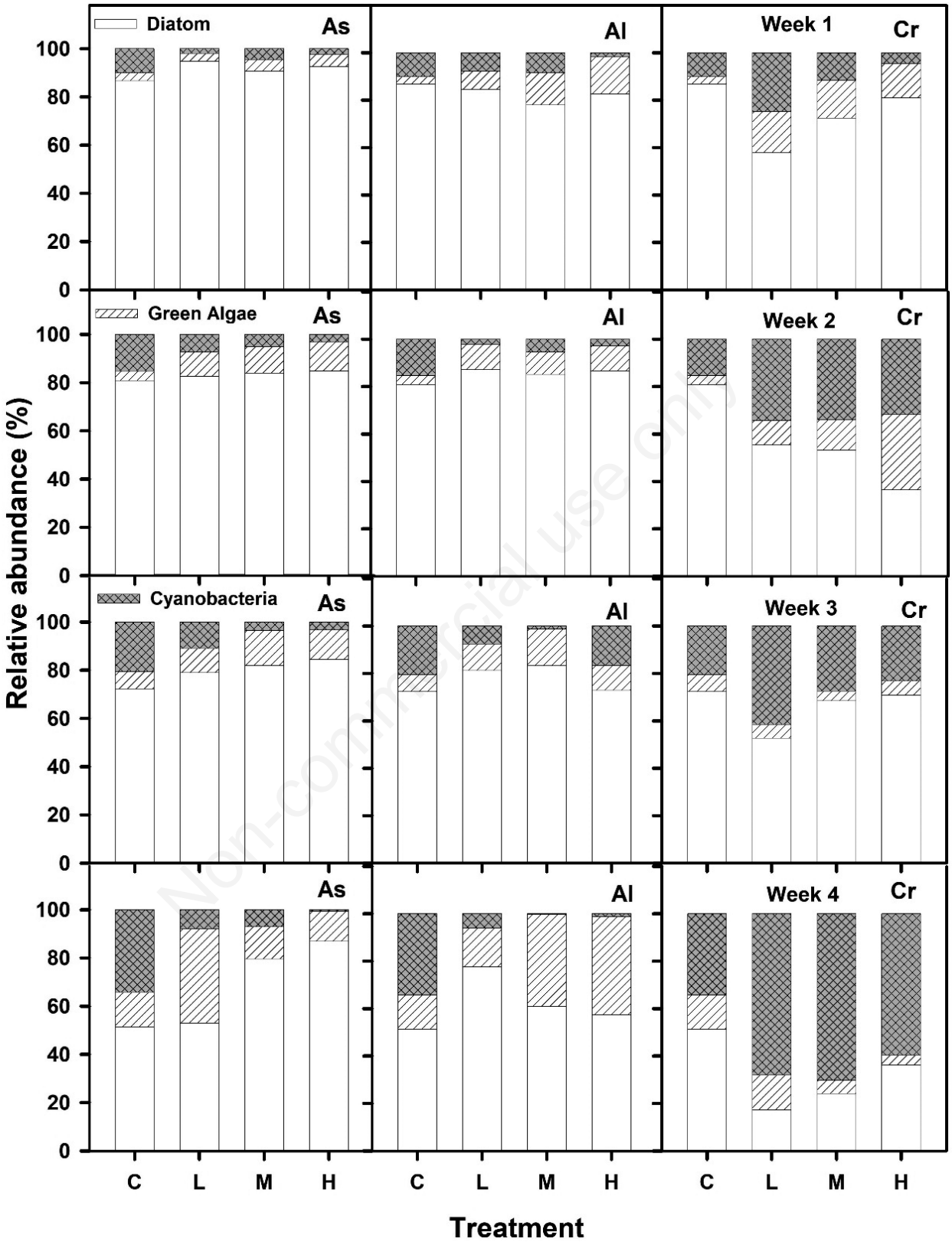

Fig. 3. Relative abundance of periphytic algal communities exposed to low, medium and high concentrations of arsenic, aluminium and chromium after 1, 2, 3 and 4 weeks of deployment of the chemical diffusing substrate in the river. C, control; L, M and H are low (1 g L^{-1}), medium (2.5 g L^{-1}) and high (5 g L^{-1}) concentrations of metal inside the chemical diffusing substrates, respectively.

