The role of algae in travertine deposition in Huanglong National Park Sichuan Province, China

Preliminary Report & Proposal for Further Investigations

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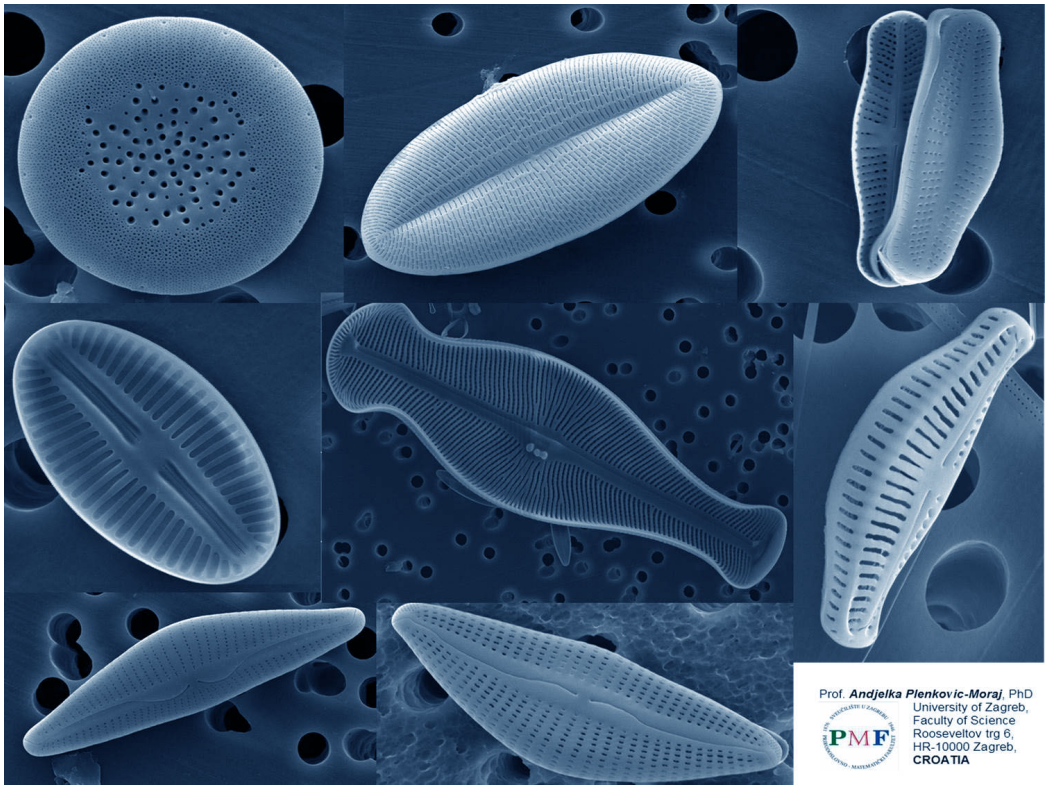

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My special thanks are extended to the Chinese Academy of Science President's International Fellowship Initiative (PIFI) for visiting scientist in 2015 and 2016 for supporting the projects: *Diatoms - Ecological Status Indicators of Jiuzhaigou Valley* and *Applied Use of Cyanobacteria and Green Algae from Jiuzhaigou Valley as Indicators of Water Quality and Ecological Status: Sampling, Analysis and Ecological Remarks,* respectively.

I would like to express my very special and great appreciation to Dr. Sun Geng for his valuable and constructive recommendations during the planning and development of the projects, theirs applications and fieldwork research. His willingness to give his time so generously has been very much appreciated.

I would also like to thank the authority staffs of the Chengdu Institute of Biology, Chinese Academy of Science and Huanglong National Park for enabling me to visit their offices, to observe their daily operations and for their professional guidance and valuable support.

The scope and purpose of visiting

According to observed strong development of green clusters in aquatic biota as well as the black biofilms on travertine barriers we were inviting to visit Huanglong in 2015. The purpose was to provide useful information, especially about freshwater algae, their ecology and biodiversity in addition to their significant management role in ecological valorization of water resources and travertine deposition. This report is explicitly preliminary in approach, largely because of the enormous scope of the issues involved, but striving to be geographically comprehensive and to use existing data and expertise to the best advantage.

