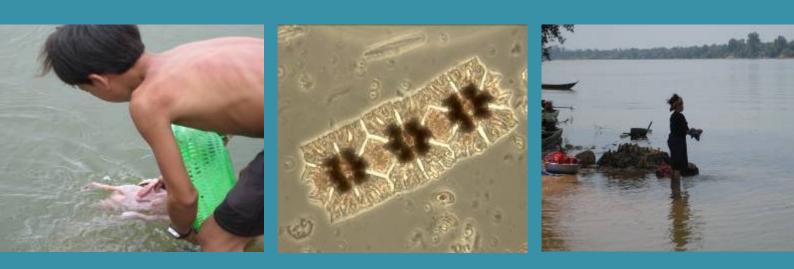


STRIVER TECHNICAL BRIEF

Strategy and methodology for improved IWRM - An integrated interdisciplinary assessment in four twinning river basins

TB No. 12



A limnological study of the Sesan River in Cambodia in the dry season: focus on toxic cyanobacteria and coliform bacteria

Chemical water analyses during the dry period in year 2008 (NE Cambodia) show that the trophic level in Sesan was oligotrophic. Surprisingly, we found that a large portion of the phytoplankton volume in the river consisted of indicator species for a mesotrophic to eutrophic environment; indicating influence from upstream sources. More specifically, cyanobacteria and especially toxic species was detected in addition to high levels of thermo- tolerant coliform bacteria.

The STRIVER Brief series translate the results from the EC FP6-funded STRIVER project into practical and useful information for policy makers and water managers



A limnological study of the Sesan River in Cambodia in the dry season: focus on toxic cyanobacteria and coliform bacteria

Anna Madeleine Tiodolf, Norwegian University of Life Sciences Per Stålnacke, Bioforsk, Norway

Abstract

The Sesan River is one of the largest tributaries of the Mekong River. The river is a major drinking water supply for the local people along river in the Ratanakiri province (North East Cambodia). In 1996, Vietnam started an expansion of hydropower plants in the Sesan River system. This has resulted in large changes downstream with uneven water flow, increased erosion, decreased fish supply and polluted water. Through interviews with inhabitants along the river we learnt that they periodically have health problems after contact with the water in the river. The symptoms are mainly gastric disorders and skin eruptions, but respiratory problems have also been reported. The aim of this study was to analyze the water quality in the Sesan River during the dry season, focusing on the presence of cyanobacteria, algae toxins and thermo-tolerant coliform bacteria (especially the indicator organism *E.coli*). In our study existence of cyanobacteria in the Se San River during the dry season was confirmed. In addition, the algae toxin microcystin was detected in the Sesan River water. In addition, the concentration of the thermo-tolerant coliform bacteria was high; the indicator organism *E.coli* being 10³-10⁶ CFU ml⁻¹ which indicate risk of water borne disease spreading.

References

This STRIVER Technical Brief is based on the following research reports and scientific literature: Tiodolf A. M. 2009 A limnological study in the Se San River in Cambodia during the dry season: focus

on toxic cyanobacteria and coliform bacteria, *Master thesis*, Norwegian University of Life Sciences (In Swedish with abstract in English and Khmer).

Method

Our field visit was conducted during a two-week period in March 2008. Water samples were taken every second day in two different areas along the Sesan River; Adong Meas, 30 kilometers downstream the Vietnamese border, and Veun Sai, about 130 kilometers downstream the Vietnamese border (Fig.1). Water samples were also taken in three Cambodian tributaries. Each study area was visited three times. Water for sampling was taken with a water-sampler or directly in a plastic container from the surface water. In addition algae-samples were taken with a 25 μ m net haul. Quantitative algae-samples and net haul samples were preserved by Lugol's solution (fytofix) for species composition. For analyzing algae toxin, 48 mm GF/C Wathman-filters were filtered with a known amount of water and dried in darkness. To detect *E.coli* selective medium, MLGA and VRBA, were used. The water-samples were filtered through 0,45 μ m cellulose membrane filters and cultivated on the selected medium in up to five days in an incubator-oven at 44°C, distributed by Colifast.