treatments has been shown Fig. 6. The relative abundance of *Phormidium ambiguum* increased abruptly in Cr treatment but the species was absent in As and Al treatments up to two weeks of the experiment. *Oscillatoria limosa* also responded sensitively to As stress and it was not found in various treatments of As up to three weeks of the experiment. Contrary to this, in Al and Cr, its relative abundance increased significantly at all the tested concentrations. *Anabaena circinalis* displayed relative abundances lower than the control but there was very little difference among the metal concentrations. The effect of concentration was particularly marked only up to two weeks in As and Al treatments and from the third week onwards, the relative abundance of the species did not differ much among different concentrations. During the fourth week, the relative abundance of species in different metal concentrations was nearly similar to the control. Another species *Anabaena cylindrica* also exhibited marked sensitivity to As and Al and it was absent in these treatments. In Cr treatments, however, the relative abundance of the species increased rapidly at all different tested concentrations. The cyanobacterium *Merismopedia punctata* exhibited increased abundance in all the metal treatments.

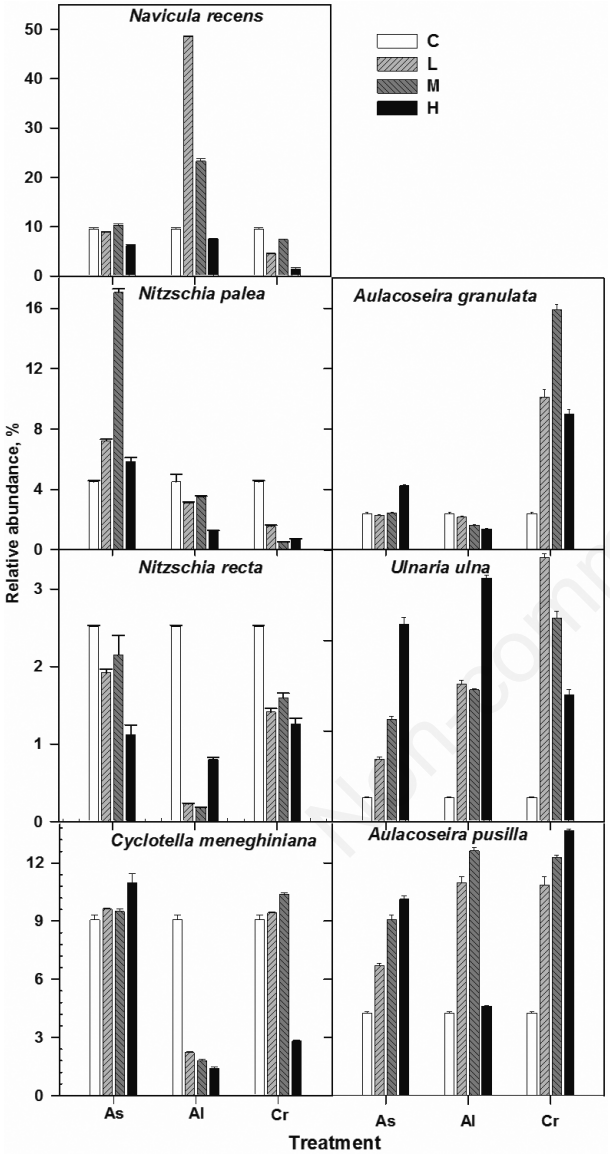

Fig. 4. Percent share of dominant diatom species in the periphytic community growing on chemical diffusing substrates filled with low, medium and high concentrations of arsenic, aluminium and chromium after 4 weeks of exposure. C, control; L, M and H are low (1 g L^{-1}), medium (2.5 g L^{-1}) and high (5 g L^{-1}) concentrations of metal inside the chemical diffusing substrates, respectively.