Introduction

In recent decades, a significant effort has been put forth all over the world to assess water quality, attending to not only to chemical parameters (nutrients, metals, pesticides, etc.), which are obviously important, but also to biological indicators. In fact, one of the undesirable consequences of pollutants is their effect on the biota. In this case, the study directed toward the effects of pollution on biota is of great interest. In this context, the European Water Framework Directive (2000) uses different biological indicators to determine the ecological status of a water body (macroinvertebrates, aquatic plants, and algae). The ecological information given by diatoms and other algal groups are usually summed up through one or more algal-based indexes, which indicate the trophic level with a single number. However, the indexes alone do not determine the water quality. Moreover, the water quality is not the only factor that determines the ecological status. Other factors such as the structure of the vegetation or the hydrodynamics of the ecosystem must also be taken into account. The sensitivity and tolerance of diatoms to a number of environmental characteristics (eutrophication, organic pollution, heavy metals, salinity, pH and pesticides) are known to differ among species. These species-specific sensitivities and tolerances can be used to infer environmental conditions in a habitat. Algae are regularly used as indicators of water quality for several reasons: they are easy to collect using well-established sampling techniques, a significant number of algal species are ubiquitous, have short generation time (one to several days) and their nutrient uptake directly from the water column allows them to act as initial indicators of the impacts of changing nutrient conditions on freshwater ecosystems. Algae respond (somewhat) predictably to changes in the aquatic systems, and are phenotypically expressed in exceptionally diverse forms with a wide range of sensitivities to water quality changes. Therefore, any short- and long-term environmental changes in freshwater ecosystems will be reflected in the composition and biomass of the algal community.

Preliminary results

The conclusions given below are of a preliminary nature, based on single surveys and comparisons with similar systems abroad, specifically with Croatians NP Plitvice Lakes and NP Krka. Under the guide of the Park personnel, the samples of travertine deposit were collected along the passes organized for visitors, which were subsequently microscopically analyzed and photo-documented. In addition, short cores were obtained for later more detailed analyses. The initial microscopic investigation has been performed at the Chengdu Institute of Biology CAS and subsequently at the University of Zagreb, Faculty of Science, Croatia.

The Huanglong system is characterized by carbonate supersaturation of the ambient waters, which contain elevated partial pressure of CO_2. It includes intense and more crystalline carbonate deposition over wide slopes, combined with series of semilunar barriers holding smaller and deeper ponds. The carbonate deposition system appears to be more closely guided by physicochemical processes, whereas the biological component is reduced to critical stages in the development and cyclic renovation of the process. The deposition of carbonate ensues due to exchange of carbon dioxide between the water and the atmosphere. Such exchange is promoted by the increase in surface-to-volume ratio in foaming waters passing through rapids. Further removal of CO_2 from the water is accomplished by photosynthetic activity of cyanobacteria, algae and mosses. The principal role of these organisms, however, is in trapping, binding of carbonate particles accompanied by local precipitation of carbonate that cements and consolidates the deposit.

The composition of dark streaks not covered by flowing water was analyzed and typical biofilm of epilithic cyanobacteria were found. The biofilm was composed predominantly by coccoid cyanobacteria *Gloeocapsa spp.* (Fig. 1), *Schizothrix* sp. (Fig. 2), and filamentous heterocystous cyanobacteria *Calothrix* sp.*, Scytonema wolleanum* (Fig. 3) and *Dichothrix* sp. It is a natural overgrowth over the carbonate crust that remains wet by capillarity after the water overflow changes its path. Dark color originates from the UV-protecting pigments gloeocapsin and scytonemin excreted by cyanobacterial cells into their extracellular gelatinous envelopes (EPS).