Meach Mean at 3SPN is acknowledged for all practical help during our field study



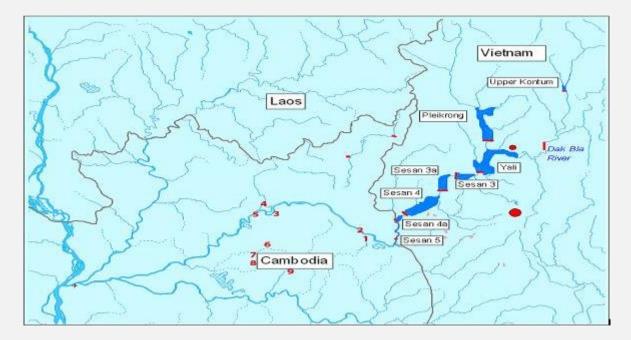


Figure 1. Map over the study area. 1. The tributary O Kup, 2. Adong Meas, 3. Kachon, 4. The tributary Vireak Chey National Park, 5. Veun Sai, 6. The tributary O Tang, 7–9 minor streams and one lake in the Ban Lung area.



Figure 2. Water sampling at Adong Meas (upper left photo: G Bun) and Kachon (upper right photo: M. Tiodolf); meeting with local people in Veun Sai 13th of March 2008 (lower left photo: M. Tiodolf); the tributary river O Kup (lower right photo: M. Tiodolf)



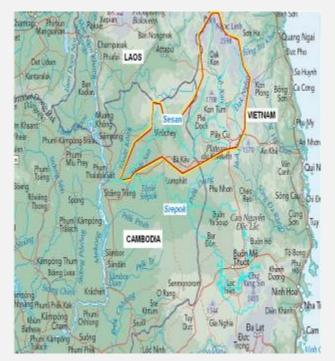


Figure 2. The catchment area of the Sesan River (Berge&Nhung, 2008).

The study area

The Sesan River is one of the largest tributaries of the Mekong River (Fig. 3) and has a drainage area of 17,000 square km, where of 11,000 square km in Vietnam and 6,000 square km in Cambodia. Most population living in the basin are made up of ethnic minority groups that rely heavily on the river for subsistence agriculture, fishing, and developing techniques suited for small-scale water utilization.

Cyanobacteria in the Sesan River

Existence of cyanobacteria in the Sesan River during the dry season was in our study confirmed (Fig. 4). More specifically, colonies of *Microcystis sp.* species (Fig. 5) was established in quantitative analysis of water samples. Colonies of *Microcystis sp.* but also cellular fibres of *Planktothrix sp.* and *Aphanizomenon sp.* was confirmed in the qualitative analysis of net haul samples. Colonies of *Pseudoanabena sp.* have been established in cultures of water samples with Blue-Green agar.

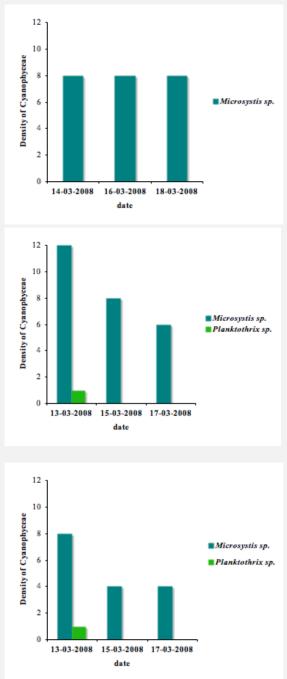


Figure 4. Average density of Cyanobacteria in the qualitative analysis of net haul samples from Adong Meas (upper panel), Kachon (middle panel) and Veun Sai (lower panel). NB! A maximum value of 32 means a total dominance of a particular algae in the sample.



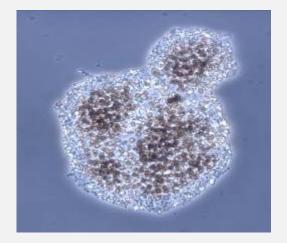


Figure 5. A colony with *Microcystis sp.* from the water samples taken in the Sesan River suggested to be *Microcystis botrys* by Associate Professor Gertrud Cronberg, Lund University. Photo: R. Ptacnik (NIVA)

Cyanotoxins in the Sesan River

Concentrations of the algae toxin microcystin (hepatoxin) (Table 1) exceeded the WHO limit for drinking water (1 μ g L⁻¹) and approach the limit for safe bathing (10 μ g L⁻¹).