Shannon diversity and species richness of the control and various metal-treated periphytic communities after 1, 2, 3, and 4 weeks of the experiment have been shown in Fig. 7. Species diversity was maximum in the case of community growing on the control CDS. The diversity declined in different metal treatments at all tested concentrations. A general pattern of concentration-dependent decline in diversity was observed in almost all the metal treatments. The maximum decline in diversity was observed in the highest tested concentration of metal, followed by medium and low. The species richness of periphytic community also followed the same general pattern as that of diversity. The species richness was found to be maximum in the control and declined in all the metal-treated CDS during the 4 weeks of the experimental period.

DISCUSSION

The test metals could very well diffuse out of the porous clay surface and the general pattern of release was similar to that encountered for nutrients and other stressors whose impact has been investigated in earlier studies (Arnegard *et al.*, 1998; Yadav *et al.*, 2018). The diffusion of the test chemicals occurred throughout the period of the study. All the test metals and metalloid, namely aluminium, chromium and arsenic, deleteriously impacted algal periphyton. There was reduction of periphytic biomass, measured as chlorophyll *a*, on substrates exposed to the mentioned elements. This very well agrees with earlier observations on many heavy metals where their high concentrations have been shown to inhibit chlorophyll *a* concentrations in periphytic biofilms (Rai *et al.*, 1981; De Filippis and Pallaghy, 1994). Nicholls *et al.* (1992) observed a statistical signification relation between aluminium concentration and total algal biovolume and number of taxa in Canadian lakes. Further, they found a decline in the taxa of Chloropyta and Bacillariophyta with an increase in aluminium concentration in water.