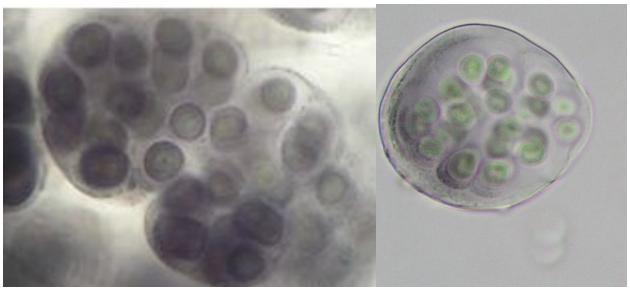

Figure 1 *Gloeocapsa* sp.

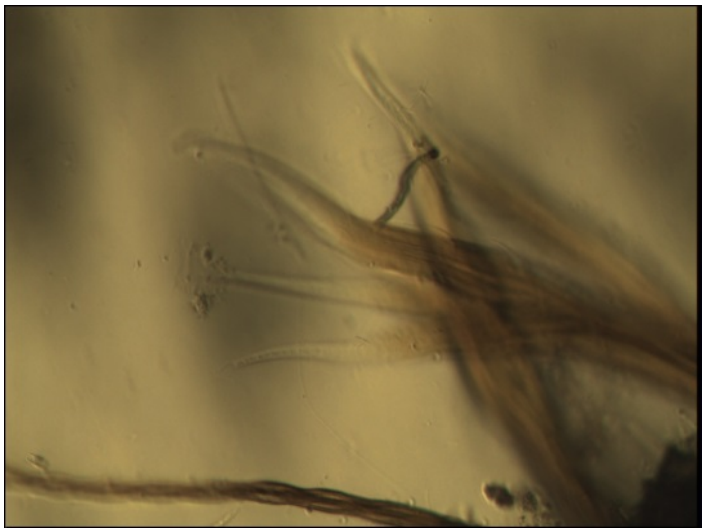

Figure 2 *Schizothrix* sp.

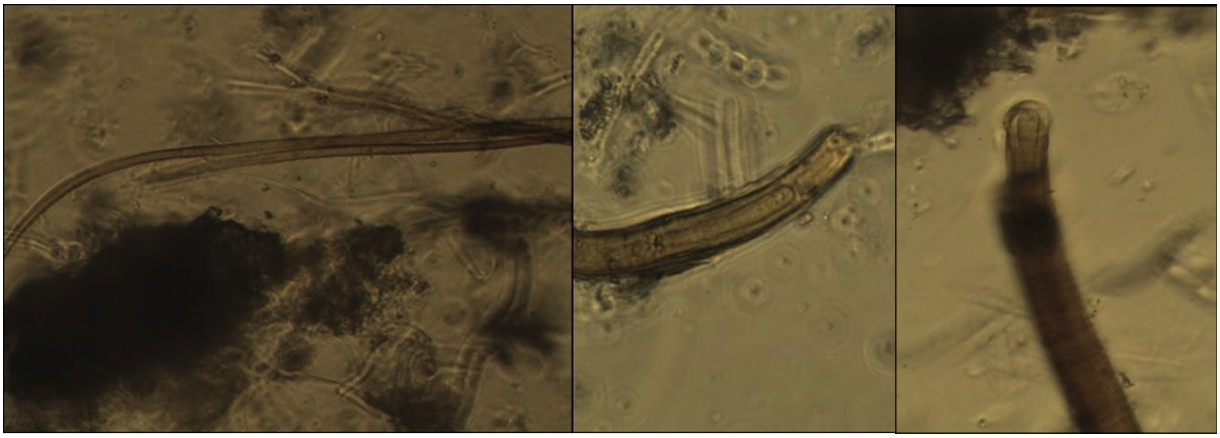

Figure 3 *Scytonema wolleanum* Forti

The subsequent analysis showed that the active deposition in light colored streaks is predominantly mineral. Dissolving the carbonate crust by dilute hydrochloric acid left a loose organic network (EPS) with embedded diatom frustules (Fig. 4). This organic matrix was gradually displaced by growing calcite crystals.