The most commonly registered symptoms shown by people exposed to cyanotoxins are allergic reactions i.e. fever, skin irritation and diarrhoea. Microcystin is toxic to the liver and can cause injury or cancer in the liver after a longer period of exposure.

Through interviews with the inhabitants along the Sesan River we learn that irritation of the skin (Fig. 6) and gastric disorders are commonly occurring after bathing in the river, especially during the dry season.

Algae composition

Both the algae and chlorophyll concentrations were different between the main stream and the local tributaries (Fig. 7).

Moreover, the algae composition in the analyzed water samples and net haul samples from the main Sesan River show predominance of the algae groups Dinophyceae, Chrysophyceae and Bacillariophyceae (Fig. 8). Many of the specific species within these groups indicate a euthrophic environment. On contrary, the samples from the local tributary rivers showed dominance of Chrysophyceae and μ -algae (Fig. 9).

Also noteworthy was that cyanobacteria made up a maximum of 3% of the algae composition in the quantitative samples from the main stream (Fig. 8; water samples from Adong Meas) while no cyanobacteria was detected in the local tributaries.



Figure 6. A child with skin-problem living in Adong Meas (2) claimed to be caused by contact with the Sesan River water. Photo: M. Tiodolf

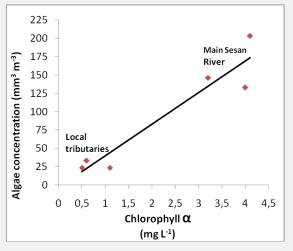
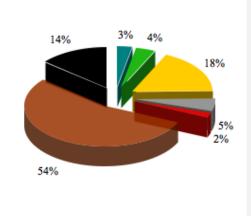
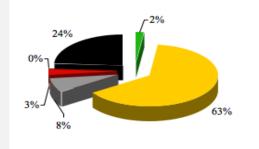
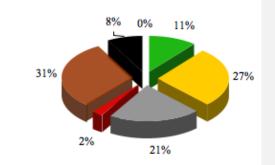


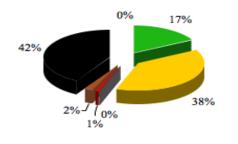
Figure 7. Difference in the algae and chlorophyll a concentrations in water samples from the Sesan River (main stream) compared to water samples from the three tributaries (March 2008).

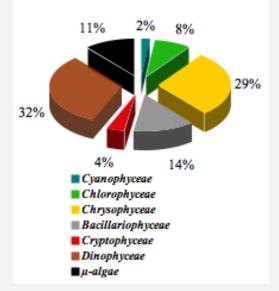


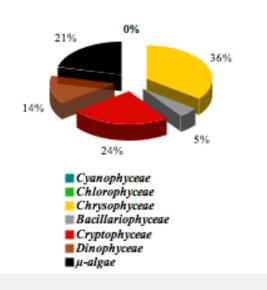












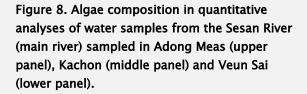


Figure 9. Algae composition in quantitative analyses of water samples from the tributaries O Kup (upper panel), Vireak Chey National Park (middle panel) and O Tang (lower panel).



Thermo-tolerant coliform bacteria

The concentration of thermo-tolerant coliform bacteria was high; the indicator organism *E.coli* being 10^3 - 10^6 CFU ml⁻¹ (Table 2).

This indicates risk of spreading of waterborne diseases. WHO's limit for *E.coli* or thermo tolerant coliform bacteria is that they should not occur in a 100 ml water sample.

Similar levels of bacteria concentration has been found through analysis of water samples in other tropical areas, for example Uganda, South Africa and India. Analyses of river water in Lebanon have shown concentrations up to

6 x 10 ⁴ CFU ml⁻¹ alongside with a significant health effect on people bathing in the river. Bathers got skin reactions, which is one of the main health problems that the locals along the Sesan River experience in connection with bathing.