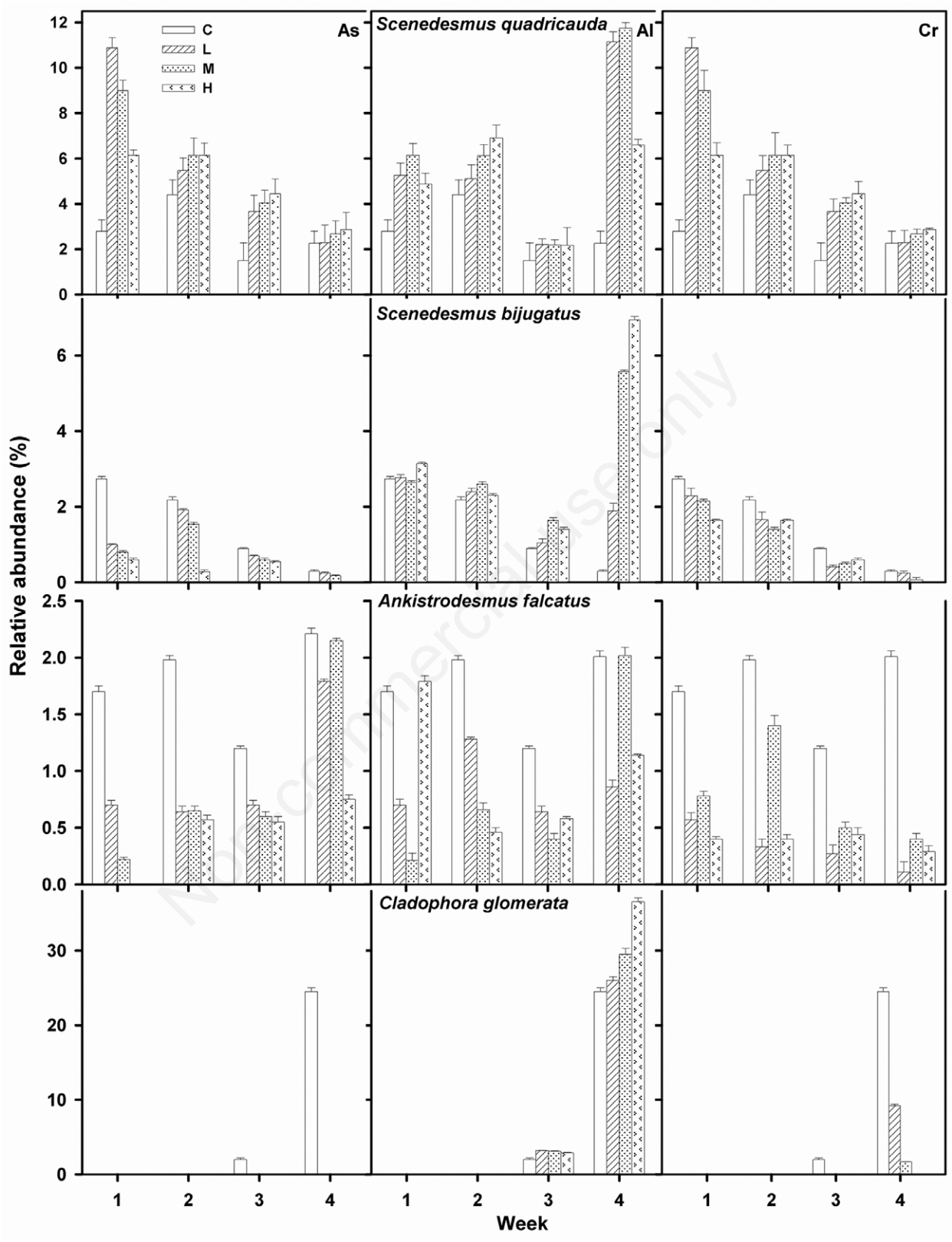

Fig. 5. Relative abundance of dominant green algae taxa in the periphytic community after 1, 2, 3 and 4 weeks of metal exposure. C, control; L, M and H are low (1 g L^{-1}), medium (2.5 g L^{-1}) and high (5 g L^{-1}) concentrations of metal within the chemical diffusing substrate.

