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Effects of road runoff on biomass and metabolic activity of periphyton in experimental streams

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Abstract

Aquatic ecosystems could be enriched with inorganic and organic matters after road runoff discharge. In this context, we studied the effects of road runoff on river biofilms (periphyton). To operate under controlled conditions the experiments were carried out in two indoor experimental streams. Glass slides were used as artificial substrates for the periphyton colonisation in the experimental streams. Current velocity was maintained at 12 cm s^{-1} (slow) in the first experimental stream and at 50 cm s^{-1} (fast) in the second one. The periphyton communities were periodically exposed to road runoff over 30 days. For this experiment, the road runoff was collected in settling basins of a motorway during rainfall events. The runoff was then characterised according to physical and chemical parameters. We tested two exposure durations (1 and 4 h) and two runoff dilutions (10% and 50%). Two laboratory experiments carried out during this study revealed that the biomass (AFDW: 0.92–2.83 g m^{-2}), the chlorophyll *a* content (6.8–78.9 mg m^{-2}) and the metabolic activity (net primary production: 61–334 mg O_2 m^{-2} h^{-1} and respiration: 17.2–68.3 mg O_2 m^{-2} h^{-1}) of the biofilms increased ($p < 0.05$). However, this was a function of current velocity, the runoff exposure duration, and the content in organic and inorganic elements present in the tested rainwater. Experimental streams constituted a simplified natural system, which did not allow the reproduction of all the environmental conditions. Thus, these experiments should be performed on natural sites.

Keywords: Road runoff; Impact; Experimental stream; Periphyton

1. Introduction

Road works can disturb aquatic environments by modifying runoff (hydraulic works, increase in drained surfaces, clogging substrats, increasing flow, etc.) and by discharging polluted effluents [1]. The French Water Act [2], as well the European Directive on urban wastewater treatment [3], require stormwater discharges to be taken into account. Studies evaluating the quality of road runoff have taken place since the 1980s, allowing the major pollutants to be defined, evaluating levels of pollution, and characterising the main factors controlling these levels [4–7]. Conversely, few studies have assessed the impact of runoff on the aquatic environment [8]. Changes were observed in a limited number of the rivers studied. These alterations were generally characterised by a decrease in the abundance, the diversity, and the number of species of benthic macroinvertebrates that were sensitive to the pollution [9–11]. Several studies also showed modifications in the structure and the metabolic activity of populations of benthic microorganisms downstream of road runoff on streams [9,12,13].

However, these studies underscore the complexity of the problem. The difficulty in evaluating and predicting the impacts is due to the intermittent characteristics of pollution, its spatio-temporal variability, and a multitude of physical, chemical, and biological factors which condition receiving water response [14,15]. The objective of the present work was to evaluate the influence of two main road runoff characteristics (content in pollutants and exposure duration) on the structural and functional response of two epilithic communities (lotic-type and lentic-type). Nevertheless, to facilitate the understanding of the mechanisms involved we decided to use an artificial environment to control the main parameters related to runoff and to the environment (hydrology, physical and chemical factors, and test organisms). Epilithic biofilm microorganisms (periphyton) represent attractive bioindicators. These communities are localised at the interface between the sediments and the surrounding

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water and can have a significant impact on biogeochemical cycles (mineralisation of the organic matter, hydrolytic enzymatic activities, etc.) and on the dynamics of the ecosystems [16,17]. Any modifications of these environmental conditions can induce adaptive modifications in the structural (taxonomic composition and biomass) and functional (photosynthetic activity and heterotrophic activity) characteristics of periphyton [18] resulting in modifications on the entire aquatic ecosystem.

2. Materials and methods

We used two indoor, flow-through, experimental streams that were 6 m long and 15 cm wide (DELTALAB). We regulated the current velocity in the first experimental stream at $12\,\mathrm{cm\,s^{-1}}$ (slow) and the water depth at 3.4 cm. The gradient of the second experimental stream (3.5‰) was set to achieve an average velocity of $50\,\mathrm{cm\,s^{-1}}$ (fast) and a depth of 2.4 cm. The water supply for the experimental streams was filtered ground water. Artificial lighting (Sylvania Gro-Lux, F58 Wigro) was used to reproduce natural light conditions as much as possible (luminosity: approximately $60\,\mu\mathrm{mol\,m^{-2}\,s^{-1}}$, light/dark cycle: 16 h of daylight and 8 h of night).

