“Antibiotics in sediments and antibiotic resistance genes in the intensive shrimp farms in Southern Vietnam” (Vietnam, SM Calls 2014 and 2015)

Swiss institution : Luiz Felippe de Alencastro & Pierre Rossi (EPFL/ENAC/Central Environmental Laboratory)
Partner institution : Quoc Tuc Dinh (VNU/ HCMUT/Faculty of Environmental and Natural Resources ) & Thu-Hang Pham (VNU/Institut for Environment and Resources)
Main present fields of work:

- Trace chemical analysis in environmental matrices.
- Development of analytical methods. Passive sampling.
- Importance of water treatment plants for the contamination of ecosystems by the micro-pollutants.
- Molecular environmental microbiology.
- Environmental pollution by microplastics.
2. GR-CEL projects with developing countries

Since 1986: participation in teaching and research projects with developing countries, like Honduras, Madagascar, Burkina-Faso, Romania and Vietnam.

*Installation of laboratories, PhD, Master students, teaching, ...*

**Projects with Vietnam**


1996–2008: CEFINEA Project (Vietnam). Developement of the South Vietnam National Center at Ho Chi Minh City for Environmental Research and Education. SDC

Réseau d’Excellence des Sciences de l’Ingénieur de la Francophonie & Centre asiatique de recherche sur l’eau
4. Vietnamese partners & others

**Dr. Quoc Tuc DINH**
Head of Dept. of Health, Safely and Environment
Faculty of Environment and Natural Resources
- Environmental pollution by organic micro-pollutants. Antibiotics
- Analytical chemistry
- Wastewater treatment

**Dr. Thi-Thu-Hang PHAM**
Head of Dept. of Environmental Biotechnology
Institute of Environment and Resources
- Biotechnology
- DNA extraction and purification techniques, PCR, fingerprinting techniques (DGGE), cloning and sequencing
- Bacterial detection. Classical culture techniques, strain isolation, biochemical test

And the collaboration of:
- **Dr. Lorenzo Spadini** (Grenoble INP - Laboratoire d'étude des Transferts en Hydrologie et Environnement)
- Clément Levasseur (Master student EPFL/SSIE) (2015)
- Nicolas Estoppey (PhD UNIL/EPFL) (2014)
3. The project

“Antibiotics in shrimp farms in Vietnam”

1) Transfer of the antibiotics from shrimp farms to the aquatic system (SM-2014 CHF 9’500)
2) Identify antibiotics resistances in the environment (SM-2015 CHF 10’000)
3. The project

Mekong Delta
3. The project

• Vietnam is one of the **largest shrimp exporters** in the world:
  - more than 640 million hectares
  - export value of US $ 3.1 billion

• **Intensive shrimp farms** uses a lot of chemical products like:
  - pesticides to sanitize the shrimp ponds
  - antibiotics for the prevention of diseases
  - effluent water from the intensive shrimp farms are released into natural water bodies without any treatment

• **Passive sampling** has been successfully used for the monitoring of polar organic compounds in aqueous systems in the last years:
  - it enables to calculate time-weighted average concentrations over the sampling periods
  - it provides precious data about pollutant bioavailability because it samples the free dissolved fraction of pollutants

• **The aims** of this study are to develop a passive sampling method with the POCIS (Polar Organic Chemical Integrative Sampler):
  - determine levels of six antibiotics and three pesticides in the surface water
  - improve the suitability of this technic for shrimp farm context
  - compare results to grab sampling and analysis in sediments
4. Method
4. Method

**Passive sampling**
- **POCIS**
  - 200 mg HLB sorbent spiked with reference compounds

**Grab sampling**
- **Sampling**
- **Filtration**
- **Solid phase extraction**

**Sediment analysis**
- **Sediments**
- **Ultrasonic extraction**
- **Solid phase extraction**

**UPLC-MS/MS**
- **Antibiotics:** Trimethoprim, Ciprofloxacin, Enrofloxacin, Erythromycin, Sulfamethoxazol, Sulfamethoxin
- **Pesticides:** Atrazine, Diuron, Metalochlor
5. Results  Antibiotics/pesticides in water in Long An Shrimp Farm

Antibiotics

- Antibiotic concentration fluctuated according to shrimp growth stages
- Sulfamethoxazole was used a large amount during production cycle
- Trimethoprim was also used during production cycle

Pesticides

- Pesticide concentration in channel was higher than shrimp pond
- ATZ, DRN could be already in surface water
5. Results  Antibiotics/pesticides in water in Can Gio Shrimp Farm

- Antibiotic concentration in shrimp pond was generally higher than channel.
- Used antibiotics and their quantity depend on shrimp farms and growth stage.
- Ciprofloxacin was used in large amounts during production cycles.
- Antibiotic quantity increased when shrimp got diseases.
6. Results