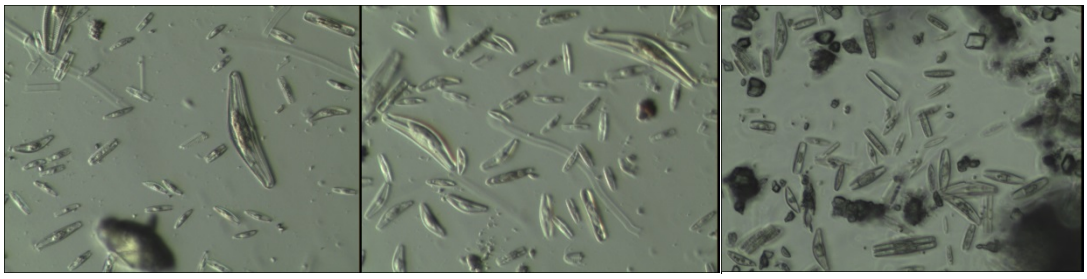

Figure 3 Diatom frustules

On upper lakes green color of water was caused by enormous number of filamentous green algae especially group Conjugatophyceae (Fig. 4). It is well known that huge number of green algae indicates on higher nutrient conditions in the aquatic ecosystems, especially in karst area. Among this group the species from genus Spirogyra (Fig. 5.) and Zygnema (Fig. 6) were predominant. Also a large number of diatoms, especially genus Cymbella (Fig. 7) were found too.

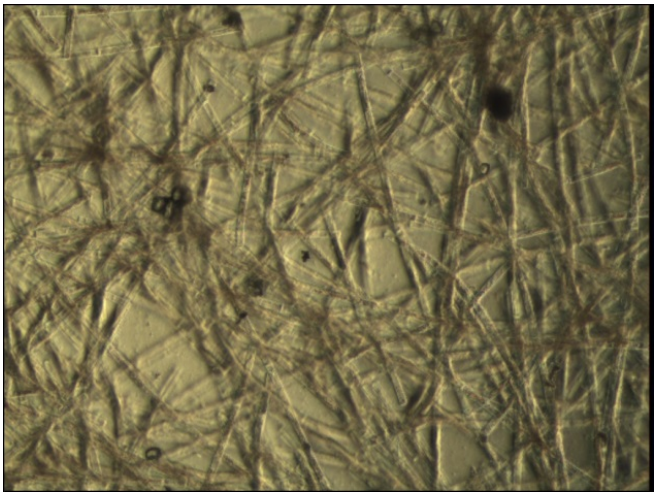

Figure 4: filamentous green algae from group Conjugatophyceae (lower magnification)

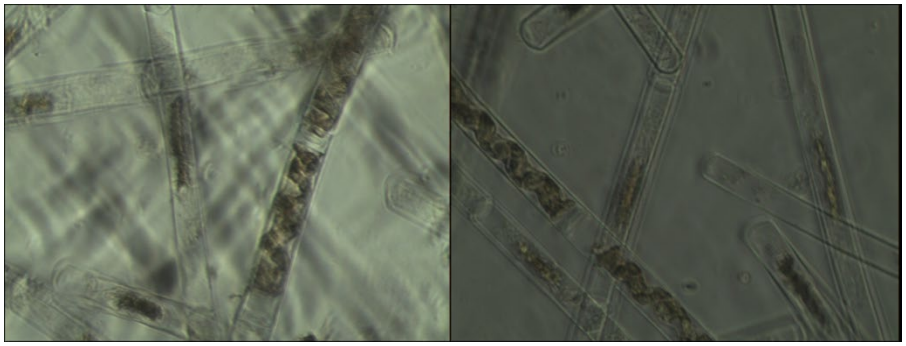

Figure 5: Filamentous green algae *Spirogyra* sp.