Road runoff samples came from storage settling basins of the national road 346 (motorway in Lyon, France). Road runoff was discharged into the basin from water tight drainage outlets, which drained two lanes over a distance of 450 m, i.e. a 5.5 ha road drainage basin. The average daily traffic at the study sites varied from 34,300 vehicles to 53,900 vehicles on the busiest days.

Road runoff samples were collected during rainfall, kept in glass flasks, and then characterised by a series of physical and chemical analyses: pH, electrical conductivity (cond.), total suspended solids (TSS), and chemical oxygen demand (COD) were measured in unfiltered raw water. The concentrations of various anions and cations (NO_3^-, SO_4^{2-}, Cl^-) and the total alkalinity (TA) of filtered (0.45 μm pore size) raw water were measured (Ionic Chromatrograph DIONEX DX100); the amounts of heavy metals (Pb, Zn, Cd, and Cu) in filtered water were measured (Atomic Absorption Spectrometer HITACHI). Furthermore, the climatic parameters were recorded when each sample was taken (duration of dry spell, duration and intensity of rainfall, and total rainfall).

Frosted glass slides were used as a substrate for periphyton (2.4 cm × 7.8 cm × 0.4 cm). This technique permitted the standardisation of communities development conditions and reduced the natural variability of the substratum, so that the effects of the road runoff could be isolated [19]. Their colonisation was assured by an inoculum composed of stones taken from a small river near to the laboratory, and placed in the upstream part of each experimental stream. The river had similar physical and chemical characteristics to the ground water supplying our experimental model. Substratum was mainly composed of sands and stones. Runs were the dominant physical structure, and average current velocity was less than $25\,\mathrm{cm\,s^{-1}}$. Fresh substrates were submerged at the beginning of the experiment to evaluate the effect of rainwater runoff on the growth dynamics of periphyton.

Two experiments were carried out: the first one between 20 June and 21 July 1997 (Experiment 1) and the second between 28 August and 25 September 1997 (Experiment 2). Road runoff were sampled before each experiment and kept at 4 °C in the dark. During previous studies, we had noted that the physical and chemical composition of runoff changed very little on storage in the fridge. Thus, during this study, we controlled only conductivity, which had remained stable during the duration of the two experiments. Thus, an aliquot could be taken at regular intervals and be exposed to the biofilms (three exposures per week). Runoff were not poured directly into the experimental stream because this would have required too large volumes (hundreds of cubic meter). Artificial substrates were delicately removed from experimental rivers and placed in different glass crystallising dishes (300 cm in diameter, 15 cm in height) containing runoff (2 l in volume). In order to ovoid biofilm pulling out, stream flows were stopped while moving the slides. For each communities (fast and slow streams), we used two dilutions (D10: 10% dilution and D50: 50% dilution) and two contact times with the runoff water for each dilution (1 and 4 h). The first contact time corresponded to runoff, which could occur after a storm, and the second after a lower intensity and longer duration rainfall. Usually, these two rainfall types carry most of the pollutants accumulated on the road [6]. At the end of the exposure, artificial substrates were replaced in their experimental streams. Each set of experimental conditions was used in triplicate (three glass slides) and compared to controls that were placed in crystallising dishes containing no road runoff. The conditions of temperature and illumination in the crystallising dishes were the same ones as those in the rivers. The agitation of water in the crystallising dishes was ensured by a magnetic stirrer (a weak agitation for lentic community and one stronger for lotic community).

The periphyton were characterised using two structural and two functional variables:

- The ash free dry weight (AFDW) was taken as an indicating parameter of the total biomass community (autotrophic and heterotrophic organisms) and chlorophyll *a* with phaeopigments as representative parameters of algal biomass. Periphyton were collected by brushing the slide surface in a known volume of water from the stream. Aliquots of the solution were filtered (Whatman GF/C glass fibre filters) for measurement of chlorophyll *a* and biomass community. For analyses of chlorophyll *a*, filters were immersed for 12 h in 90% acetone and the absorbance of the extracts was read in a spectrophotometer [20]. This method also allows phaeopigments analysis, which correspond to chlorophyll degradation products. Biomass community (AFDW) was measured as the difference in weight between filters dried at 60 °C for 24 h (dry weight: DW) and combusted at 500 °C for 2 h.
- Net primary production (NPP) and respiration (R) were measured as functional variables to represent community autotrophic and heterotrophic activity. The light and dark bottle oxygen method is as described by Ref. [21]. Every slide sample was delicately placed in airtight Pyrex flasks (BOD flasks) filled with stream water. Initial dissolved oxygen levels in each flask were measured (WTW oxymeter Oxi 538, Stir-