AB 1 multiresistant bacterial isolates: identification and characterization of their resistance capacity.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number of ARGs</th>
<th>Plasmid (kbp)</th>
<th>Plasmid numbers</th>
<th>Best match</th>
<th>% Identity</th>
<th>NCBI accession number</th>
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<td>4</td>
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<td>Corynebacterium sp.</td>
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- Presence of large plasmids encoding for multiple resistances
- Pathogenic strains (shrimps and man), such as Klebsiella sp. and Corynebacterium sp.
- Resistances against sulfonamide, β-lactams, aminoglycosides, macrolides, tetracyclines, fluoroquinolones, phenics, polymyxins, pleuromutilins, lincosamides, sulfamides, diaminopyrimidine (trimethoprim)
6. Results

Quantified *sul2* genes in total DNA directly extracted from environmental samples.

Copy number of the resistance gene *Sul2* (resistance to sulfonamide) in environmental samples. (A) Sludge, (B) surface water. C: effluent channel of the shrimp farm to the river, R: Vam Co Dong River, an effluent of the Mekong River.
6. Conclusion

- POCIS shown to be suitable for monitoring antibiotics and pesticides in shrimp farm context
- Compared to grab sampling, POCIS provided average concentrations over the sampling period and took into account episodic pollution
- Sampler protection must however be improved to avoid theft
- Used antibiotics and their quantity depend on shrimp farms and growth stage
- Pesticides were found in water and sediments. Monitoring during pond water treatment should be carried out
- Results of sediments analysis provide additional information (potential previous emissions)
- High concentration of antibiotics were detected and potential risk for public heath (antibiotic resistance) should be considered as pond water is discharged to the environment at the end of shrimp growth
- High amounts of AB resistance genes were quantified, as well as a large variety of genes targetting a large panel of ABs.
7. Valorisation


**TdM EPFL à HCMC:** *Suivi de la pollution des eaux due à l’élevage de crevettes dans le Sud du Vietnam : Cas des antibiotiques et des pesticides.* Clément Levasseur, 07/2015

**TdM à HCMC:** *Occurrence and fate of antibiotics in the Saigon river.* Nguyễn Trọng An. 12/2015


**Articles in preparation:**
- *Frequency and diversity of antibiotic multiresistant bacteria in the effluent of a shrimp farming facility.* Long An, Viet Nam. Thi-Thu-Hang Pham, Quoc-Tuc Đinh, Pierre Rossi and Luiz Felippe de Alencastro.
- *Determination of pesticides and antibiotics in shrimp farms in South Vietnam using POCIS.* Clément Lavasseur, Quoc-Tuc Đinh, Thi-Thu-Hang Pham, Nicolas Estoppey, Luiz Felippe de Alencastro.
8. From Seed Money to “BIGGER” projects...

According to Vietnamese partners, after our SM-14/15 they obtained the following results:

Dr. Tuc Dinh:
- "Bourse d’excellence de la Confédération Suisse" à Le Nguyen Thien Kim, internship at EPFL from 09/2015 till 09/2016. 24’000 CHF. Done.
- Project “Development the Passive Sampling for analyse of antibiotics in waters”. Submitted to the Vietnam National University, 2015. 23’000 US$. Accepted.
- Project “Assessment of transfer of trace metals and antibiotics in the Saigon River: water, sediment and biota ». Submitted to DORE HCM Ville, 2015. 50’000 US$. Rejected.

Dr. Thu-Hang Pham:

All togethther:
- Project « Quantitative occurrence of bacterial antibiotic multi-resistance genes in the delta of the Mekong River (Viet Nam) as a consequence of shrimp farming industry ». Submitted to the Seed Money projects with selected Asian countries funded by the Swiss State Secretariat for Education, Research and Innovation (SERI). 2016. 10’000 CHF. Accepted.
9. About the collaboration

- Excellent!
- Vietnamese partners well formed
- Partners very interested by the collaboration
- Happy to accept master students or internships from EPFL in VNU
- Contact between students very profitable for their students. Improvement of English!
- Rich exchanges

Some difficulties:
- Labs and equipment not always easy to access.
- Old facilities, old equipment.
- Chemicals and consumables not easily available.
- Language.
- Planning of the work.
Acknowledgements

. CODEV - EPFL Cooperation & Development Center

. EPFL - RESCIF

. EtuRescif

. Faculty of Environment and Natural Resources
  Ho Chi Minh City University of Technology (HCMUT)

. CARE (Centre asiatique de recherche sur l’eau)

. Mes collègues du GR-CEL
Thanks for your attention