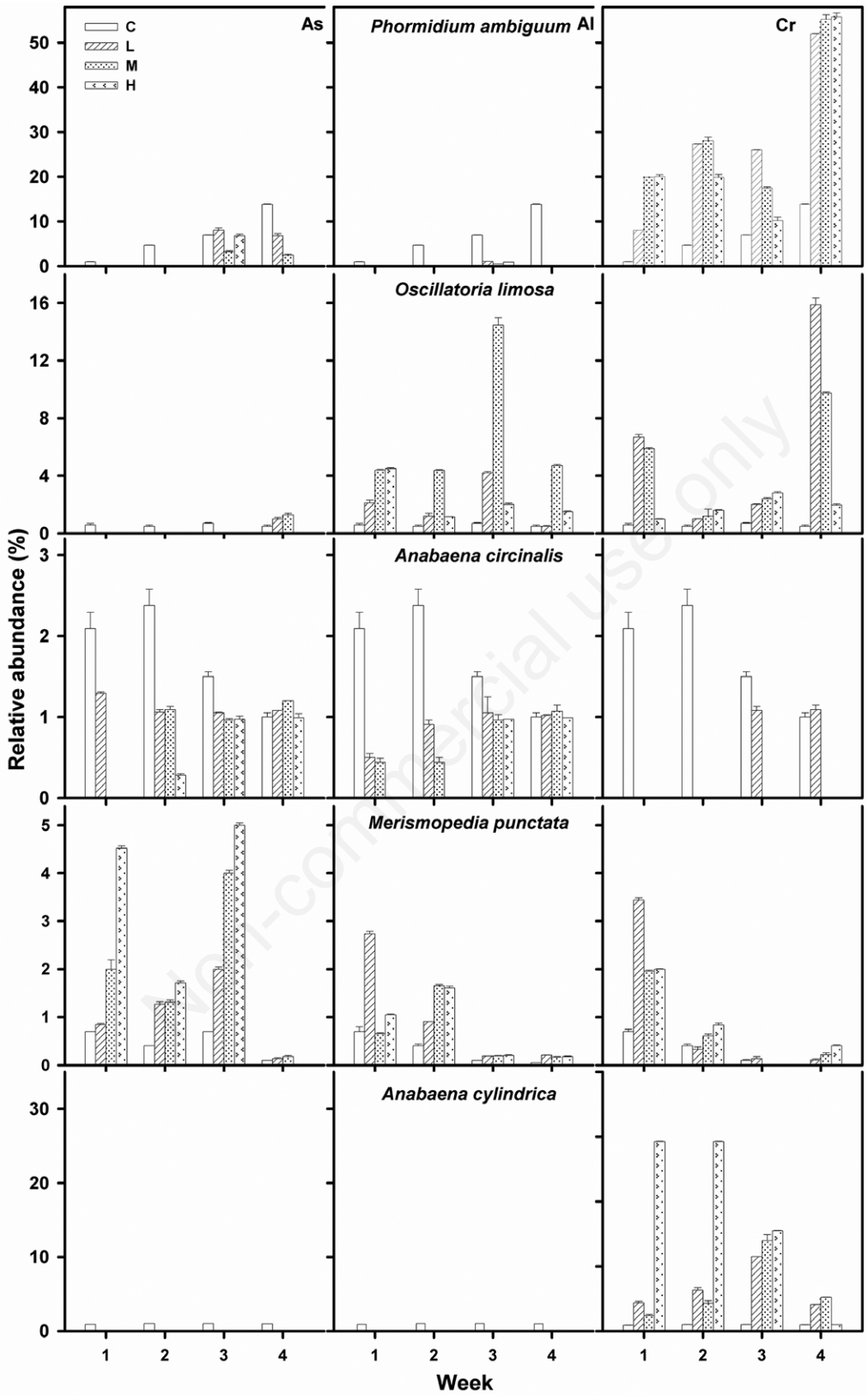

Fig. 6. Percent share of dominant cyanobacterial species in the periphytic community after 1, 2, 3 and 4 weeks after metal exposure. C, control; L, M and H are low (1 g L^{-1}), medium (2.5 g L^{-1}) and high (5 g L^{-1}) concentrations of metal within the chemical diffusing substrate.

Paleolimnological studies have been carried out to relate the abundance of diatoms with various factors, including the concentration of aluminium and many of these studies do reveal a definite relationship with the concentration of aluminium (Gensemer and Plyle, 1999). However, these latter authors further mention that these are mere statistical correlations of the abundance of diatoms and other algae with environmental factors, which of course includes aluminium as one of the factors. Insofar as aluminium toxicity to algae and their communities is concerned, a majority of studies focus on acidification rather than aluminium toxicity itself (Gensemer and Plyle, 1999). Such studies do not clearly discern the effect of aluminium from that of low pH. However, many earlier authors conclude that acid-tolerant algae are generally aluminium-tolerant (Havens and Decosta, 1987). Upreti *et al.* (2013) also noted a decrease in the biomass of phytoplankton and periphyton under aluminium stress.

The present observations are in consonance with Tuulaikhuu *et al.* (2015) who noted decreased chl *a* in indoor experimental channels exposed to arsenate. Recently, Barral-Fraga *et al.* (2016) observed a reduction of the total biovolume of a diatom community exposed to arsenic stress in a laboratory stream.

Various periphytic groups varied with regard to their tolerance to metals. The most important observation of the present study has been the abundance of cyanobacteria on chromium-diffusing substrates. This obviously is a reflection of their tolerance to chromium. Patrick (1978) also noted an abundance of cyanobacteria in a lab-grown periphytic assemblage subjected to Cr enrichment. Cyanobacteria are prokaryotes and greater tolerance of diverse prokaryotes to chromium has already been indicated (see Viti and Giovannetti, 2007). The present observation on Cr does not agree with Licursi and Gomez (2013) who noted a decline in cyanobacteria together with