rOx G probe), then the flasks were sealed airtight and placed in a thermostat bath adjusted to stream water temperature. Lighting was provided by two florescent Mazda Fluor Presiflux tubes giving approximately 60 μE m^{-2} s^{-1} of light. After 2 h of incubation, dissolved oxygen content was measured again. The net primary production was estimated through the difference with initial oxygen content. The flasks were resealed and wrapped in aluminium foil for artificial darkness then incubated for a 2 h incubation in the bath after which a final measurement of dissolved oxygen content was taken. Oxygen consumed by respiration was determined by the difference with the second measurement. The results were expressed in quantity of gas produced (NPP) or consumed (R) per unit of time and surface of biofilm (mg O_2 m^{-2} h^{-1}).

Various ratios were calculated from these parameters [22]:

- AFDW/DW as a measure of the extent of the biotic component compared to the abiotic material deposits within the periphyton mat.
- Phaeopigments/chlorophyll *a* as a measure of the proportion of dead or dying algae cells within the biofilm.
- Autotrophic Index (AFDW/chlorophyll *a*) used in determining the relationship between the biomass of the autotrophic (algae) and the heterotrophic (bacteria and organic matter) elements of the community.
- Gross primary production/respiration (GPP/R) characterised metabolic activity of the community ($GPP/R < 1$: heterotrophic community; $GPP/R > 1$: autotrophic community) [21].

The aim of this study was to uncover the main and interaction effects of categorical independent variables (exposure conditions: dilution, time exposure, current velocity) on an interval dependent variables (structural and functional parameters). The number of acquired results from each experiment and artificial stream (several measurement dates, two contact times, three dilutions, and three replicates) allowed us to carry out statistical analyses on each set of data. An analysis of variance (ANOVA) was used. The means of the groups formed by values of the independent variables differed significantly if $p \leq 0.05$ (Fisher test). Differences with $0.05 \leq p \leq 0.10$ were considered marginally significant.

3. Results

3.1. Results of the physicochemical analysis

The rain runoff in the first experiment contained low concentrations of mineral and organic elements (Table 1). Conversely, in the second experiment the rainwater was characterised by an acid pH and high levels of organic matter (COD) and heavy metals. Road runoff TSS, COD, and heavy metals concentrations were larger than in groundwater (Table 1). Conversely, conductivity and nitrate concentrations are lower. Runoff discharged into the storage settle basins presented high variability (Table 1). The values of the second experiment were generally

Table 1
Physical and chemical characteristics of the groundwater supply for the experimental streams (from June to September1997)

	pH	Cond. (μS cm^{-1})	TSS (mg l^{-1})	COD (mg O_2 l^{-1})	NO_3^- (mg l^{-1})	Cl^- (mg l^{-1})	SO_4^{3-} (mg l^{-1})	Pb^{2+} (μg l^{-1})	Zn^{2+} (μg l^{-1})	Cu^{2+} (μg l^{-1})
Groundwater										
Mean	7.3	490	<1	0.8	12.2	16.1	31	<1	<1	<1
Standard deviation	0.1	5	–	0.05	3.9	1.9	2.9	–	–	–
Road runoff										
Experiment 1	7.2	198	20	76	3.5	13	23	4.5	60	39.5
Experiment 2	5.5	236	50	345	6.6	20	41	10	800	127
Storage settling basins										
Mean	7.3	244	141	164	6.0	22	24	2,1	153	49.1
Standard deviation	0.7	134	154	155	6.5	20	14	3,5	245	36.5
Maximum	7.9	532	421	545	22.6	68	54	10	800	127
Minimum	5.5	92	18	47	1.4	3	5	0	40	11

The road runoff sampled for experiments (Experiment 1: 13 June 1997; Experiment 2: 17 July 1997), and road runoff discharged into the storage settling basins (from April to September1997—nine samples). Cond., conductivity; TSS, total suspend solid; COD, chemical oxygen demand.

situated within a range comprising of between the average (conductivity, NO_3^-, Cl^-) and the greatest values (COD, Pb, Zn, and Cu) observed from April to September 1997 (COD, Pb, Zn, and Cu).