> "When waters are released, sediment of construction materials, such as rock, soil, concrete and dead animals, such as small fish, snakes, turtles and frogs passed through turbines are factors with long-lasting effects on local people's life downstream That's why the water is called 'cement water' "

CHRES. 2001. Study into impacts of Yali Falls Dam on resettled and downstream communitie. Vietnam National University

Water chemistry

Chemical water analyses show that the Sesan Rivers nutrient level is oligotrophic during the dry period (Table 3). However, the water samples from the tributaries O Kup and O Tang show higher phosphorous concentrations and in some cases indicate a eutrophic trophic level.



Figure 10. Sampling of foam floating on the Sesan River (March 2008). Photo: P. Stålnacke

Conclusions

The following conclusions can be drawn from the sampling in March 2008:

- Occurrence of cyanobacteria and cyanotoxins in the main stream of the Sesan River was confirmed.
- No cyanobacteria in the water samples taken in three different tributaries were detected.
- The algae composition in the main stream of the Sesan River indicate a mesotrophic to eutrophic environment. The reason for this could be that the algae originate from more stagnant water, with higher nutrient concentrations, i.e., originating further upstream in the Sesan River.
- In contrast to the algae composition results, the water chemistry analyses in the main stream of Sesan River show an oligotrophic trophic level.
- According to the WHO limits, the results of this study show the drinking quality of the water in the Sesan River is not suitable for human consumption. More specifically, the content of thermo-tolerant coliform bacteria and the indicator organism *E.coli* along with the levels of cyanotoxin microcystins were found to be too high.



Supplementary data tables

Table 1. Concentrations of the cyanotoxin microcystin in water samples analyzed by an anti-adda ELISA kit (ABRAXIS, product no. 520011) at the NIVA-laboratory (Norway).

| Locality | Date | Filtrated amount of water | Concentration of Microcystin | | |
|--------------------------------------|-------|------------------------------|---------------------------------|--|--|
| | March | | | | |
| | 2008 | (L) | (µg L ⁻¹) | | |
| Tributary | | | | | |
| O Kup (1) | 14 | 1,27 | * | | |
| O Kup (1) | 14 | 1,05 | * | | |
| O Kup (1) | 16 | 1,00 | * | | |
| O Kup (1) | 16 | 1,08 | * | | |
| O Kup (1) | 18 | 1,00 | * | | |
| O Kup (1) | 18 | 1,00 | * | | |
| Se San | | | | | |
| Adong Meas (2) | 14 | 1,20 | 5,23 | | |
| Adong Meas (2) | 14 | 1,30 | 8,39 | | |
| Adong Meas (2) | 16 | 1,62 | * | | |
| Adong Meas (2) | 16 | 1,88 | 6,96 | | |
| Adong Meas (2) | 18 | 1,00 | * | | |
| Adong Meas (2) | 18 | 1,00 | * | | |
| Se San | | | | | |
| Kachon (3) | 13 | 1,00 | * | | |
| Kachon (3) | 13 | 1,00 | * | | |
| Kachon (3) | 15 | 1,17 | * | | |
| Kachon (3) | 15 | 1,52 | 5,58 | | |
| Kachon (3) | 17 | 1,00 | * | | |
| Kachon (3) | 17 | 1,00 | ł | | |
| tributary | | | | | |
| Vireak Chey National Park (4) | 15 | 0,75 (?) | * | | |
| Vireak Chey National Park | 15 | 0,75(1) | | | |
| $\begin{pmatrix} 4 \\ \end{pmatrix}$ | 15 | 0,75 | * | | |
| Vireak Chey National Park (4) | 17 | 0,75 | * | | |
| Vireak Chey National Park | | | | | |
| (4) | 17 | 0,75 | * | | |
| Se San | | | | | |
| Veun Sai (5) | 13 | 0,73 | * | | |
| Veun Sai (5) | 15 | 1,00 | * | | |
| Veun Sai (5) | 15 | 1,05 | 4 | | |
| Veun Sai (5) | 17 | 0,85 | 4 | | |
| Veun Sai (5) | 17 | 0,85 | 4 | | |
| Tributary | | | | | |
| O Tang (6) *) Concentrations be | 13 | 1,00 | * | | |