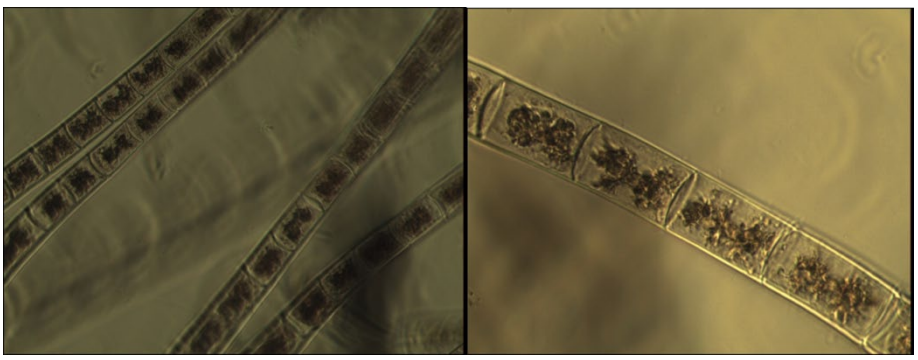

Figure 6: Filamentous green algae *Zygnema* sp.

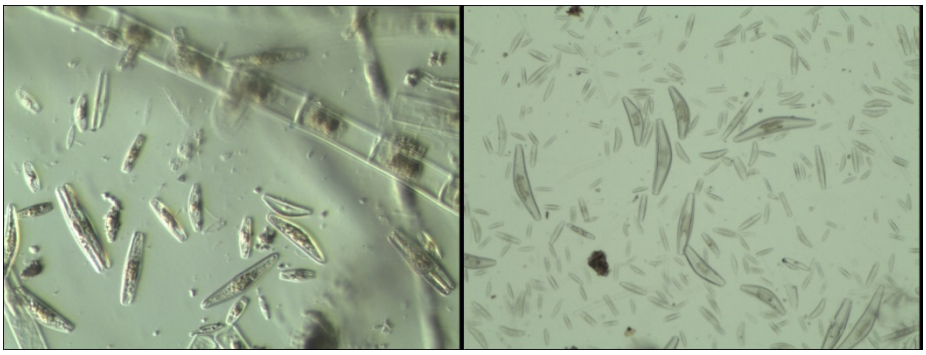

Figure 7: Huge number of diatom especially genus Cymbella

Proposed Future Research

The aim

The aim of further research will be to provide a firm foundation for subsequent, more detailed assessments of a wider usage of algae in ecological valorization of water resources, education of personnel in accordance with a long-term strategy for development of investigations and environmental protection.

Within this general context, the further investigations will have several principal objectives:

1. The main objective will be to determinate possible correlations between the physical and chemical water parameters and abundance of algae in phytoplankton and benthos/periphyton communities in order to establish useful indices in water quality management and their role in travertine deposits.
2. To produce a preliminary ecological status of water quality assessment according to diatoms and other groups of algae in phytoplankton and periphyton communities, with the aim to strengthen ecological valorization of Huanglong water quality management.
3. To develop a stronger framework of co-operation between China and Croatia and to be more effective in future investigations of monitoring techniques based on the ecosystem services
4. To present a global overview (Check list of algal species) of freshwater algae biodiversity in Huanglong.

Materials and Methods

Phytoplankton, periphyton and phytobenthos samples will be collected according the Protocols given below and suggested Timetable (Table 1.)

Table 1:

Time table

黄龙

Samples of phytolankton (the water from the lake) take at June 4 and June 25, 2016
Periphyton samples from several locations pick up each time you are picking up glass slide
May 7, 2016 - bricks with glass slides will be placed in water

No. of days - exposiure of glass slides	Date of picking up glass slides	No. of botlles
7	May 14	3
14	May 21	3
21	May 28	3
28	June 4	3
35	June 11	3
42	June 18	3
49	June 25	21
Total number of samples from glass slides		**39**

Periphyton samples from exposed artificial substrates – glass slides (Fig. 8) will be collected at intervals of every 7 days with replicates from 7th of May, until the 25th of June 2016.