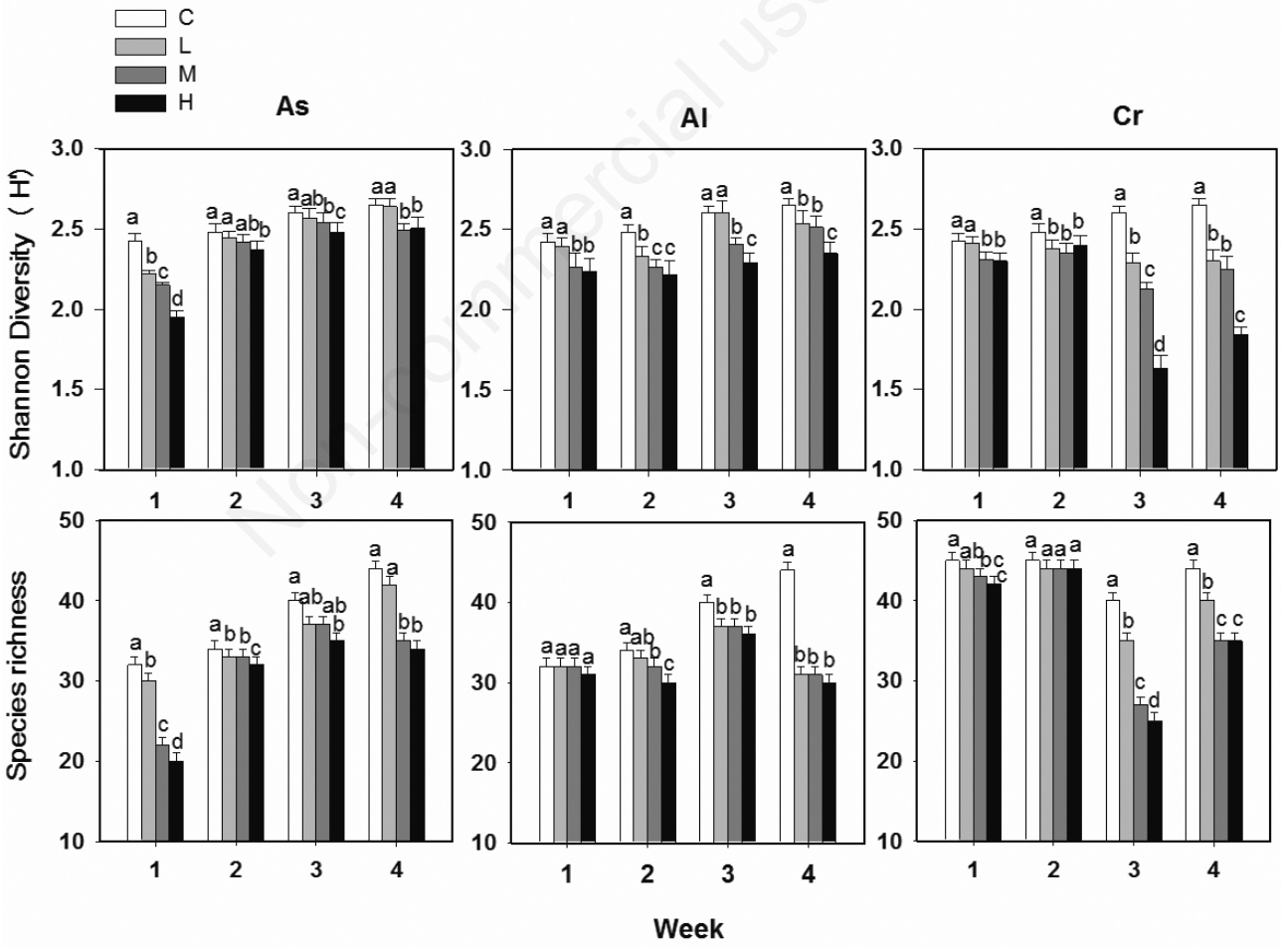

Fig. 7. Shannon diversity (H') and species richness of the periphytic algal assemblage exposed to low, medium and high concentrations of the test metals after 1, 2, 3 and 4 weeks of deployment of the experiment. C, control; L, M and H are low (1 g L^{-1}), medium (2.5 g L^{-1}) and high (5 g L^{-1}) concentrations of metal within the chemical diffusing substrate. Data means bearing different letters are significantly different from each other at $p<0.05$ (Tukey's HSD test).

an increased abundance of diatoms and green algae under Cr stress. The differences observed in the results are probably due to variations in algal species compositions between the two experiments. Licursi and Gomez (2013) used an experimental mesocosm to assess the effect of Cr on an existing algal community, where some algal taxa non-tolerant to Cr were present in the community. On the other hand, the present study was conducted *in situ*, in a large river. The natural periphytic assemblage of river differed substantially from the community existing in the experimental mesocosms (Licursi and Gómez, 2013). It exhibited greater species diversity and displayed dominance of some different algal taxa that were more tolerant to Cr and thrived during community development. Thus, the apparent difference in Cr tolerance might be due to differences in tolerant algal species in the two studies investigated.