| Locality | Date | Cultivating | Thermo-tolerant | coliform | bacteria | |
|-------------------------------|-------|-------------|----------------------------|----------------------------|----------------------------|--|
| | March | days | yellow colonies | green colonies | total | |
| | 2008 | 42-44° C | (CFU 100ml ⁻¹) | (CFU 100ml ⁻¹) | (CFU 100ml ⁻¹) | |
| Tributary | | | | | | |
| O Kup (1) | 14 | 3 | * | 33 x 10 ⁵ | 33 x 10 ⁵ | |
| O Kup (1) | 16 | 5 | * | 20 x 10 ⁵ | $20 \ge 10^{5}$ | |
| O Kup (1) | 18 | 1 | | | ## | |
| Se San | | | | | | |
| Adong Meas (2) | 14 | 3# | * | 10 x 10 ⁶ | 10 x 10 ⁶ | |
| Adong Meas (2) | 16 | 5 | * | $17 \ge 10^{5}$ | 17 x 10 ⁵ | |
| Adong Meas (2) | 18 | 2 | 40 x10 ⁵ | * | 40 x10 ⁵ | |
| Se San | | | | | | |
| Kachon (3) | 13 | 2,5# | 21×10^{4} | * | 21×10^{4} | |
| Kachon (3) | 15 | 2 | * | 21 x 10 ⁴ | 21 x 10 ⁴ | |
| Kachon (3) | 17 | 4 | | | ## | |
| Tributary | | | | | | |
| Vireak Chey National park (4) | 15 | 2 | * | 70 x 10 4 | 70 x 10 4 | |
| Vireak Chey National park (4) | 17 | 2 | 9 x 10 ^5 | 8x 10 5 | $17 \ge 10^{5}$ | |
| Se San | | | | | | |
| Veun Sai (5) | 13 | 2# | 40 x 10 ⁴ | * | 40 x 10 ⁴ | |
| Veun Sai (5) | 15 | 2 | * | $20 \ge 10^{5}$ | $20 \ge 10^{5}$ | |
| Veun Sai (5) | 17 | 2 | | | ## | |
| Tributary | | | | | | |
| O Tang (6) | 13 | 2,5# | 85 x 10 ⁴ | * | 85 x 10 4 | |
| Other watersystems in | | | | | | |
| the Ban Lung area | | | | | | |
| Stream water (8) | 17 | 1 | 14 x 10 ⁴ | $1 \ge 10^{-4}$ | 15 x 10 ⁴ | |
| Kan San (9) | 16 | 5 | 90 x 10 ⁴ | * | 90 x 10 ⁴ | |

Table 2. Water samples cultivated on the selective medium MLGA. Green colonies are *E.coli* and yellow colonies are thermo-tolerant coliform bacteria, which are not *E.coli*.

*) No growth of thermo-tolerant coliform bacteria.

#) Samples cultivated in 25-35°C.

##) Not countable cultures due to too high concentrations of bacterial colonies.



| Locality | Date March 2008 | Chl a $(mg L^{-1})$ | TOC $(mg L^{-1})$ | Tot-N (μgN L ⁻¹) | Tot-P (μg P L ⁻¹) | Turbidity (NTU) | Conductivity (mS m ⁻¹) |
|---------------------------------------------------------------|--------------------|---------------------|--------------------------|----------------------------------------|-----------------------------------------|---------------------------|---------------------------------------|
| | | | | | | | |
| O Kup (1) | 14 | 0,5 | 1,4 | 130 | 31,6 | 12,1 | 4,3 |
| O Kup (1) | 16 | 0,4 | 1,5 | 230 | 32 | 15,7 | 4,5 |
| O Kup (1) | 18 | 0,5 | 1,6 | 116 | 23,2 | 6,9 | 4,4 |
| Se San | | | | | | | |
| Adong Meas (2) | 14 | 3,2 | 0,8 | 160 | 3,5 | 1,8 | 4,0 |
| Adong Meas (2) | 16 | 3 | 0,8 | 160 | 3,9 | 2,9 | 4,1 |
| Adong Meas (2) | 18 | 2,7 | 1,1 | 190 | 4,9 | 1,3 | 4,2 |
| Se San | | | | | | | |
| Kachon (3) | 13 | 4 | 0,8 | 160 | 4,2 | 1,6 | 3,9 |
| Kachon (3) | 15 | 4,2 | 0,7 | 140 | 3,2 | 3,1 | 3,8 |
| Kachon (3) | 17 | 3,8 | 0,8 | 150 | 4,2 | 2,2 | 3,9 |
| Tributary | | | | | | | |
| Vireak Chey national park (4) Vireak Chey national park | 15 | 1,1 | 0,8 | 110 | 3,5 | 1,9 | 2,5 |
| (4) | 17 | 1,4 | 0,9 | 110 | 3,5 | 2,4 | 2,5 |
| Se San | | | | | | | |
| Veun Sai (5) | 13 | 4,1 | 0,8 | 170 | 4,6 | 2,8 | 3,9 |
| Veun Sai (5) | 15 | 4,2 | 0,7 | 130 | 3,9 | 3,0 | 4,0 |
| Veun Sai (5) | 17 | 3,9 | 0,7 | 150 | 4,2 | 1,7 | 4,0 |
| Tributary | | | | | | | |
| O Tang (6) | 13 | 0,6 | 1,6 | 200 | 10,5 | 3,8 | 10,1 |