Figure 8: Experimental artificial plates (bricks)

In situ specimens will be preserved by the addition of 4% formaldehyde. The identification of diatom species will be performed with a light microscope Leicka DMLB at Chengdu Institute of Biology, Chinese Academy of Science. The algae species will be identified to the lowest taxonomical level according to relevant literature. For the purpose of quantitative estimation of phytoplankton and periphyton, the cells of each algal species will be counted on a millimeter grid with an area of 1 cm^2 and a volume of 0.05 ml. The relative abundance of taxa within the algal community will be determined by the valve percentage representation of each taxon relative to 400 numbered valve at every permanent slide. Three different microhabitats for tracking algal colonization will be defined. Artificial substrates (glass slides) will be placed

horizontally and oriented parallel to the current flow, 10 cm beneath the water surface. The microhabitats will be made of seven glass slides that will be fixed on the upper side of a brick.

The diatom-based assessment of the ecological status of NP Huanglong will be based on a multimetric approach and includes the Trophic Status Module, which assesses nutrient load and The Saprobic Status Module, which assesses organic load. Computer software OMNIDIA 5.1 and Cro-D will be used to calculate diatom indices, according to their abundance, in order to determine the water quality. Computer program PRIMER 6.1.10 for Windows will be utilized for calculation of species richness, evenness, and diversity indices, and for statistical data analyses (PCA, CAP) with pertaining figures. Graphical displays will be made using Microsoft Office Excel 2007, Microsoft Word 2007 and Photoshop 10.0. Species will be photographed by using AxioCam MRC camera (Carl Zeiss Microscopy GmbH, 2006). The photographs will be processed with digital image processing software AxioVision Rel. 4.6.3. For SEM microphotographs the scanning microscope Tescan Mira 3 will be used.

General notes for each field work
BE SURE to bring or care about:

- Recommended Timetable
- A field notebook
- Rubber boots
- Plastic bucket (marked with a quantity of 5 L of water) or plastic vessels (marked with a quantity of 1 L of water) that on the field you can filtered 10 L of lake water (only for phytoplankton samples)
- Microscopic glass slides to replace (only for periphyton)
- Brush or knife for scraping phytobenthos from rocks or aquatic plants
- Plastic bottles for storing of microscopic glass slides (only for periphyton)
- Plastic bottles for storing plankton
- Plastic bottles for storing the benthic organisms
- **Important:** in note book you are able to write with pen but on the sampled bottles **PLEASE WRITE WITH LEAD PENCIL**
- When you will return from the field **DO NOT FORGET** and **BE SURE** to fix all samples. Put into each vial with sample 15-20 drops of formaldehyde
- All of the collected samples stored in a cold place (refrigerator)

Protocol for collecting plankton samples

Plankton = the small and microscopic organisms drifting or floating in the sea or fresh water.

- Samples of phytoplankton (the water from the lake) take at June 4 and June 25, 2016
- In the field notebook **REQUIRED to WRITE** the number of the sample, date and place of sampling.
- On the bottle, in which the sample will be saved, also COMPULSORY to WRITE the number of the sample, date and place of sampling.
- 10 L of lake water filtered through a phytoplankton net like on the figure 1

Figure 1: Filtering

- After filtration of 10 liters pond water, add to the filtrate on phytoplankton net some lake water. Shaken it several times as shown in Figure 2

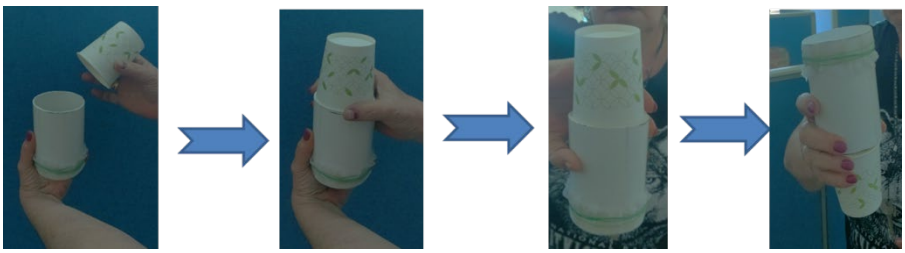

Figure 2: How to shake phytoplankton samples

- The sample of phytoplankton from plastic cup saves **into properly marked bottle** as in Figure 3.