In experimental stream 1 (slow current velocity), the measurements were stopped on days 20 (first experiment) and 26 (second experiment) of the colonisation due to the development of filamentous algae, which became detached when the glass slides were manipulated. In experimental stream 2 (fast current velocity), the stationary phase of growth was reached later due to the more restrictive hydraulic condition. Therefore, the measurements were stopped on days 31 (first experiment) and 29 (second experiment).

3.2. Impacts on the periphyton

The biomass of periphyton, expressed as ash free dry mass, was comprised of between 0.92 and 2.83 g m^{-2} (Fig. 1). During the first experiment, it was generally greater in the artificial stream having a faster current velocity ($p = 0.0004$), but it was not influenced by the road runoff ($p = 0.3052$). In September (test 2), biomass was generally greater in the experimental stream having a lower current velocity ($p = 0.0001$), but the effect of road runoff was at the limit of being statistically significant ($p = 0.0531$).

The proportion of biotic materials within the periphyton (AFDW/DW) was comprised of between 0.63 and 0.91 (Fig. 1). Overall, the weakest values were observed where biofilms were submitted to the longest durations of exposure (test 1: $p = 0.0022$; test 2: $p = 0.0419$). Similarly, in the experimental stream having the slowest velocity (12 cm s^{-1}), AFDW/DW was significantly weaker when the biofilms were submitted to road runoff diluted by 50% (test 1: $p = 0.0007$; test 2: $p = 0.0358$).

The algal biomass, expressed in chlorophyll *a* (Fig. 2), was comprised of between 24.8 and 78.9 mg m^{-2} during the first experiment, then between 6.8 and 35.9 mg m^{-2} in the second. Its response in relation to the exposure conditions was identical to that of the total biomass (AFDW). Nevertheless, during the second experiment, contrary to the previous parameters, the values of chlorophyll *a* were generally greater in the biofilms growing in the artificial stream having the fastest current speed ($p = 0.0032$).

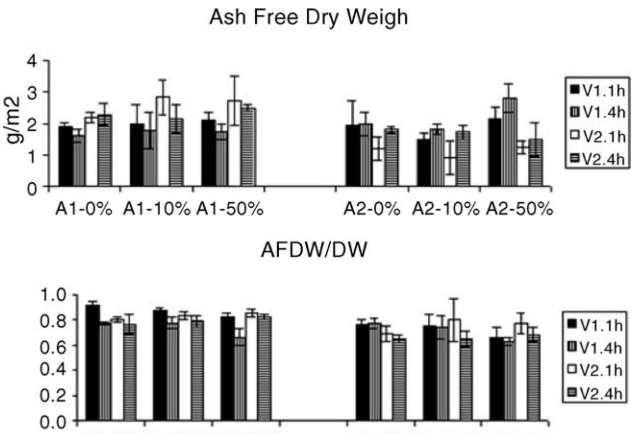

Fig. 1. Mean ash free dry weight (AFDW) and ash proportion (AFDW/DW) in periphyton at the end of the Experiment 1 (A1) and Experiment 2 (A2), as determined in artificial streams with low current velocity (V_1) and fast current velocity (V_2), remaining at different dilutions of road runoff (0%: control reference, 10%: dilution 10%, 50%: dilution 50%) after 1 and 4 h exposures (three exposures per week). Bars represented the standard deviation.