In general, the present study showed greater tolerance of green algae to Al and As. This observation very well agrees with several earlier studies on other heavy metals. Takamura *et al.* (1989) in a large-scale survey observed greater tolerance of freshwater benthic green algae to heavy metals. Genter *et al.* (1987) similarly observed greater tolerance of green algae in a periphytic community to zinc in a stream mesocosm. A number of other researchers have similarly noted the tolerance of green algae to heavy metals (Whitton, 1970; Foster, 1982). Whereas cyanobacteria showed tolerance against chromium, they were sensitive to Al and As stresses. The greater sensitivity of cyanobacteria to metal pollutants agrees well with some earlier reports (Takamura *et al.*, 1989; Singh and Rai 1990; Nirmala Kumari *et al.*, 1991). On the contrary, Bhattacharya and Pal (2011) interestingly found *Oscillatoria princeps*, *O. limosa*, *Anabaena* sp. and *Phormidium laminosum* to be tolerant to arsenic. Maeda *et al.* (2004) also reported *Phormidium* to be resistant to arsenic.

The present study showed that different periphytic species varied in their sensitivity to Al, As and Cr. Some species were sensitive to all three stresses whereas some showed tolerance to all of them. Still, there were species which showed sensitivity to one metal but were resistant to others. Obviously, tolerance to one particular metal stress does not automatically confer tolerance to others as there are obviously different mechanisms for the tolerance against different metals (Gaur and Rai, 2001). *Ulnaria ulna* was found to be tolerant to all three tested metals. Barral-Fraga *et al.* (2016) also found its tolerance against As. It deserves mention that *U. ulna* is one of the most metal-tolerant species of diatoms (Blanck *et al.*, 2003; Duong *et al.*, 2008). Castro *et al.* (2015) found *Nitzschia palea* to be sensitive to arsenate, whereas the present study found it to be tolerant. The present observations also do not agree with Bhattacharya and Pal (2011) who found *Oscillatoria limosa* to be tolerant to As. The present study found this cyanobacterium to be sensitive to As but tolerant to Cr as well as Al. The tolerance of *Scenedesmus quadricauda* to all the test metals in the present case is in agreement with the report on its greater copper tolerance (Oliveira, 1985). However, the other species *Scendesmus bijugatus* showed sensitivity to test metals. So even within a genus, different species respond differently to metal stress.

Al, As and Cr had lowered species richness and diversity of the periphytic algal community. This apparently resulted due to the elimination or reduced abundance of some of the sensitive species followed by an increased abundance of tolerant forms. Changes in these parameters were not large due perhaps to the reason that these three are amongst the least toxic metals/metalloids. The declination of species richness agrees well with several earlier reports (Upreti *et al.*, 2013; Barral-Fraga, 2016). Shannon index also underwent a decline under metal stress in a concentration-dependent manner. This observation agrees with several other reports (Upreti *et al.*, 2013; Morin *et al.*, 2008a, 2008b; Arini *et al.*, 2012a, 2012b; Barral-Fraga, 2016). Unlike the present observations, Hirst *et al.* (2002) could not relate species diversity and other community parameters with a metal concentration in stream water.

CONCLUSIONS

Our work clearly shows that the studied contaminants arsenic, aluminium and chromium have deleterious effects on the biomass and composition of periphyton. The study indicated differential response of various periphytic groups to different metal treatments. The metal stresses lowered the species richness and Shannon diversity and increase the abundance of tolerant species and the disappearance or decrease of sensitive species. The discharge of high concentration of metal contaminant in the fluvial ecosystem have deleterious effects on algae and hence affect the primary productivity of waterbodies, which may directly or indirectly affect the organisms of different trophic levels (through food chain), mainly humans.

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