Figure 3: Saving the phytoplankton sampling

- **Do not forget the way back from the field to fix samples with formaldehyde.**

Protocol for collecting the glass slides

- Microscope glass slides collect **according to timetable**
- Make sure **to pick up the slides in right order** (from left to right) as shown in Figure 4

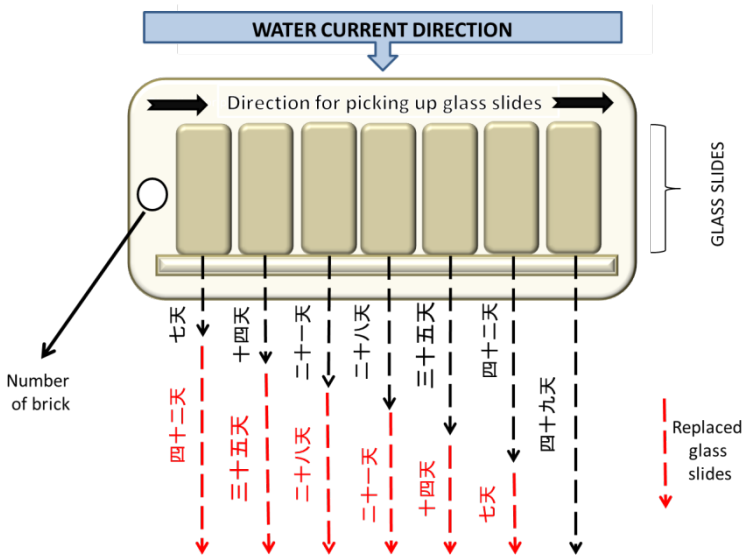

Figure 4: Sampling glass slides from the brick

- Each time during collecting the glass slides **REQUIRED to WRITE** in the field notebook: a number of the brick, date of sampling, and the number of days of glass slides exposure
- On the bottle, in which will be glass slides stored, also **OBLIGATORY TO WRITE** the number of the brick, date of sampling, and the number of days of exposure of glass slides
- Make sure that the surface of the slides on which is the periphyton (top side of glass) do not accidentally delete by your fingers. Try to gently push the slide out of the slot.
- In the bottle for saving glass slide is plastic barrier. During saving glass slides into bottle **ENSURE** that the top of the slide with periphyton remains above the barrier plastic as in Figure 5.

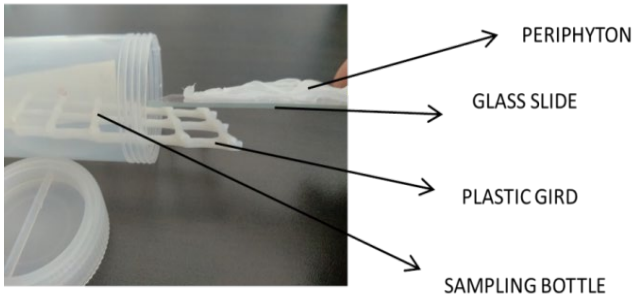

Figure 5: Saving glass slide with periphyton into bottle

- When you are properly settled glass slide into the bottle, add some water into bottle to cover the height of glass slides.
- **Do not forget the way back from the field to fix samples with formaldehyde.**

Protocol for sampling periphyton

Periphyton = small organisms attached to or clinging to plants and other objects projecting above the bottom sediments.

- Each time during collecting periphyton **REQUIRED to WRITE** in the field notebook: a number of samples, date of sampling, and location.
- On the sampling bottle it is also **OBLIGATORY TO WRITE** the number of samples, date of sampling, and location.
- Periphyton samples **from several locations** pick up each time you are picking up glass slides.
- Collect periphyton by scraping the stones or aquatic plants. For scraping use a toothbrush or knife (Fig. 6). Put the stone or aquatic plants in a plastic tub. Add water and scrap surface of the stone. Leave a few minutes to settle scraped material. If there is more water than fit into the bottle, carefully poured off them. The sample of periphyton saves in properly labeled bottle.

Figure 6: Scraping stone surface

- **Do not forget the way back from the field to fix samples with formaldehyde.**