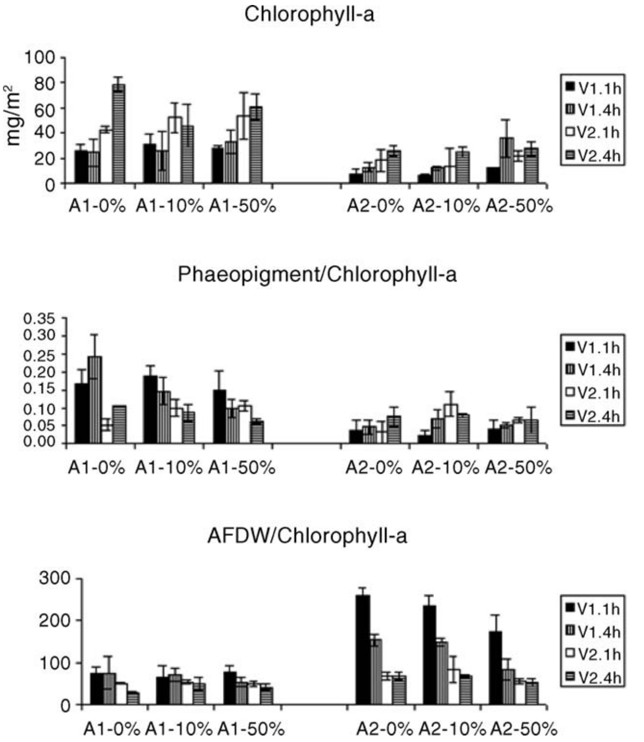

Fig. 2. Mean chlorophyll *a*, phaeopigment proportion (phaeopigment/chlorophyll *a*) and Autotrophic Index (AFDW/chlorophyll *a*) in periphyton at the end of the Experiment 1 (A1) and Experiment 2 (A2), as determined in artificial streams with low current velocity (V_1) and fast current velocity (V_2), remaining at different dilutions of road runoff (0%: control reference, 10%: dilution 10%, 50%: dilution 50%) after 1 and 4 h exposures (three exposures per week). Bars represented the standard deviation.

The proportion phaeopigments/chlorophyll *a* was comprised of between 0.02 and 0.24 (Fig. 2). The weakest values were observed during the second experiment (0.02–0.11), and more particularly in the experimental stream having the slowest current velocity ($p = 0.003$). Inversely, during the first experiment, the proportion was smaller in the artificial stream having the fastest velocity (50 cm s^{-1}) when the biofilms where submitted to road runoff diluted by 50% ($p = 0.0260$).

In July (test 1), the road runoff did not have any effect on the structure of the biofilm, the values of the Autotrophic Index (AFDW/chlorophyll *a*) being comparable ($p = 0.7699$). These were comprised between 29 and 77 (Fig. 2), but the strongest values were observed in the experimental stream having the slowest velocity ($p = 0.0003$). During the second experiment, the difference between the two experimental streams was much more noticeable, the index reading values comprised between 84 and 259 in the experimental stream having the slowest current speed. However, the proportion was significantly weaker when the biofilms were submitted to road runoff diluted by 50% ($p = 0.0001$).

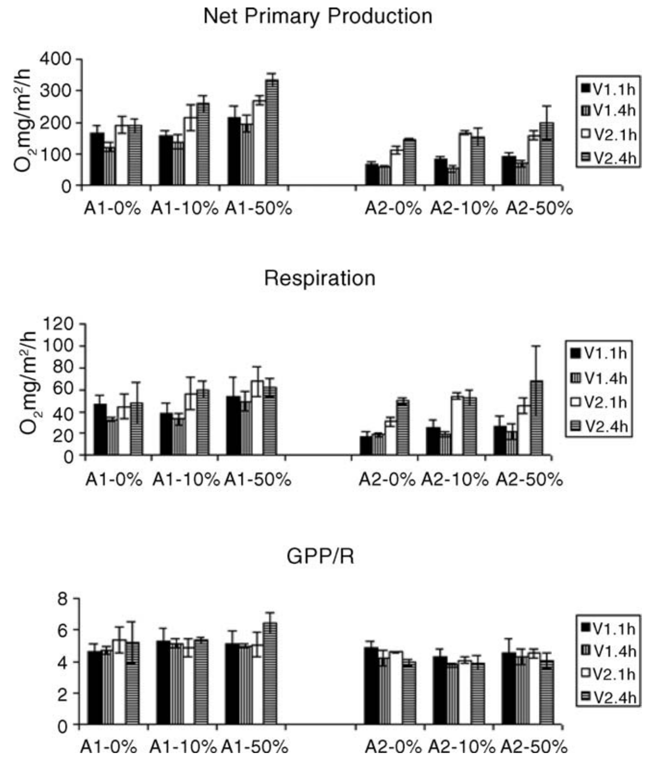

Fig. 3. Mean net primary production (NPP), respiration (R) and the gross primary production/respiration ratio (GPP/R) in periphyton at the end of the Experiment 1 (A1) and Experiment 2 (A2), as determined in artificial streams with low current velocity (V_1) and fast current velocity (V_2), remaining at different dilutions of road runoff (0%: control reference, 10%: dilution 10%, 50%: dilution 50%) after 1 and 4 h exposures (three exposures per week). Bars represented the standard deviation.