Table 3. Chemical analyses of water quality at various sites (March 2008). Laboratory analyses performed at Norwegian University of Life Sciences.

Table 4. Selected results from metal compounds at various sites (March 2008). Laboratory analyses performed at Norwegian University of Life Sciences.

| Locality | Date | Aluminium | Copper | Iron | Lead | Zink |
|-------------------------------|-------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| | | Al | Cu | Fe | Pb | Zn |
| | March | OES | MS | OES | MS | MS |
| | 2008 | (µg L ⁻¹) | (µg L ⁻¹) | (mg L ⁻¹) | (µg L ⁻¹) | (µg L ⁻¹) |
| Tributaries | | | | | | |
| O Kup (1) | 14 | 80 | 1,4 | 0,94 | 0,21 | 2,9 |
| O Kup (1) | 16 | 120 | 2,4 | 1,1 | 0,36 | 3,9 |
| O Kup (1) | 18 | 120 | 4,5 | 1,3 | 0,45 | 12 |
| Vireak Chey National Park (4) | 15 | 30 | 0,3 | 0,55 | 0,35 | 2,1 |
| Vireak Chey National Park (4) | 17 | 40 | 0,4 | 0,55 | 0,18 | 1,2 |
| Se San | | | | | | |
| Veun Sai (5) | 13 | 100 | 0,9 | 0,2 | 0,22 | 0,9 |
| Veun Sai (5) | 15 | 90 | 0,8 | 0,21 | 0,24 | 1,4 |
| Veun Sai (5) | 17 | 50 | 0,8 | 0,12 | 0,13 | 1,1 |





The STRIVER Policy and Technical Brief series translate the results from the project into practical and useful information for policy makers and water managers.

The Briefs are also available online: www.striver.no

About STRIVER

STRIVER- Strategy and methodology for improved IWRM - An integrated interdisciplinary assessment in four twinning river basins is a three year EC funded project 2006-2009 under the 6th framework programme (FP6) coordinated jointly by Bioforsk and NIVA. The point of departure for STRIVER is the lack of clear methodologies and problems in operationalisation of Integrated Water Resource Management (IWRM) as pointed out by both the scientific and management communities.13 partners from 9 countries participate as contractual partners in addition to an external advisory board.

Title of project:

Strategy and methodology for improved IWRM - *An integrated interdisciplinary assessment in four twinning river basins* (STRIVER)

Instrument: SUSTDEV-2005-3.II.3.6: Twinning European/thirdcountries river basins.Contract number: 037141Start date of project: July 2006Duration: 36 months

Project funded by the European Commission within the Sixth Framework Programme (2002-2006)

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Front-cover photos: *Rinsing a chicken along the Sesan River in Cambodia (Left photo: M. Tiodolf), Micrasterias foliacea algae in the Sesan River (Middle photo: R. Ptacnik), local woman washing clothes along the Sesan River (Right photo: M. Tiodolf)*

Launch-date: 17 August, 2009