The net primary production of periphytic communities was comprised of between 61 and 334 mg O_2 m^{-2} h^{-1} (Fig. 3). During the two experiments, it was significantly greater in the experimental stream having the fastest velocity ($p = 0.0001$). Similarly, whatever the dilution, the net primary production increased significantly when the biofilm was submitted to road runoff (test 1: $p = 0.0001$; test 2: $p = 0.0022$). Nevertheless, the duration of exposure did not influence the response (test 1: $p = 0.62$; test 2: $p = 0.88$).

The respiration, R of the periphyton varied in a way comparable to the net primary production in relation to the conditions of exposure. The values were comprised of between 17.2 and 68.3 mg O_2 m^{-2} h^{-1} (Fig. 3), with as previously stronger values during the first experiment. However, the proportion of GPP/R was not modified by the tested conditions of exposure (Fig. 3) and is comprised of between 3.8 and 6.4.

4. Discussion

The rain runoff mineral and organic matters in the first experiment were low compared to the average values normally recorded [4,5,23]. In the second experiment, values were generally situated within a range comprised between the average (conductivity, TSS, COD, NO_3^-, Cl^-, and SO_4^{3-}) and the greatest values (Zn and Cu) generally observed. This probably results from a longer dry-weather duration before the discharge (16 days) than that corresponding to the first experiment (1 day). Thus, accumulated pollution on the road surface was more important.

The road runoff small exposure conditions could cause an increase in biomass (AFDW, chlorophyll) of the periphyton. This response could be noticed when the communities were submitted to rain runoff that was the most concentrated in organic and mineral matter, the least diluted (50%) and subjected to the longest periods of exposure (4 h). The notable decrease of the Autotrophic Index (AFDW/chlorophyll *a*) indicated that these road runoff exposure conditions could stimulate preferentially the autotrophic organisms of the biofilm [22]. The photosynthetic activity of biofilms constituted a more sensitive indicator to disturbance. The net primary production and respiration were affected by road discharge whatever their concentration in pollutants (Experiments 1 and 2) and their level of dilution (10% and 50%). This result confirms the importance of using conjointly of structural and functional parameters of the periphyton in order to evaluate the effects of a disturbance [18,24,25]. The stimulation of microorganisms by road runoff water has already been shown in several studies. By observing the effects of winter salting of roads, Gjessing et al. [26] observed that the degradation of organic matter (BOD) by bacteria was increased two-fold when the rainwater was diluted by 20% and three-fold at a 90% dilution. They also observed a slight growth stimulation of two green algae (*Selenastrum capricornutum* and *Synedra acus*) at high rainwater dilutions (50%), although few effects were observed at a weak dilution (10%). Furthermore, in seven English rivers Maltby et al. [9] observed a slight increase in the diversity and biomass of chlorophyll in the benthic algae downstream of motorway runoff.

Many substances present in runoff water can stimulate the growth of benthic microorganisms, such as weak concentrations of hydrocarbons [27,28]. Conversely, the presence of heavy metals in rainwater can be inhibitory. Only the levels of copper in Experiment 2 (runoff and D50 dilution) were greater than the EC 50–96 h of the green algae *Chlorella vulgaris* [29]. However, we did not observe a metabolic activity decrease on our periphytic community. The mode and time of exposure used in our experiments (dynamic conditions, length of exposure: 1–4 h) were different to those used in the ecotoxicological experiments (static conditions, length of exposure: 24–96 h). The periods of exposure were low and the pollutants did not have time to penetrate deeply in the biofilm [30]. The experiments were carried out on a community most probably made up of organisms with differing sensitivities to pollutants. Furthermore, the chemical stress could modify the taxonomic composition of algal populations, causing a reduction in the number of sensitive species and an increase in the number of tolerant species [25,30–32]. However, a chemical stress does not automatically induce structural and/or functional modifications, as numerous regulatory physiological processes may be produced [25,33,34]. For example, micro-organisms may synthesize chelates which decrease the bioavailability of heavy metals and in consequence of their toxicity [19,31,32].

Our results showed that the effect of road runoff can be relatively limited and may not cause an important increase in biomass. Chlorophyll remained inferior to the threshold value of 150 mg l^{-1} from which algae generate harmful effects [35]. Road runoff disturbances can be compared with those of regular floods which periodically and temporarily increase the concentration of organic and mineral elements in the water [36,37]. Peterson et al. [38] showed that short-term enrichments could stimulate the growth of periphyton. However, the effect is clearer when the biofilms are thin (lotic areas), in this case a high concentration in nutrients is not required to stimulate growth. When the biofilms are thick, the increase in growth remains low, because only the cells in the outer layers have access to the nutrients. Furthermore, an exposure time of four hours corresponds to one or two cell divisions, which is not enough time for the cells to store enough nutrients for future generations and to allow a large increase in biomass. In this case, the effect is clearer when the enrichment lasts longer [38].

Generally the biomass and photosynthetic activity were greater in the experimental stream having higher current velocity (50 cm s^{-1}). However, during the second experiment the biomass (AFDW) were greater in the slow experimental stream (12 cm s^{-1}). This response could be attributed to sedimentation of suspended materials of road runoff. The effluent was more loaded with suspended solids (TSS: 50 mg l^{-1}) and the highest biomass were noticed where the exposure conditions were the most favourable: greatest concentration of effluent (50%) and longest duration of exposure (4 h). Moreover, the weakest values of AFDW/DW were obtained (63%) demonstrating that was essentially mineral particles which had deposited sediment and/or were trapped in the biofilm [39]. This accumulation of mineral particles was equally highlighted by the value of Autotrophic Index. The highest values (AI: 84–259) were obtained in these exposure conditions, revealing a lesser dominance of the autotrophic community in favour of the heterotrophic and/or detritic component [40]. However, the respiration of the community (R) not being more important in these conditions, comforts the hypothesis of the trapping in the biofilm of detritic materials of essentially mineral origin emanating from road runoff [41].

Many other factors can modify the effect of a pollutant and condition an organism's response: the characteristics and properties of the runoff (concentration, duration, amplitude, and frequency) [15], the physical and chemical characteristics of the water in the receiving ecosystem (pH, hardness, temperature, TSS), the physical characteristics of the pollutants, the synergetic, and antagonistic effects of other pollutants [7,42,43], the time of year [44]. For example, on the A11 motorway, Legret et al. [23] observed that more than 50% of heavy metals and nearly 40% of hydrocarbons are in fraction with a diameter of less than 250 μm, which is equivalent of 18% of suspended matter. As a consequence, it is probable that in our case the absence of any inhibition effect was partly due to the fixation of pollutants on the suspended particles in the runoff, which reduces their bioavailability and their toxicity [26,45].

Our experimental streams constituted a simplified natural system. Particularly, flow regime and substrata (glass slides) were less heterogeneous, and they could under-predict periphyton responses to runoff discharges. Consequently, any extrapolation of these results to whole aquatic systems should be made with care.

5. Conclusions

Our experiments, in experimental streams and in controlled conditions, showed that the biomass and the photosynthetic activity of periphytic biofilms could increased when they were exposed periodically to road runoff. This effect could be noticed more especially when the runoff was only slightly diluted and when the exposure duration with the pollutants was longest. This result has already been shown in a few studies observing the stimulation of microorganisms by road runoff water. Conversely, other studies also have assessed alterations of aquatic communities (the absence of inhibitory effects may be because our experiments were not carried out in the most extreme conditions, the dilution of the water never exceeded 50%). However, rare were these studies carried out in an indoor artificial stream. Indeed, our experimental streams constituted a simplified natural system, which did not allow the reproduction of all the environmental conditions, found in the natural environment. Some important mechanisms can be minimised or even become negligible, such as transfer, dispersion, retention, and transformation processes that affect the pollutants in the different compartments of an ecosystem (sediments, trophic networks, . . .). These processes may influence the communities response, and our experimental system did not allow us to evaluate them. Maltby et al. [34] was able to demonstrate the extent of these phenomena in the response of biocenoses exposed to rainwater runoff. Furthermore, our experiments did not allow the reconstitution of any mechanical shock due to the sudden increase in flow following runoff. Thus, these experiments should be performed on other sites (experimental and/or natural) where all the conditions will be correct for the runoff to have a major impact on the natural environment (good quality rivers and low flow, much traffic, low dilution factor for the runoff, etc.). This would then allow a better forecast of impact on less disturbed situations.

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